# Models 392 and 394
# DNA/RNA Synthesizers

## USER'S MANUAL

Applied Biosystems

# *MODELS 392 and 394 DNA/RNA SYNTHESIZERS*
# USER'S MANUAL

Part Number 901237
Revision C
May 1991

Applied Biosystems

## How To Start

Begin by reading Section 1, Introduction. It contains vital information and will help you understand how the manual is organized and how to use it. Next, refer to Section 2, Operation, for instructions on how to prepare for and start a synthesis.

**The Model 392/394 is designed for research purposes only and is not intended for diagnostic use.**

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# SYNTHESIZER FUNCTIONS BY TYPE

## Applied Biosystems Model 392/394 5-Base Instruments

### CONTROL

103 - Wait
104 - Interrupt
105 - Start Detrityl
106 - Begin
107 - End
108 - Block to Col
109 - Waste - Port
110 - Waste - Bottle
112 - Trityl Advance
113 - End Advance
120 - Advance FC
121 - Relay 1 On
122 - Relay 1 Off
123 - Relay 1 Pulse*
124 - Relay 2 On
125 - Relay 2 Off
126 - Relay 2 Pulse*
127 - Relay 3 On
128 - Relay 3 Off
129 - Relay 3 Pulse*
130 - Relay 4 On
131 - Relay 4 Off
132 - Relay 4 Pulse*
133 - Relay 5 On
134 - Relay 5 Off

### BOTTLE CONTROL

150 - Bottle01 On
151 - Bottle01 Off
152 - Bottle02 On
153 - Bottle02 Off
154 - Bottle03 On
155 - Bottle03 Off
156 - Bottle04 On
157 - Bottle04 Off
158 - Bottle05 On
159 - Bottle05 Off

### VENTING

100 - 10 Vent
111 - Block Vent
114 - Vent Bases

### PRIME DELIVERY LINES

50 - A to Waste
51 - G to Waste
52 - C to Waste
53 - T to Waste
54 - 5 to Waste
58 - Tet to Waste
59 - 10 to Waste
60 - 11 to Waste
61 - 12 to Waste
62 - 14 to Waste
63 - 15 to Waste
64 - 18 to Waste
101 - Phos Prep
102 - Cap Prep
115 - Prep 10

### ARGON FLUSHES

1 - Block Flush
2 - Reverse Flush
3 - Trityl Flush
4 - Flush to Waste
5 - Flush to Collect
6 - Flush to A
7 - Flush to G
8 - Flush to C
9 - Flush to T
10 - Flush to 5
14 - Flush to Bases
15 - Flush to Tet
16 - Flush to 10
17 - Flush to 11+12
18 - Flush to 14+15
19 - Flush to 18

### COLUMN CONTROL

140 - Column 1 On
141 - Column 1 Off
142 - Column 2 On
143 - Column 2 Off
144 - Column 3 On
145- Column 3 Off
146 - Column 4 On
147 - Column 4 Off

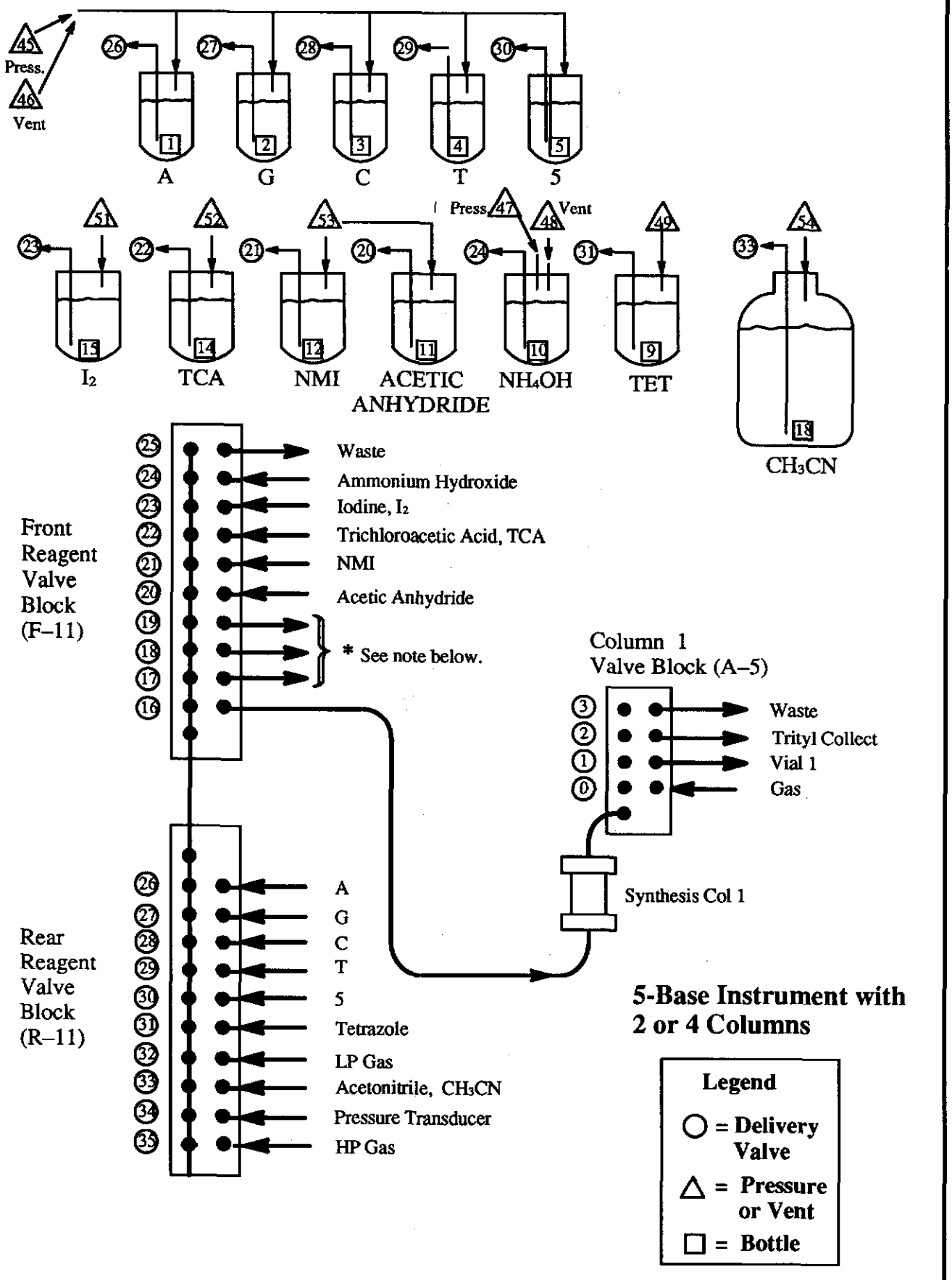
### REAGENTS TO RESERVOIRS

70 - 18 to A
71 - 18 to G
72 - 18 to C
73 - 18 to T
74 - 18 to 5
78 - 18 to Tet
79 - 18 to 10
80 - 18 to 11
81 - 18 to 12
82 - 18 to 11+12
83 - 18 to 14
84 - 18 to 15
85 - 18 to 14+15
86 - 18 to Bases
90 - 15 to A
91 - 15 to G
92 - 15 to C
93 - 15 to T
94 - 15 to Bases

### REAGENTS TO COLUMN

25 - A to Column
26 - G to Column
27 - C to Column
28 - T to Column
29 - 5 to Column
33 - B+Tet to Column
34 - Tet to Column
35 - 5+Tet to Column
36 - 10 to Collect
37 - 11 to Column
38 - 12 to Collect
39 - Cap to Column
40 - 14 to Column
41 - 15 to Column
42 - 18 to Column
43 - Push to Column

* Pulsed relay functions require setting pulse duration using the Main Menu Configuration Relay option.


45
Press.
46
Vent
26
27
28
29
30
1
2
3
4
5
A
G
C
T
5
51
52
53
Press.
47
48
Vent
49
54
23
22
21
20
24
31
33
15
14
12
11
10
9
18
I2
TCA
NMI
ACETIC ANHYDRIDE
NH4OH
TET
CH3CN
Front Reagent Valve Block (F–11)
25
24
23
22
21
20
19
18
17
16
Waste
Ammonium Hydroxide
Iodine, I2
Trichloroacetic Acid, TCA
NMI
Acetic Anhydride
* See note below.
Column 1 Valve Block (A–5)
3
2
1
0
Waste
Trityl Collect
Vial 1
Gas
Synthesis Col 1
Rear Reagent Valve Block (R–11)
26
27
28
29
30
31
32
33
34
35
A
G
C
T
5
Tetrazole
LP Gas
Acetonitrile, CH3CN
Pressure Transducer
HP Gas
5-Base Instrument with 2 or 4 Columns
Legend
○ = Delivery Valve
△ = Pressure or Vent
□ = Bottle
* Note: Only the column valve block for Column 1 is shown. Your synthesizer will be equipped with either two or four columns.

# SYNTHESIZER FUNCTIONS BY TYPE
## Applied Biosystems Model 392/394 8-Base Instruments

### CONTROL
103 - Wait
104 - Interrupt
105 - Start Detrityl
106 - Begin
107 - End
108 - Block to Col
109 - Waste - Port
110 - Waste - Bottle
112 - Trityl Advance
113 - End Advance
120 - Advance FC
121 - Relay 1 On
122 - Relay 1 Off
123 - Relay 1 Pulse*
124 - Relay 2 On
125 - Relay 2 Off
126 - Relay 2 Pulse*
127 - Relay 3 On
128 - Relay 3 Off
129 - Relay 3 Pulse*
130 - Relay 4 On
131 - Relay 4 Off
132 - Relay 4 Pulse*
133 - Relay 5 On
134 - Relay 5 Off

### BOTTLE CONTROL
150 - Bottle01 On
151 - Bottle01 Off
152 - Bottle02 On
153 - Bottle02 Off
154 - Bottle03 On
155 - Bottle03 Off
156 - Bottle04 On
157 - Bottle04 Off
158 - Bottle05 On
159 - Bottle05 Off
160 - Bottle06 On
161 - Bottle06 Off
162 - Bottle07 On
163 - Bottle07 Off
164 - Bottle08 On
165 - Bottle08 Off

140 - Column 1 On
141 - Column 1 Off
142 - Column 2 On

### PRIME DELIVERY LINES
50 - A to Waste
51 - G to Waste
52 - C to Waste
53 - T to Waste
54 - 5 to Waste
55 - 6 to Waste
56 - 7 to Waste
57 - 8 to Waste
58 - Tet to Waste
59 - 10 to Waste
60 - 11 to Waste
61 - 12 to Waste
62 - 14 to Waste
63 - 15 to Waste
64 - 18 to Waste
101 - Phos Prep
102 - Cap Prep
115 - Prep 10

### VENTING
100 - 10 Vent
111 - Block Vent
114 - Vent Bases

### ARGON FLUSHES
1 - Block Flush
2 - Reverse Flush
3 - Trityl Flush
4 - Flush to Waste
5 - Flush to Collect
6 - Flush to A
7 - Flush to G
8 - Flush to C
9 - Flush to T
10 - Flush to 5
11 - Flush to 6
12 - Flush to 7
13 - Flush to 8
14 - Flush to Bases
15 - Flush to Tet
16 - Flush to 10
17 - Flush to 11+12
18 - Flush to 14+15
19 - Flush to 18

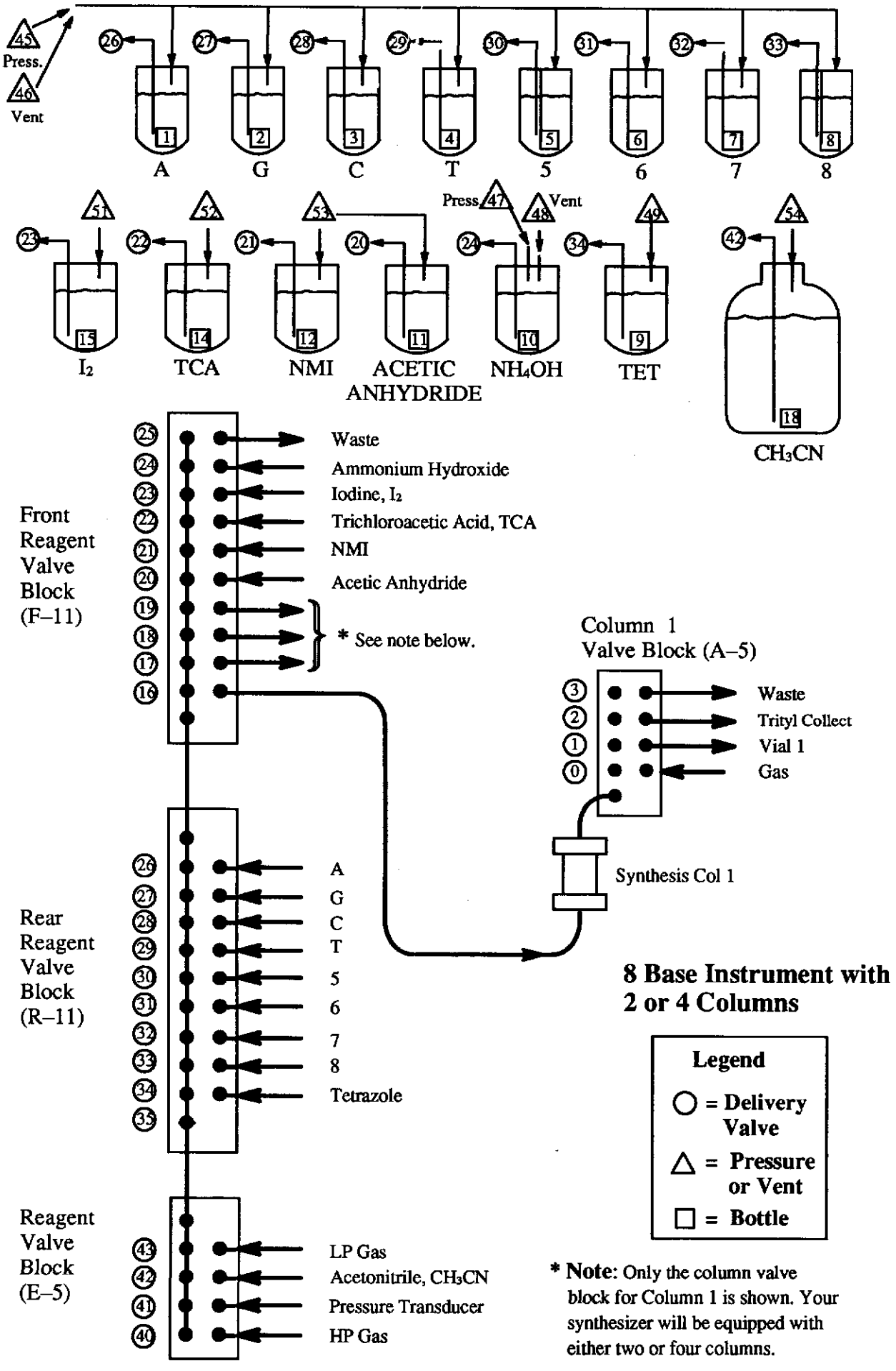
### COLUMN CONTROL
143 - Column 2 Off
144 - Column 3 On
145- Column 3 Off

### REAGENTS TO RESERVOIRS
70 - 18 to A
71 - 18 to G
72 - 18 to C
73 - 18 to T
74 - 18 to 5
75 - 18 to 6
76 - 18 to 7
77 - 18 to 8
78 - 18 to Tet
79 - 18 to 10
80 - 18 to 11
81 - 18 to 12
82 - 18 to 11+12
83 - 18 to 14
84 - 18 to 15
85 - 18 to 14+15
86 - 18 to Bases
90 - 15 to A
91 - 15 to G
92 - 15 to C
93 - 15 to T
94 - 15 to Bases

### REAGENTS TO COLUMN
25 - A to Column
26 - G to Column
27 - C to Column
28 - T to Column
29 - 5 to Column
30 - 6 to Column
31 - 7 to Column
32 - 8 to Column
33 - B+Tet to Column
34 - Tet to Column
35 - 5+Tet to Column
36 - 10 to Collect
37 - 11 to Column
38 - 12 to Collect
39 - Cap to Column
40 - 14 to Column
41 - 15 to Column
42 - 18 to Column
43 - Push to Column

146 - Column 4 On
147 - Column 4 Off

* Pulsed relay functions require setting pulse duration using the Main Menu Configuration Relay option.


Press.
Vent
A
G
C
T
5
6
7
8
I2
TCA
NMI
ACETIC ANHYDRIDE
NH4OH
TET
CH3CN
Front Reagent Valve Block (F–11)
Waste
Ammonium Hydroxide
Iodine, I2
Trichloroacetic Acid, TCA
NMI
Acetic Anhydride
* See note below.
Column 1 Valve Block (A–5)
Waste
Trityl Collect
Vial 1
Gas
Synthesis Col 1
Rear Reagent Valve Block (R–11)
A
G
C
T
5
6
7
8
Tetrazole
Reagent Valve Block (E–5)
LP Gas
Acetonitrile, CH3CN
Pressure Transducer
HP Gas
8 Base Instrument with 2 or 4 Columns
Legend
= Delivery Valve
= Pressure or Vent
= Bottle
* Note: Only the column valve block for Column 1 is shown. Your synthesizer will be equipped with either two or four columns.

# MODEL 392/394 USER'S SAFETY INFORMATION

Part No. 901702, Rev. A
May 1991

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# MODEL 392/394 USER'S SAFETY INFORMATION

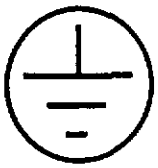
## MODEL 392/394 SAFETY SYMBOLS DEFINED

WARNING: The symbols below are affixed to the Model 392/394. Read and understand the explanations of the symbols before you interact with the instrument in any way.

### Electrical

This symbol indicates the on position of the main power switch.

This symbol indicates the off position of the main power switch.



This protective grounding terminal must be connected to earth ground before any other electrical connections are made to the instrument.

This terminal either receives or delivers alternating current or voltage.

This terminal can receive or supply an alternating and a direct current or voltage.

This terminal may be connected to another instrument's signal ground reference. This is not a protective ground terminal.

For continued protection against the risk of fire, replace fuses only with Listed and Certified fuses of the specified type and rating as shown on the rear of the instrument.

## Hazardous Chemicals

This instrument contains hazardous chemicals in bottles, lines, and valve blocks. Do not operate or interact with the instrument until you have read all the Material Safety Data Sheets for chemicals associated with this instrument.

Contents of the numbered chemical bottles are as follows:

| Bottle | Volume, mL | Chemical |
|---|---|---|
| 1, 2, 3, and 4 | 30 | Anhydrous acetonitrile (for dissolving phosphoramidites |
| 9 | 45 | Tetrazole/acetonitrile |
| 11 | 45 | Acetic anhydride/lutidine/tetradydrofuran |
| 12 | 45 | 1-Methylimidazole/tetrahydrofuran |
| 14 | 450 | Trichloroacetic acid/dichlormethane |
| 15 | 200 | Iodine, water, pyridine, and tetrahydrofuran or tetraethylthiuram disulfide |

The Material Safety Data Sheets for hazardous chemicals supplied by Applied Biosystems, Inc. are presented on p. 7 through p. 35.

## Waste Output

This instrument produces hazardous liquid and vapor waste. Ensure that the waste container is correctly installed, as shown on pp. 5 and 6.

Ensure that harmful vapors are vented to a fume hood or similar facility by means of a vent line as shown on pp. 5 and 6.

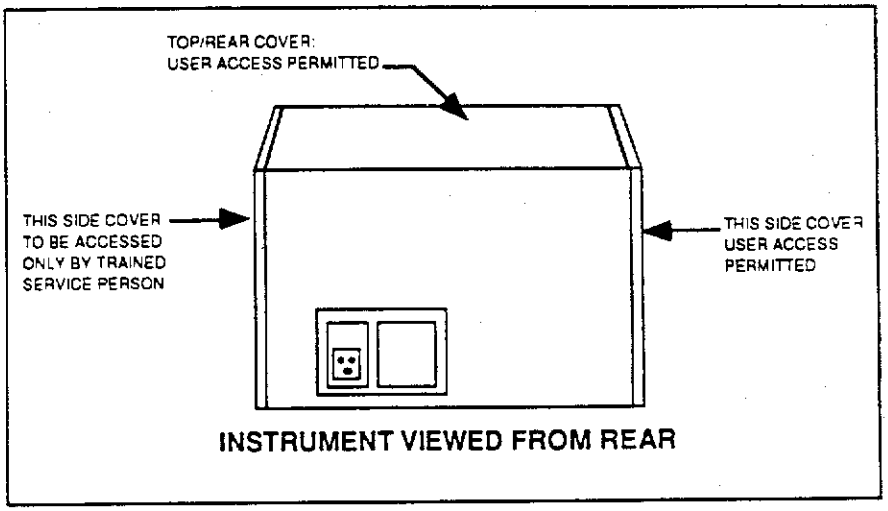
Do not handle or dispose of hazardous waste until you have read the Waste Profile on p. 36. The Waste Profile gives information on composition, physical data, reactivity, spill and leak cleanup, fire and explosion data, health hazards, waste disposal, special protective equipment, and special precautions. Dispose of hazardous waste in accord with all local, state, and federal regulations.

# SECONDARY CONTAINMENT

Provide secondary containment for all hazardous materials and waste.

# ACCESS

The figure below indicates instrument access:



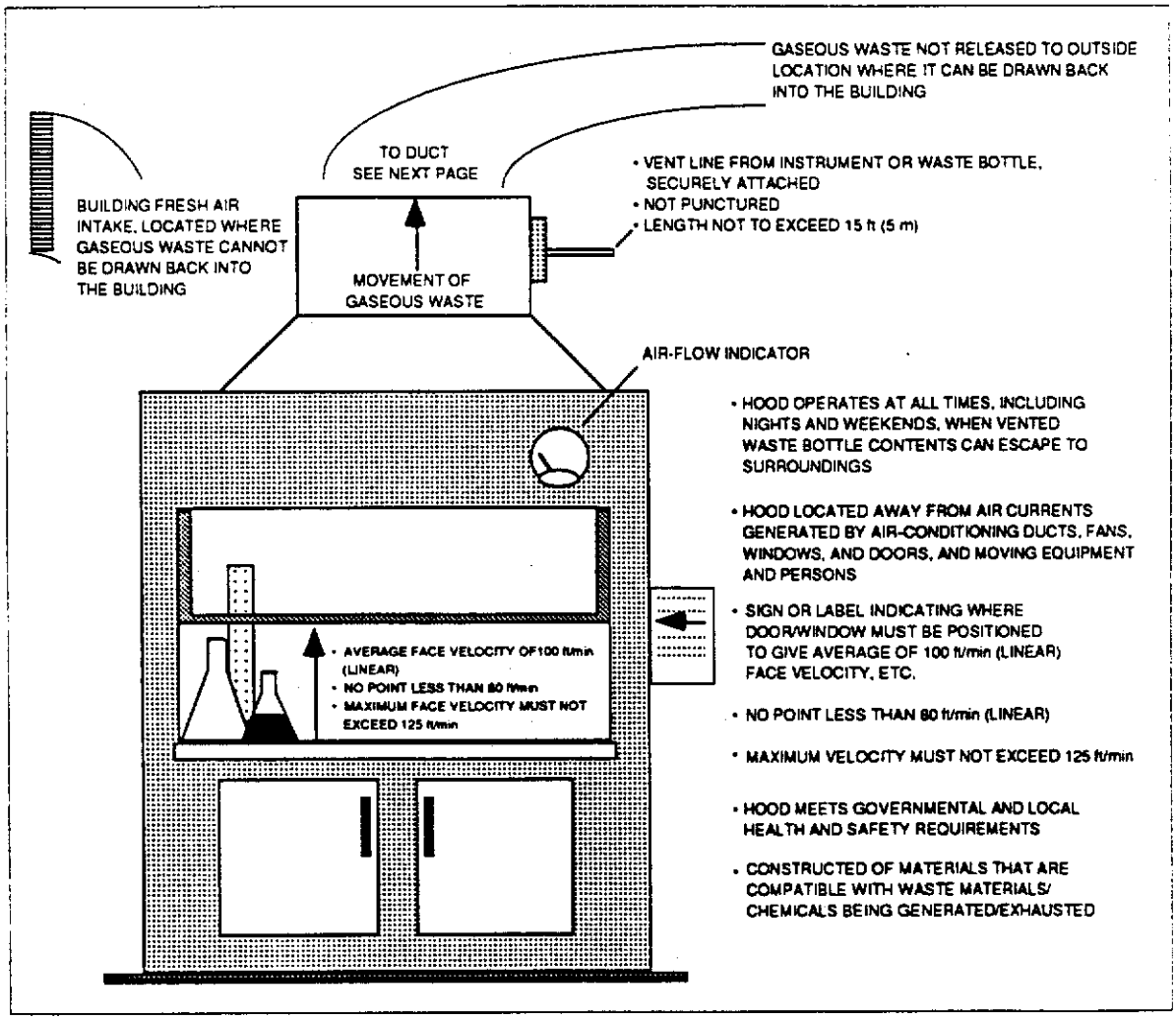
# LABORATORY VENTILATION REQUIREMENTS FOR APPLIED BIOSYSTEMS, INC. INSTRUMENTS

The information presented here reflects U.S. regulations and practices for venting all Applied Biosystems, Inc. instruments to a fume hood or to a duct.

> WARNING: Do not operate a vented instrument unless it is connected in accord with all the requirements presented here. The wastes produced by certain chemicals in Applied Biosystems instruments are hazardous and can cause injury, illness, or death. Handle all liquid, solid, and gaseous products of the instrument as potentially hazardous. Read all applicable material safety data sheets and waste profiles. Dispose of wastes in accord with all applicable health and safety regulations and laws. Always mix/prepare hazardous materials beneath a fume hood.

## Duct for Venting Gaseous Waste Produced by Instrument

*10/90*




- Periodically check and record face velocity (at least yearly).
- Periodically inspect and maintain exhaust system, including fans and motors (at least yearly).

# Duct for Venting Gaseous Waste Produced by Instrument

690

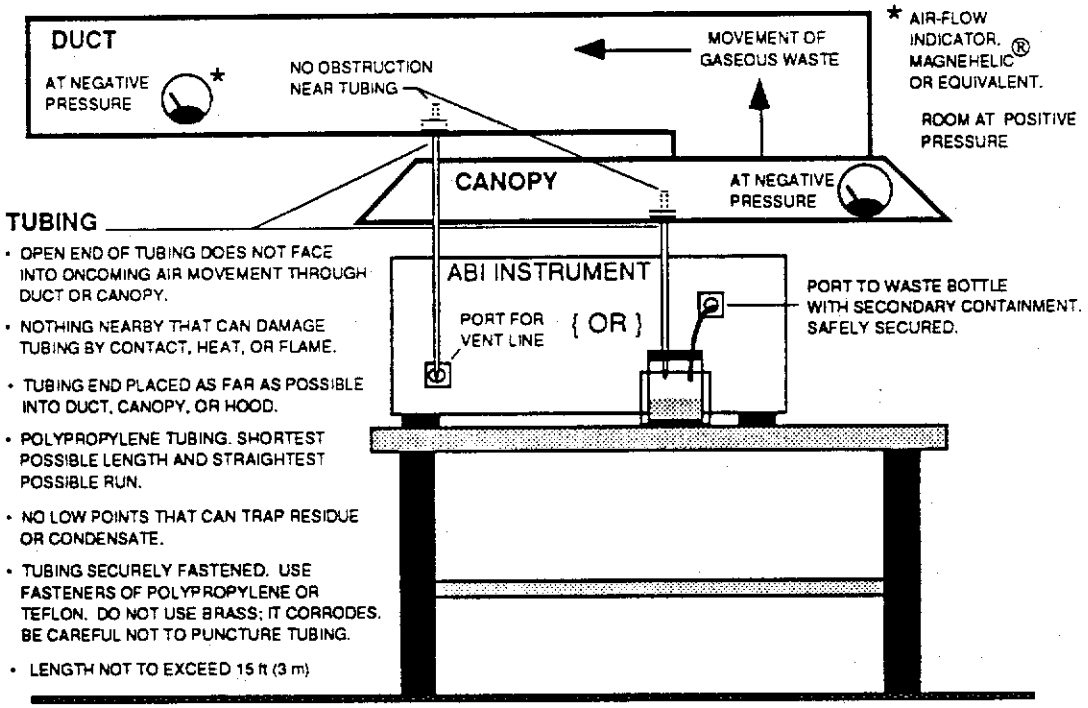
**DUCT-WORK**

- CONSTRUCTED OF MATERIALS COMPATIBLE WITH WASTE MATERIALS BEING GENERATED.
- DUCT EXHAUST SYSTEM OPERATES AT ALL TIMES, INCLUDING NIGHTS AND WEEKENDS, WHEN VENTED WASTE BOTTLE CONTENTS CAN ESCAPE TO SURROUNDINGS.
- NO STRONG OXIDIZERS, BASES, OR OTHER CHEMICALS THAT ARE INCOMPATIBLE WITH GASEOUS WASTE
- VAPOR OR GAS MOVEMENT USUALLY MEASURED AT 1000-2000 fpm*

* Velocities may be considerably less for nonmetal ducts.

**CANOPY**

- AIR EXHAUST OPERATES AT ALL TIMES, INCLUDING NIGHTS AND WEEKENDS, WHEN VENTED WASTE BOTTLE CONTENTS CAN ESCAPE TO SURROUNDINGS.
- DO NOT CONNECT TO A DUCTLESS HOOD OR SYSTEM THAT PURIFIES/FILTERS AIR AND RETURNS IT TO THE ROOM.




**TUBING**

- OPEN END OF TUBING DOES NOT FACE INTO ONCOMING AIR MOVEMENT THROUGH DUCT OR CANOPY.
- NOTHING NEARBY THAT CAN DAMAGE TUBING BY CONTACT, HEAT, OR FLAME.
- TUBING END PLACED AS FAR AS POSSIBLE INTO DUCT, CANOPY, OR HOOD.
- POLYPROPYLENE TUBING. SHORTEST POSSIBLE LENGTH AND STRAIGHTEST POSSIBLE RUN.
- NO LOW POINTS THAT CAN TRAP RESIDUE OR CONDENSATE.
- TUBING SECURELY FASTENED. USE FASTENERS OF POLYPROPYLENE OR TEFLON. DO NOT USE BRASS; IT CORRODES. BE CAREFUL NOT TO PUNCTURE TUBING.
- LENGTH NOT TO EXCEED 15 ft (3 m)

- Periodically inspect and maintain fans and motors (at least yearly).
- Visually inspect tubing periodically for (corrosion, if metal) breakage, crimping, or other damage. Replace the tubing if necessary.
- Remove buildup of condensate or particulates by flushing vent line with pure, dry pressurized gas. Handle and dispose of all wastes as if they were hazardous.
- Always wear a laboratory coat, eye protection, and gloves that resist waste chemicals when you work on waste bottles, exhaust lines/tubing, and liquid traps.

# MATERIAL SAFETY DATA SHEETS

## Acronyms Used

| | |
|---|---|
| ACGIH: | American Conference of Governmental Industrial Hygienisis |
| CAS#: | Chemical Abstract Service Reference Numbers for Specific Pure Chemicals |
| IDLH: | Immediate Danger to Life and Health |
| LEL: | Lower Explosion Limit |
| MSHA: | Mine Safety and Health Administration |
| NIOSH: | National Institute for Occupational Safety and Health |
| OSHA: | Occupational Safety and Health Administration |
| PEL: | Permissible Exposure Limit. The federal OSHA limit usually expressed as a TWA for an 8-hour work shift |
| PPM: | Parts Per Million |
| SCBA: | Self-Contained Breathing Apparatus |
| TLV: | Threshold Limit Value. The ACGIH recommended TWA, usually for an 8-hour work shift. |
| TWA: | Time Weighted Average |
| UEL: | Upper Explosive Limit |

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# MATERIAL SAFETY DATA SHEET

**EMERGENCY PHONE NUMBER** (USA) 415-570-6667, ext. 999 (UK) 0925-825650

## I. Identification

| | | | |
|---|---|---|---|
| PRODUCT NAME | Anhydrous Acetonitrile, 500 ml | PART NUMBER | 400262 |
| CHEMICAL NAME | Acetonitrile | CHEMICAL FAMILY | Nitrile |
| FORMULA | $CH_3CN$ | MOLECULAR WEIGHT | 41 |
| SYNONYMS | DNA Synthesis Reagents #13,16 & 18, methyl cyanide, ethanenitrile, cyanomethane | | |
| DEPARTMENT OF TRANSPORTATION | HAZARD CLASSIFICATION | 3, Flammable liquid, poisonous, DOT12A, UN 1648 | |
| | SHIPPING NAME | Methyl Cyanide, flammable liquid, poisonous | |

## II. Ingredients

| MATERIAL | % | TLV (Units) | PEL (Units) | CAS NUMBER |
|---|---|---|---|---|
| Acetonitrile | 100 | 40 ppm | 40 ppm | 75-05-8 |
| | | | | |
| | | | | |
| | | | | |
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| | | | | |
| | | | | |
| | | | | |

## III. Physical Data

| | | | |
|---|---|---|---|
| BOILING POINT, 760 mmHg | 81.6°C (179°F) | FREEZING POINT | -45°C (-49°F) |
| SPECIFY GRAVITY ($H_2O = 1$) | 0.787 | VAPOR PRESSURE, mmHg | 89 at 25°C |
| VAPOR DENSITY (Air = 1) | 1.3 | SOLUBILITY IN WATER, % BY WEIGHT | miscible |
| PERCENT VOLATILE BY VOLUME | 100% | EVAPORATION RATE (Butyl Acetate = 1) | 5.8 |
| APPEARANCE AND ODOR | Colorless liquid with a sharp ether-like odor | | |

## IV. Fire and Explosion Hazard Data

| | | | | |
|---|---|---|---|---|
| FLASH POINT (method used) | 5.6°C (42°F) | | | (closed cup) |
| FLAMMABLE LIMITS | Lower: | 4.4% | Upper: | 16.0% |
| FIRE EXTINGUISHING MEDIA | Dry chemical, alcohol foam, carbon dioxide or Halon | | | |
| SPECIAL FIRE FIGHTING PROCEDURES | Use self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. | | | |
| UNUSUAL FIRE AND EXPLOSION HAZARDS | Water spray may be ineffective except in cooling containers exposed to fire. | | | |

## V. Health Hazard Data

| | |
|---|---|
| EXPOSURE LIMITS | The current OSHA standard for acetonitrile exposure is 40 ppm in air averaged over 8 hours. See Note 1. |

**EFFECTS OF ACUTE OVEREXPOSURE**

| | |
|---|---|
| SWALLOWING | High concentrations may cause nausea, vomiting, respiratory depression, weakness, chest or abdominal pain, vomiting of blood, convulsions, shock, unconsciousness and death. |
| SKIN | Exposure to the liquid may cause skin irritation. |
| INHALATION | May cause a slight flushing of the face, a feeling of chest tightness and irritation of the nose and throat. |
| EYES | May cause irritation. |

**EFFECTS OF CHRONIC OVEREXPOSURE**

No chronic disease has been reported. May cause respiratory irritation.

**OTHER HEALTH HAZARDS**

Points of systemic attack include kidneys, liver, lungs, skin, eyes, central nervous system and cardiovascular system. See Note 2.

| CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN | NTP No | IARC No | OSHA No |
|---|---|---|---|

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY CHEMICAL OVEREXPOSURE

A history of fainting spells or convulsive disorders may present an added risk to persons working with toxic nitriles.

**EMERGENCY AND FIRST AID PROCEDURES**

| | |
|---|---|
| SWALLOWING | If conscious, give large quantities of water immediately and induce vomiting. Get medical attention immediately. Do not induce vomiting if unconscious. |
| SKIN | Remove contaminated clothing. Flush the contaminated area with large volumes of water for 15 minutes. Seek medical attention immediately. |
| INHALATION | Provide fresh air and rest. If breathing is difficult, provide oxygen and get medical attention immediately. |
| EYES | Flush eyes immediately with large amounts of water for 15 minutes. Get medical attention immediately. |

| | |
|---|---|
| NOTES TO PHYSICIAN | See Note 2. |

## VI. Reactivity Data

STABILITY:

STABLE [X]

UNSTABLE [ ]

CONDITIONS TO AVOID

Avoid contact with strong oxidizers.

INCOMPATIBILITY (materials to avoid) Contact with strong oxidizers may cause fire or explosion.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS Burning may release toxic vapors and gases, including carbon monoxide, oxides of nitrogen, and hydrogen cyanide.

HAZARDOUS POLYMERIZATION

MAY OCCUR [ ]

WILL NOT OCCUR [X]

CONDITIONS TO AVOID

See Section IX.

## VII. Spill or Leak Procedures

STEPS TO BE TAKEN

Avoid inhalation and skin contact. Wear protective clothing. Remove all ignition sources. Ventilate area of spill or leak. See Note 3.

WASTE DISPOSAL METHOD This reagent should be disposed of as a regulated hazardous waste by a permitted hazardous waste management facility in accordance with federal, state, and local regulations.

## VIII. Special Protective Equipment

RESPIRATORY PROTECTION (Specify Type)

An MSHA- or NIOSH-approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for emergencies and high concentrations.

VENTILATION

Handle within a well-ventilated area. Minimize open exposure to air.

PROTECTIVE GLOVES

Butyl rubber or similar type resistant to solvents and petroleum products.

EYE PROTECTION

Safety glasses with side shields, monogoggles or face shield.

OTHER PROTECTIVE EQUIPMENT

As necessary to prevent skin contact.

## IX. Special Precautions

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE

Handle as a flammable liquid. Use with adequate ventilation. Do not breathe vapor. Store in a secure, well ventilated location suitable for flammable materials. Keep away from heat, sparks and flame.

OTHER PRECAUTIONS

Eye wash and safety shower should be in area of use. This reagent will attack many forms of rubber, plastics, coatings and finishes.

## X. Additional Information

Note 1: For acetonitrile the STEL is 60 ppm in air. The IDLH is 4,000 ppm. The $LD_{50}$ orally in rats is 3.8 g/kg. Prolonged exposure may cause irritation of the skin.

Note 2: In most cases of systemic acetonitrile poisoning there is a latent period of several hours between exposure and onset of symptoms. It has been thought that the delayed response is due to the slow release of cyanide.

Note 3: Small spills and leaks may be collected with absorbent towels or pads and removed to a well ventilated area away from ignition sources. Acetonitrile must not be allowed to enter confined spaces because of the possibility of explosion.

CAUTION!

THIS PRODUCT IS STRICTLY INTENDED FOR LABORATORY RESEARCH PURPOSES ONLY.
DISPOSE OF EMPTY BOTTLES PROPERLY.

Material Safety Data Sheet Number: 920262

PREPARED BY: B B Moon

Date Issued: 11/18/85

The opinions expressed herein are those of qualified experts within Applied Biosystems, Inc., information from our suppliers and data presented in various technical publications. We believe that the information contained herein is current as of the date of this Material Safety Data Sheet. It is the user's responsibility to determine the suitability of this information for the adoption of safety precautions as may be necessary. We reserve the right to revise Material Safety Data Sheets from time to time as new technical information becomes available.

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# MATERIAL SAFETY DATA SHEET

**EMERGENCY PHONE NUMBER** (USA) 415-570-6667, ext. 999 (UK) 0925-825650

## I. Identification

| | | | |
|---|---|---|---|
| PRODUCT NAME | Tetrazole/Acetonitrile, 180 ml | PART NUMBER | 400606 |
| CHEMICAL NAME | N/A | CHEMICAL FAMILY | Mixture |
| FORMULA | NHN:NCH:N in $CH_3CN$ | MOLECULAR WEIGHT | N/A |
| SYNONYMS | DNA Synthesis Reagent #9, Activator, Tetrazole Reagent | | |
| DEPARTMENT OF TRANSPORTATION | HAZARD CLASSIFICATION: 3, Flammable liquid, poisonous, DOT12A, UN 1992 | | |
| | SHIPPING NAME: Flammable liquid, poisonous, n.o.s. | | |

## II. Ingredients

| MATERIAL | % | TLV (Units) | PEL (Units) | CAS NUMBER |
|---|---|---|---|---|
| Tetrazole | 4 | N/A | N/A | N/A |
| Acetonitrile | 96 | 40 ppm | 40 ppm | 75-05-8 |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

## III. Physical Data

| | | | |
|---|---|---|---|
| BOILING POINT, 760 mmHg | N/A | FREEZING POINT | N/A |
| SPECIFY GRAVITY ($H_2O$ = 1) | N/A | VAPOR PRESSURE, mmHg | N/A |
| VAPOR DENSITY (Air = 1) | 1.3 (acetonitrile) | SOLUBILITY IN WATER, % BY WEIGHT | 100% |
| PERCENT VOLATILE BY VOLUME | 96% | EVAPORATION RATE (Butyl Acetate = 1) | 5.8 |
| APPEARANCE AND ODOR | Colorless liquid with an ether-like odor | | |

## IV. Fire and Explosion Hazard Data

| | | | | |
|---|---|---|---|---|
| FLASH POINT (method used) | Acetonitrile only: 5.6°C (42°F) | | | (closed cup) |
| FLAMMABLE LIMITS | Lower: 4.4% | | Upper: | 16.0% |
| FIRE EXTINGUISHING MEDIA | Dry chemical, alcohol foam, carbon dioxide or Halon | | | |
| SPECIAL FIRE FIGHTING PROCEDURES | Use self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. | | | |
| UNUSUAL FIRE AND EXPLOSION HAZARDS | Water spray may be ineffective except in cooling containers exposed to fire. | | | |

## V. Health Hazard Data

| | |
|---|---|
| EXPOSURE LIMITS | The current OSHA standard for acetonitrile exposure is 40 ppm in air averaged over 8 hours. See Note 1. |

### EFFECTS OF ACUTE OVEREXPOSURE

| | |
|---|---|
| SWALLOWING | High concentrations may cause nausea, vomiting, respiratory depression, weakness, chest or abdominal pain, vomiting of blood, convulsions, shock, unconsciousness and death. |
| SKIN | Exposure to the liquid may cause irritation. |
| INHALATION | May cause a slight flushing of the face, a feeling of chest tightness and irritation of the nose and throat. |
| EYES | May cause irritation. |

### EFFECTS OF CHRONIC OVEREXPOSURE

No chronic disease has been reported for acetonitrile or tetrazole.

### OTHER HEALTH HAZARDS

Points of attack include kidneys, liver, lungs, skin, eyes, central nervous system and cardiovascular system. See Note 2.

| CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN | NTP No | IARC No | OSHA No |
|---|---|---|---|

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY CHEMICAL OVEREXPOSURE

A history of fainting spells or convulsive disorders may present an added risk to persons working with toxic nitriles.

### EMERGENCY AND FIRST AID PROCEDURES

| | |
|---|---|
| SWALLOWING | If conscious, give large volumes of water immediately and induce vomiting. Get medical attention immediately. Do not induce vomiting if unconscious. |
| SKIN | Remove contaminated clothing. Flush the contaminated area with large volumes of water for 15 minutes. Seek medical attention immediately. |
| INHALATION | Provide fresh air and rest. If breathing is difficult, provide oxygen and get medical attention immediately. |
| EYES | Flush eyes immediately with large amounts of water for at least 15 minutes. Seek medical attention immediately. |

| | |
|---|---|
| NOTES TO PHYSICIAN | See Note 2. |

## VI. Reactivity Data

STABILITY:

STABLE [X]

UNSTABLE [ ]

CONDITIONS TO AVOID

Avoid contact with strong oxidizers, metals, or metal salts. Tetrazole may form explosive metal salts.

INCOMPATIBILITY (materials to avoid) Contact with strong oxidizers may cause fire or explosion.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS Burning may release toxic vapors and gases, including carbon monoxide, oxides of nitrogen, and hydrogen cyanide.

HAZARDOUS POLYMERIZATION

MAY OCCUR [ ]

WILL NOT OCCUR [X]

CONDITIONS TO AVOID

See Section IX.

## VII. Spill or Leak Procedures

STEPS TO BE TAKEN Avoid inhalation and skin contact. Wear protective clothing. Remove all ignition sources. Ventilate area of spill or leak. See Note 3.

WASTE DISPOSAL METHOD This reagent should be disposed of as a regulated hazardous waste by a permitted hazardous waste management facility in accordance with federal, state, and local regulations.

## VIII. Special Protective Equipment

| | |
|---|---|
| RESPIRATORY PROTECTION (Specify Type) | An MSHA- or NIOSH-approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for emergencies and high concentrations. |
| VENTILATION | Handle within a well-ventilated area. Minimize open exposure to air. |
| PROTECTIVE GLOVES | Butyl rubber or similar type resistant to solvents and petroleum products. |
| EYE PROTECTION | Safety glasses with side shields, monogoggles or face shield. |
| OTHER PROTECTIVE EQUIPMENT | As necessary to prevent skin contact. |

## IX. Special Precautions

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE

Handle as a flammable liquid. Use with adequate ventilation. Do not breathe vapor. Store in a secure, well ventilated location suitable for flammable materials. Keep away from heat, sparks and flame.

OTHER PRECAUTIONS Eye wash and safety shower should be in area of use. This reagent will attack many forms of rubber, plastics, coatings and finishes.

## X. Additional Information

Note 1: For acetonitrile the STEL is 60 ppm in air. The IDLH is 4,000 ppm. The $LD_{50}$ orally in rats is 3.8 g/kg. Prolonged exposure may cause irritation of the skin. No specific data is available for tetrazole.

Note 2: In most cases of systemic acetonitrile poisoning there is a latent period of several hours between exposure and onset of symptoms. It has been thought that the delayed response is due to the slow release of cyanide.

Note 3: Small spills and leaks may be collected with absorbent towels or pads and removed to a well ventilated area away from ignition sources. Acetonitrile must not be allowed to enter confined spaces because of the possibility of explosion.

CAUTION!

THIS PRODUCT IS STRICTLY INTENDED FOR LABORATORY RESEARCH PURPOSES ONLY.
DISPOSE OF EMPTY BOTTLES PROPERLY.

Material Safety Data Sheet Number: 920606

PREPARED BY: R B Moon

Date Issued 11/05/86

The opinions expressed herein are those of qualified experts within Applied Biosystems, Inc., information from our suppliers, and data presented in various technical publications. We believe that the information contained herein is current as of the date of this Material Safety Data Sheet. It is the user's responsibility to determine the suitability of this information for the adoption of safety precautions as may be necessary. We reserve the right to revise Material Safety Data Sheets from time to time as new technical information becomes available.

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# MATERIAL SAFETY DATA SHEET

**EMERGENCY PHONE NUMBER** (USA) 415-570-6667, ext. 999 (UK) 0925-825650

## I. Identification

| | | | |
|---|---|---|---|
| PRODUCT NAME | Acetic Anhydride/Lutidine/tetrahydrofuran, 180 ml | PART NUMBER | 400607 |
| CHEMICAL NAME | N/A | CHEMICAL FAMILY | Mixture |
| FORMULA | $(CH_3CO)_2O/(CH_3)_2C_5H_3N/C_4H_8O$ | MOLECULAR WEIGHT | N/A |
| SYNONYMS | DNA Synthesis Reagent #11, Cap A, Acetic Anhydride Reagent | | |
| DEPARTMENT OF TRANSPORTATION | HAZARD CLASSIFICATION | 3, Flammable liquid, corrosive, DOT12A, UN 2924 | |
| | SHIPPING NAME | Flammable liquid, corrosive, n.o.s. | |

## II. Ingredients

| MATERIAL | % | TLV (Units) | PEL (Units) | CAS NUMBER |
|---|---|---|---|---|
| Acetic anhydride | 10 | 5 ppm | 5 ppm | 108-24-7 |
| 2,6-Lutidine | 10 | N/A | N/A | 108-48-5 |
| Tetrahydrofuran (THF) | 80 | 200 ppm | 200 ppm | 109-99-9 |
| | | | | |
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| | | | | |
| | | | | |
| | | | | |

## III. Physical Data

| | | | |
|---|---|---|---|
| BOILING POINT, 760 mmHg | THF only: 66°C (151°F) | FREEZING POINT | N/A |
| SPECIFY GRAVITY ($H_2O = 1$) | N/A | VAPOR PRESSURE, mmHg | N/A |
| VAPOR DENSITY (Air = 1) | 2.5 (THF) | SOLUBILITY IN WATER, % BY WEIGHT | 100% |
| PERCENT VOLATILE BY VOLUME | 100% | EVAPORATION RATE (Butyl Acetate = 1) | 800 (THF) |
| APPEARANCE AND ODOR | Colorless liquid with a sharp ether-like odor | | |

## IV. Fire and Explosion Hazard Data

| | | | | |
|---|---|---|---|---|
| FLASH POINT (method used) | THF only: -14°C (6°F) | | | (closed cup) |
| FLAMMABLE LIMITS | (THF) Lower: 1.8% | | Upper: | 11.8% |
| FIRE EXTINGUISHING MEDIA | Dry chemical, alcohol foam, carbon dioxide or Halon | | | |
| SPECIAL FIRE FIGHTING PROCEDURES | Use self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. | | | |
| UNUSUAL FIRE AND EXPLOSION HAZARDS | Water spray may be ineffective. Reagent vapor may travel considerable distance to source of ignition. | | | |

## V. Health Hazard Data

| | |
|---|---|
| EXPOSURE LIMITS | The current OSHA standard for THF exposure is 200 ppm averaged over 8 hours. See Note 1. |

### EFFECTS OF ACUTE OVEREXPOSURE

| | |
|---|---|
| SWALLOWING | Harmful if swallowed. May cause severe burns to throat and mucous membranes. May cause liver or kidney damage. |
| SKIN | Causes irritation. May cause severe burns if not washed away immediately. |
| INHALATION | Irritation of mucous membranes and upper respiratory tract. Exposure to high concentrations may cause severe damage to the lungs. |
| EYES | May cause serious burns. |

### EFFECTS OF CHRONIC OVEREXPOSURE

Liver and kidney damage have been reported in laboratory animals exposed to high concentrations of THF. Acetic anhydride vapor may cause chronic eye irritation.

### OTHER HEALTH HAZARDS

Points of attack include eyes, skin, respiratory system and central nervous system. Allergic sensitization of the skin may occur.

| CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: | NTP No | IARC No | OSHA No |
|---|---|---|---|

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY CHEMICAL OVEREXPOSURE

No specific information is available.

### EMERGENCY AND FIRST AID PROCEDURES

| | |
|---|---|
| SWALLOWING | If conscious, give large volumes of water immediately and induce vomiting. Get medical attention immediately. Do not induce vomiting if unconscious. |
| SKIN | Remove contaminated clothing. Flush the contaminated area with large volumes of water for 15 minutes. Seek medical attention immediately. |
| INHALATION | Provide fresh air and rest. If breathing is difficult, provide oxygen and get medical attention immediately. |
| EYES | Flush eyes immediately with large amounts of water for at least 15 minutes. Seek medical attention immediately. |

| | |
|---|---|
| NOTES TO PHYSICIAN | Special attention should be given to skin, eyes and respiratory tract. |

## VI. Reactivity Data

STABILITY:

STABLE [X]

UNSTABLE [ ]

CONDITIONS TO AVOID

Contact with strong oxidizers may cause fire or explosion.

INCOMPATIBILITY (materials to avoid): Water, alcohols, strong aqueous bases, strong oxidizers.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS: Burning may release toxic vapors and gases, including organic acids, carbon monoxide, and oxides of nitrogen.

HAZARDOUS POLYMERIZATION

MAY OCCUR [ ]

WILL NOT OCCUR [X]

CONDITIONS TO AVOID

See Section IX.

## VII. Spill or Leak Procedures

STEPS TO BE TAKEN: Avoid inhalation and skin contact. Wear protective clothing. Remove all ignition sources. Ventilate area of spill or leak. See Note 2.

WASTE DISPOSAL METHOD: This reagent should be disposed of as a regulated hazardous waste by a permitted hazardous waste management facility in accordance with federal, state, and local regulations.

## VIII. Special Protective Equipment

RESPIRATORY PROTECTION (Specify Type): An MSHA- or NIOSH-approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for emergencies and high concentrations.

VENTILATION: Handle within a well-ventilated area. Minimize open exposure to air.

PROTECTIVE GLOVES: Butyl rubber or similar type resistant to THF.

EYE PROTECTION: Safety glasses with side shields, monogoggles or face shield.

OTHER PROTECTIVE EQUIPMENT: As necessary to prevent skin contact.

## IX. Special Precautions

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE

Handle as a flammable liquid. Use with adequate ventilation. Do not breathe vapor. Store in a secure, well ventilated location suitable for flammable materials. Keep away from heat, sparks and flame.

OTHER PRECAUTIONS: Eye wash and safety shower should be in area of use. This reagent will attack many forms of rubber, plastics, coatings and finishes. HANDLE WITH CARE!

## X. Additional Information

Note 1: For the THF the STEL is 250 ppm in air. The IDLH is 20,000 ppm. There is no STEL data for acetic anhydride; the IDHL level is 1000 ppm. There is no specific exposure limit data for 2,6-Lutidine.

Note 2: Small spills and leaks may be collected with absorbent towels or pads and removed to a well ventilated area away from ignition sources. This reagent must not be allowed to enter confined spaces because of the possibility of vapor explosion.

CAUTION!

THIS PRODUCT IS STRICTLY INTENDED FOR LABORATORY RESEARCH PURPOSES ONLY.
DISPOSE OF EMPTY BOTTLES PROPERLY.

Material Safety Data Sheet Number: 920607

Date Issued: 11/05/86

PREPARED BY

The opinions expressed herein are those of qualified experts within Applied Biosystems, Inc., information from our suppliers, and data presented in various technical publications. We believe that the information contained herein is current as of the date of this Material Safety Data Sheet. It is the user's responsibility to determine the suitability of this information for the adoption of safety precautions as may be necessary. We reserve the right to revise Material Safety Data Sheets from time to time as new technical information becomes available.

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Tel: 81-3-264-8191 • Facsimile 81-3-264-8821
Telex 781-02324174

# MATERIAL SAFETY DATA SHEET

**EMERGENCY PHONE NUMBER** (USA) 415-6667, ext. 3333 (UK) 0925-825650

## I. Identification

| | | | |
|---|---|---|---|
| PRODUCT NAME | 1-Methylimidazole/Tetrahydrofuran, 180mL | PART NUMBER | 400785 |
| CHEMICAL NAME | 1-Methylimidazole in Tetrahydrofuran | CHEMICAL FAMILY | Mixture |
| FORMULA | $C_4H_6N_2$ in $C_4H_8O$ | MOLECULAR WEIGHT | N/A |
| SYNONYMS | DNA Synthesis Reagent #12, Cap B, N-Methylimidazole Reagent | | |
| DEPARTMENT OF TRANSPORTATION | HAZARD CLASSIFICATION: Flammable liquid | | |
| | SHIPPING NAME: Tetrahydrofuran, UN2056 | | |

## II. Ingredients

| MATERIAL | % | TLV (Units) | PEL (Units) | CAS NUMBER |
|---|---|---|---|---|
| 1- Methylimidazole (NMI) | 16 | N/A | N/A | 616-47-7 |
| Tetrahydrofuran (THF) | 84 | 200ppm | 200ppm | 109-99-9 |
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| | | | | |

## III. Physical Data

| | | | |
|---|---|---|---|
| BOILING POINT, 760 mmHg | THF only: 66°C (151°F) | FREEZING POINT | N/A |
| SPECIFY GRAVITY ($H_2O$ = 1) | N/A | VAPOR PRESSURE, mmHg | N/A |
| VAPOR DENSITY (Air = 1) | 2.5 (THF) | SOLUBILITY IN WATER, % BY WEIGHT | 100% |
| PERCENT VOLATILE BY VOLUME | 84% | EVAPORATION RATE (Butyl Acetate = 1) | 800 (THF) |
| APPEARANCE AND ODOR | Colorless liquid with an ether-like odor. | | |

## IV. Fire and Explosion Hazard Data

| | | | |
|---|---|---|---|
| FLASH POINT (method used) | THF only: -14°C (6°F) | closed cup | |
| FLAMMABLE LIMITS (THF) | Lower: 1.8% | Upper: 11.8% | |
| FIRE EXTINGUISHING MEDIA | Dry chemical, alcohol foam, carbon dioxide or Halon. | | |
| SPECIAL FIRE FIGHTING PROCEDURES | Use self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. See Note 1. | | |
| UNUSUAL FIRE AND EXPLOSION HAZARDS | Water spray may be ineffective except in cooling containers exposed to fire. | | |

## V. Health Hazard Data

| | |
|---|---|
| EXPOSURE LIMITS | The current OSHA standard for THF exposure is 200ppm averaged over 8 hours. There is no federal standard for NMI. See Note 2. |

### EFFECTS OF ACUTE OVEREXPOSURE

| | |
|---|---|
| SWALLOWING | Harmful if swallowed. High concentrations may cause nausea, dizziness, headaches, liver or kidney damage. |
| SKIN | Exposure to liquid may cause skin irritation. |
| INHALATION | May cause irritation of the upper respiratory tract, dizziness, nausea, headaches. |
| EYES | May cause irritation. |

### EFFECTS OF CHRONIC OVEREXPOSURE

Liver and kidney damage have been reported in laboratory animals exposed to high concentrations of THF.

### OTHER HEALTH HAZARDS

Points of attack include eyes, skin, respiratory system and central nervous system. Allergic sensitization of the skin may occur.

| CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: | NTP No | IARC No | OSHA No |
|---|---|---|---|

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY CHEMICAL OVEREXPOSURE

No specific information is available.

### EMERGENCY AND FIRST AID PROCEDURES

| | |
|---|---|
| SWALLOWING | If conscious, give large volumes of water immediately. Get medical attention. |
| SKIN | Remove contaminated clothing. Flush the contaminated area with water for 15 minutes. Seek medical attention. |
| INHALATION | Provide fresh air and rest. If breathing is difficult, provide oxygen and get medical attention immediately. |
| EYES | Flush eyes immediately with large amounts of water for at least 15 minutes. Seek medical attention immediately. |

NOTES TO PHYSICIAN

Special attention should be given to skin, eyes and respiratory tract.

## VI. Reactivity Data

STABILITY:
STABLE [X]
UNSTABLE [ ]

CONDITIONS TO AVOID
Avoid contact with strong acids or oxidizers.

INCOMPATIBILITY (materials to avoid)
Contact with strong oxidizers may cause fire or explosion.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS
Burning may release toxic vapors and gases, including carbon monoxide and oxides of nitrogen.

HAZARDOUS POLYMERIZATION:
MAY OCCUR [ ]
WILL NOT OCCUR [X]

CONDITIONS TO AVOID
See Section IX.

## VII. Spill or Leak Procedures

STEPS TO BE TAKEN
Avoid inhalation and skin contact. Wear protective clothing. Remove all ignition sources. Ventilate area of spill or leak. See Note 3.

WASTE DISPOSAL METHOD
This reagent should be disposed of as a regulated hazardous waste by a permitted hazardous waste management facility in accordance with federal, state, and local regulations.

## VIII. Special Protective Equipment

RESPIRATORY PROTECTION (Specify Type)
An MSHA- or NIOSH-approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for emergency or high concentrations.

VENTILATION
Handle within a well-ventilated area. Minimize open exposure to air.

PROTECTIVE GLOVES
Latex or Neoprene rubber gloves are recommended.

EYE PROTECTION
Safety glasses with side shields, monogoggles or face shield.

OTHER PROTECTIVE EQUIPMENT
As necessary to prevent skin contact.

## IX. Special Precautions

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE

Handle as a flammable liquid. Use with adequate ventilation. Do not breathe vapor. store in a secure, well-ventilated location suitable for flammable materials. Keep away from heat, sparks and flame.

OTHER PRECAUTIONS
Eye wash and safety shower should be in area of use. This reagent will attack many forms of rubber, plastics, coatings and finishes.

## X. Additional Information

NOTE 1: NFPA 704M rating: Health-2, Flammability-3, Reactivity-1.

NOTE 2: For THF, the STEL is 250ppm in air, and the IDLH is 20,000ppm. There is no specific information available for NMI.

NOTE 3: Small spills and leaks may be collected with absorbent towels or pads and removed to a well- ventilated area away from ignition sources. This reagent must not be allowed to enter confined spaces because of the possibility of vapor explosion.

**CAUTION!**

**THIS PRODUCT IS STRICTLY INTENDED FOR LABORATORY RESEARCH PURPOSES ONLY. DISPOSE OF EMPTY BOTTLES PROPERLY.**

Material Safety Data Sheet Number: 920785 PREPARED BY: RB Moon

Date Issued: 11-2-87

The opinions expressed herein are those of qualified experts within Applied Biosystems, Inc., information from our suppliers, and data presented in various technical publications. We believe that the information contained herein is current as of the date of this Material Safety Data Sheet. It is the user's responsibility to determine the suitability of this information for the adoption of safety precautions as may be necessary. We reserve the right to revise Material Safety Data Sheets from time to time as new technical information becomes available.

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# MATERIAL SAFETY DATA SHEET

**EMERGENCY PHONE NUMBER** (USA) 415-570-6667, ext. 3333 (UK) 0925-825650

## I. Identification

| | | | |
|---|---|---|---|
| PRODUCT NAME | Iodine/Water/Pyridine/Tetrahydrofuran, 200 ml | PART NUMBER | 400753 |
| CHEMICAL NAME | N/A | CHEMICAL FAMILY | Mixture |
| FORMULA | $I_2/H_2O/C_5H_5N/C_4H_8O$ | MOLECULAR WEIGHT | N/A |
| SYNONYMS | DNA Synthesis Reagent #15, Iodine Oxidation Reagent | | |
| DEPARTMENT OF TRANSPORTATION | HAZARD CLASSIFICATION | 3, Tetrahydrofuran, UN2056 | |
| | SHIPPING NAME | Tetrahydrofuran | |

## II. Ingredients

| MATERIAL | % | TLV (Units) | PEL (Units) | CAS NUMBER |
|---|---|---|---|---|
| Iodine | 3 | 0.1 ppm | 0.1 ppm | 7553-56-2 |
| Water | 2 | --- | --- | --- |
| Pyridine | 20 | 5 ppm | 5 ppm | 110-86-1 |
| Tetrahydrofuran (THF) | 75 | 200 ppm | 200 ppm | 109-99-9 |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

## III. Physical Data

| | | | |
|---|---|---|---|
| BOILING POINT, 760 mmHg | THF only: 66°C (151°F) | FREEZING POINT | N/A |
| SPECIFY GRAVITY ($H_2O$ = 1) | N/A | VAPOR PRESSURE, mmHg | N/A |
| VAPOR DENSITY (Air = 1) | 2.5 (THF) | SOLUBILITY IN WATER, % BY WEIGHT | N/A |
| PERCENT VOLATILE BY VOLUME | 97% | EVAPORATION RATE (Butyl Acetate = 1) | 800 (THF) |
| APPEARANCE AND ODOR | Brown liquid with a pungent, disagreeable odor | | |

## IV. Fire and Explosion Hazard Data

| | | | | |
|---|---|---|---|---|
| FLASH POINT (method used) | THF only: -14°C (6°C) | | | (closed cup) |
| FLAMMABLE LIMITS | THF | Lower: 1.8% | Upper: | 11.8% |
| FIRE EXTINGUISHING MEDIA | Dry chemical, alcohol foam, carbon dioxide or Halon | | | |
| SPECIAL FIRE FIGHTING PROCEDURES | Use self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. See note 1. | | | |
| UNUSUAL FIRE AND EXPLOSION HAZARDS | Water spray may be ineffective except in cooling containers exposed to fire. | | | |

## V. Health Hazard Data

| | |
|---|---|
| EXPOSURE LIMITS | The current OSHA standards for THF and pyridine exposure are 200 ppm and 5 ppm, respectively, averaged over 8 hours. See Note 2. |

### EFFECTS OF ACUTE OVEREXPOSURE

| | |
|---|---|
| SWALLOWING | Harmful if swallowed. High concentrations may cause nausea, dizziness, headache, liver or kidney damage. |
| SKIN | Exposure to liquid may cause serious skin irritation. |
| INHALATION | May cause acute irritation of the upper respiratory tract, dizziness, nausea, headaches. |
| EYES | May cause severe irritation. |

### EFFECTS OF CHRONIC OVEREXPOSURE

Liver and kidney damage have been reported in laboratory animals exposed to high concentrations of THF. Liver and kidney injury have been reported in humans given pyridine as an oral medication.

### OTHER HEALTH HAZARDS

Points of attack include eyes, skin, respiratory system and central nervous system. Allergic sensitization of the skin may occur.

| CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: | NTP No | IARC No | OSHA No |
|---|---|---|---|

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY CHEMICAL OVEREXPOSURE

No specific information is available.

### EMERGENCY AND FIRST AID PROCEDURES

| | |
|---|---|
| SWALLOWING | If conscious, give large volumes of water immediately. Get medical attention. |
| SKIN | Remove contaminated clothing. Flush the contaminated area with large volumes of water for 15 minutes. Seek medical attention. |
| INHALATION | Provide fresh air and rest. If breathing is difficult, provide oxygen and get medical attention immediately. |
| EYES | Flush eyes immediately with large amounts of water for at least 15 minutes. Seek medical attention immediately. |

| | |
|---|---|
| NOTES TO PHYSICIAN | Special attention should be given to skin, eyes and respiratory tract. |

## VI. Reactivity Data

| STABILITY: | CONDITIONS TO AVOID |
|---|---|
| STABLE [X] | Avoid contact with strong oxidizers. |
| UNSTABLE [ ] | |

INCOMPATIBILITY (materials to avoid) Contact with strong oxidizers may cause fire or explosion.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS Burning may release toxic vapors and gases, including iodine, hydrogen iodide, carbon monoxide, oxides of nitrogen, and toxic cyanides.

| HAZARDOUS POLYMERIZATION | CONDITIONS TO AVOID |
|---|---|
| MAY OCCUR [ ] | See Section IX. |
| WILL NOT OCCUR [X] | |

## VII. Spill or Leak Procedures

STEPS TO BE TAKEN Avoid inhalation and skin contact. Wear protective clothing. Remove all ignition sources. Ventilate area of spill or leak. See Note 3.

WASTE DISPOSAL METHOD This reagent should be disposed of as a regulated hazardous waste by a permitted hazardous waste management facility in accordance with federal, state, and local regulations.

## VIII. Special Protective Equipment

RESPIRATORY PROTECTION (Specify Type) An MSHA- or NIOSH-approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for emergencies and high concentrations.

| | |
|---|---|
| VENTILATION | Handle within a well-ventilated area. Minimize open exposure to air. |
| PROTECTIVE GLOVES | Neoprene or nitrile rubber gloves are recommended. |
| EYE PROTECTION | Safety glasses with side shields, monogoggles or face shield. |
| OTHER PROTECTIVE EQUIPMENT | As necessary to prevent skin contact. |

## IX. Special Precautions

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE

Handle as a flammable liquid. Use with adequate ventilation. Do not breathe vapor. Store in a secure location suitable for flammable materials. Keep away from heat, sparks and flame.

OTHER PRECAUTIONS Eye wash and safety shower should be in area of use. This reagent will attack many forms of rubber, plastics, coatings and finishes.

## X. Additional Information

Note 1: NFPA 704M rating: Health-2, Flammability-3, Reactivity-1.

Note 2: For THF the STEL is 250 ppm in air, and the IDLH is 20,000 ppm. For pyridine the STEL in air is 10 ppm and IDLH level is 3,600 ppm. There is no STEL data for iodine.

Note 3: Small spills and leaks may be collected with absorbent towels or pads and removed to a well ventilated area away from ignition sources. This reagent must not be allowed to enter confined spaces because of the possibility of vapor explosion.

**CAUTION!**

**THIS PRODUCT IS STRICTLY INTENDED FOR LABORATORY RESEARCH PURPOSES ONLY.**
**DISPOSE OF EMPTY BOTTLES PROPERLY**

Material Safety Data Sheet Number: 920753

PREPARED BY: RBMoon

Date Issued: 9-9-87

The opinions expressed herein are those of qualified experts within Applied Biosystems, Inc., information from our suppliers and data presented in various technical publications. We believe that the information contained herein is current as of the date of this Material Safety Data Sheet. It is the user's responsibility to determine the suitability of this information for the adoption of safety precautions as may be necessary. We reserve the right to revise Material Safety Data Sheets from time to time as new technical information becomes available.

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# MATERIAL SAFETY DATA SHEET

**EMERGENCY PHONE NUMBER** (USA) 415-570-6667, ext. 999 (UK) 0925-825650

## I. Identification

| | | | |
|---|---|---|---|
| PRODUCT NAME | Trichloroacetic Acid/Dichloromethane, 450 ml | PART NUMBER | 400236 |
| CHEMICAL NAME | N/A | CHEMICAL FAMILY | Mixture |
| FORMULA | $CCl_3COOH$ in $CH_2Cl_2$ | MOLECULAR WEIGHT | N/A |
| SYNONYMS | DNA Synthesis Reagent #14, TCA Detritylation Reagent | | |
| DEPARTMENT OF TRANSPORTATION | HAZARD CLASSIFICATION | 8, corrosive liquid, poisonous, DOT12A, UN 2922 | |
| | SHIPPING NAME | Corrosive liquid, poisonous, n.o.s. | |

## II. Ingredients

| MATERIAL | % | TLV (Units) | PEL (Units) | CAS NUMBER |
|---|---|---|---|---|
| Trichloroacetic acid (TCA) | 2 | 1 ppm | 1 ppm | 76-03-9 |
| Dichloromethane (DCM) | 98 | 100 ppm | 500 ppm | 75-09-2 |
| | | | | |
| | | | | |
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| | | | | |
| | | | | |
| | | | | |

## III. Physical Data

| | | | |
|---|---|---|---|
| BOILING POINT, 760 mmHg | DCM only: 40°C (104°F) | FREEZING POINT | N/A |
| SPECIFY GRAVITY ($H_2O = 1$) | N/A | VAPOR PRESSURE, mmHg | 349 @ 20°C (DCM) |
| VAPOR DENSITY (Air = 1) | 2.9 (DCM) | SOLUBILITY IN WATER, % BY WEIGHT | 3% |
| PERCENT VOLATILE BY VOLUME | 98% | EVAPORATION RATE (Butyl Acetate = 1) | N/A |
| APPEARANCE AND ODOR | Colorless liquid with a pleasant aromatic odor | | |

## IV. Fire and Explosion Hazard Data

| | | | |
|---|---|---|---|
| FLASH POINT (method used) | None | | |
| FLAMMABLE LIMITS | Lower: N/A | Upper: | N/A |
| FIRE EXTINGUISHING MEDIA | Dry chemical, foam, carbon dioxide, or Halon | | |
| SPECIAL FIRE FIGHTING PROCEDURES | Use self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. | | |
| UNUSUAL FIRE AND EXPLOSION HAZARDS | May form flammable vapor/air mixtures above 100°C. | | |

## V. Health Hazard Data

| | |
|---|---|
| EXPOSURE LIMITS | The current OSHA standard for DCM exposure is 500 ppm averaged over 8 hours. See Note 1. |

### EFFECTS OF ACUTE OVEREXPOSURE

| | |
|---|---|
| SWALLOWING | Harmful if swallowed. DCM is a mild narcotic. Effects of intoxication include headache, giddiness, stupor, irritability, numbness, and tingling in the limbs. |
| SKIN | Exposure to liquid may cause irritation. Continued contact with the skin may cause burns. |
| INHALATION | Severe exposure may cause halucinations, pulmonary edema, coma and death. |
| EYES | May cause severe irritation. |

### EFFECTS OF CHRONIC OVEREXPOSURE

Exposure to DCM may cause elevated carboxy-hemoglobin levels which may be significant in smokers or workers with anemia or heart disease.

### OTHER HEALTH HAZARDS

Points of attack include eyes, skin, respiratory system and central nervous system. Recent studies suggest that DCM is a potential carcinogen.

| CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: | NTP No | IARC No | OSHA No |
|---|---|---|---|

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY CHEMICAL OVEREXPOSURE

Anemias or cardiovascular disease.

### EMERGENCY AND FIRST AID PROCEDURES

| | |
|---|---|
| SWALLOWING | If conscious, give large volumes of water immediately and induce vomiting. Do not induce vomiting if unconscious. |
| SKIN | Remove contaminated clothing. Flush the contaminated area with large volumes of water for 15 minutes. Seek medical attention. |
| INHALATION | Provide fresh air and rest. If breathing is difficult, provide oxygen and get medical attention immediately. |
| EYES | Flush eyes immediately with large amounts of water for at least 15 minutes. Seek medical attention immediately. |

NOTES TO PHYSICIAN

None

## VI. Reactivity Data

STABILITY:

STABLE [X]

UNSTABLE [ ]

CONDITIONS TO AVOID

Contact with strong oxidizers may cause fire or explosion.

INCOMPATIBILITY (materials to avoid) Strong oxidizers, strong bases, and chemically active metals such as aluminum or magnesium powders, sodium or potassium.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS Burning may release toxic vapors and gases, including phosgene, hydrogen chloride, and carbon monoxide.

HAZARDOUS POLYMERIZATION:

MAY OCCUR [ ]

WILL NOT OCCUR [X]

CONDITIONS TO AVOID

See Section IX.

## VII. Spill or Leak Procedures

STEPS TO BE TAKEN

Avoid inhalation and skin contact. Wear protective clothing. Ventilate area of spill or leak. Collect reagent with absorbent towels or pads and remove to a well ventilated area.

WASTE DISPOSAL METHOD This reagent should be disposed of as a regulated hazardous waste by a permitted hazardous waste management facility in accordance with federal, state, and local regulations.

## VIII. Special Protective Equipment

RESPIRATORY PROTECTION (Specify Type)

An MSHA- or NIOSH-approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for high concentrations and emergencies.

VENTILATION

Handle within a well-ventilated area. Minimize open exposure to air.

PROTECTIVE GLOVES

Butyl rubber or similar type resistant to DCM and other chlorinated solvents.

EYE PROTECTION

Safety glasses with side shields, monogoggles or face shield.

OTHER PROTECTIVE EQUIPMENT

As necessary to prevent skin contact.

## IX. Special Precautions

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE

Use with adequate ventilation. Do not breathe vapor. Avoid skin contact. Store in a secure, well ventilated area away from heat.

OTHER PRECAUTIONS

Eye wash and safety shower should be in area of use. This reagent will attack aluminum surfaces and many forms of rubber, plastics, coating, and finishes.

## X. Additional Information

Note 1: For methylene chloride the STEL is 1000 ppm with a maximum peak exposure of 2000 ppm for 5 minutes in any 2 hour period. NIOSH has recommended a PEL of 75 ppm with a ceiling level of 500 ppm averaged over a 15 minute period. NIOSH recommends a further PEL reduction where carbon monoxide is present since a major metabolic product of methylene chloride is carbon monoxide. The ACGIH has proposed a TWA of 100 ppm and an STEL of 500 ppm. The IDLH level is 5000 ppm.

CAUTION!

THIS PRODUCT IS STRICTLY INTENDED FOR LABORATORY RESEARCH PURPOSES ONLY.
DISPOSE OF EMPTY BOTTLES PROPERLY .

Material Safety Data Sheet Number 920236

PREPARED BY: B B Moon

Date Issued 11/18/85

The opinions expressed herein are those of qualified experts within Applied Biosystems Inc., information from our suppliers, and data presented in various technical publications. We believe that the information contained herein is current as of the date of this Material Safety Data Sheet. It is the users responsibility to determine the suitability of this information for the adoption of safety precautions as may be necessary. We reserve the right to revise Material Safety Data Sheets from time to time as new technical information becomes available.

# MATERIAL SAFETY DATA SHEET

850 LINCOLN CENTRE DRIVE
FOSTER CITY, CA 94404

EMERGENCY PHONE NUMBER
(USA) 415-570-6667, ext. 3333
(UK) 0925-825650

## I. Identification

| | | | |
|---|---|---|---|
| PRODUCT NAME | TETD/Acetonitrile, 180 mL | PART NUMBER | 401147 |
| CHEMICAL NAME | Tetraethylthiuram disulfide/Acetonitrile | MOL. WEIGHT | TETD - 296.54 acetonitrile - 41 |
| FORMULA | TETD - $C_{10}H_{20}N_2S_4$ Acetonitrile - $C_2H_3N$ (See Note 2) | STORAGE TEMP | 20-30°C |
| CHEMICAL FAMILY | TETD - thiuram Acetonitrile - nitrile | SHELF LIFE | 1 yr |
| SYNONYMS | sulfurizing reagent, Antabuse, Disulfuram, DNA synthesis, Methyl cyanide, Cyanomethane, Ethylnitrile | | |
| TRANSPORTATION INFORMATION: | Hazard Classification - 3 flammable liquid, poisonous | | |
| GROUND (DOT)<br>AIR (ICAO) | Acetonitrile, Flammable Liquid, NA 1648, Ltd Qty<br>Methyl Cyanide, 3, UN 1648, 6.1, Flammable Liquid | | |
| | TETD: NIOSH Registry Number: J01225000<br>DOT ID Number: NA9188<br>DOT Hazard Classification: ORM-E | | |
| TARIFF CODE | Not Applicable | | |

## II. Ingredients

| MATERIAL | % | TLV (Units) | PEL (Units) | CAS NUMBER |
|---|---|---|---|---|
| Acetonitrile | 85 | 40 ppm | 40 ppm | 75-05-8 |
| Tetraethylthiuram disulfide (TETD) | 15 | 2 mg/m$^3$ | 2 mg/m$^3$ | 97-77-8 |
| | | | | |
| | | | | |
| | | | | |

## III. Physical Data

| | | | |
|---|---|---|---|
| BOILING POINT | >82°C | FREEZING POINT | 0°C |
| SPECIFIC GRAVITY | .833 g/mL | VAPOR PRESSURE mmHg | Not Measured |
| VAPOR DENSITY | 1.3 (acetonitrile) | SOLUBILITY IN WATER, % BY WEIGHT | Miscible |
| PERCENT VOLATILE BY VOLUME | 85% | EVAPORATION RATE (Butyl Acetate=1) | 5.8 |
| APPEARANCE AND ODOR | Colorless liquid with an ether-like odor. | | |

## IV. Fire and Explosion Hazard Data

| | |
|---|---|
| FLASH POINT | Acetonitrile only: 5.6°C (42°F) (closed cup) |
| FLAMMABLE LIMITS | Lower: 4.4% Upper: 16.0% |
| FIRE EXTINGUISHING MEDIA | Dry chemical, alcohol foam, carbon dioxide or Halon™ |
| SPECIAL FIRE FIGHTING PROCEDURES | Use self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. |
| UNUSUAL FIRE AND EXPLOSION HAZARDS | Water spray may be ineffective except in cooling containers exposed to fire. Vapor is heavier than air and may travel considerable distance to ignition source. |

## V. Health Hazard Data

| | |
|---|---|
| EXPOSURE LIMITS | OSHA standard for acetonitrile is 40 ppm in air averaged over 8 hours, STEL is 60 ppm in air, IDLH is 4000 ppm. |

## EFFECTS OF ACUTE EXPOSURE

| | |
|---|---|
| SWALLOWING | In most cases of systemic acetonitrile poisoning, there is a latent period of several hours between exposure and onset of symptoms, perhaps due to the slow release of cyanide. (Also, See Note 1). |
| SKIN | Prolonged exposure to TETD may cause irritation of the skin or allergic dermatitis. |
| INHALATION | May cause flushing of the face, a feeling of chest tightness and irritation of the nose and throat. In high concentrations may cause nausea, vomiting, gastric distress, respiratory depression, elevated blood cyanide and possible death. |
| EYES | May cause irritation. |

## EFFECTS OF CHRONIC EXPOSURE

No chronic diseases have been reported for acetonitrile or TETD. May cause respiratory irritation.

## OTHER HEALTH HAZARDS

Teratogencity and embryotoxicity was reported in several animal studies for acetonitrile at very high doses.

| CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: | NTP NO | IARC NO | OSHA NO |
|---|---|---|---|

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY CHEMICAL OVEREXPOSURE

TETD, also known as Antabuse, is useful in the treatment of alcoholism. No chronic affect has been reported. May cause respiratory irritation. Preclude from exposure those individuals with diseases of central nervous systems, heart and lungs.

## EMERGENCY AND FIRST AID PROCEDURES

| | |
|---|---|
| SWALLOWING | If conscious, give large volumes of water immediately. Get medical attention immediately. If not breathing, give artificial respiration. |
| SKIN | Remove contaminated clothing. Flush the contaminated area with large volumes of water for 15 minutes. Seek medical attention immediately. Wash skin with soap. |
| INHALATION | Provide fresh air and rest. If breathing is difficult, provide oxygen at once and get medical attention immediately. |
| EYES | Flush eyes immediately with large amounts of water for at least 15 minutes. Seek medical attention immediately. |

| | |
|---|---|
| NOTES TO PHYSICIAN | Do not rely on determination of blood cyanide or urinary thiocyanate for evidence of exposure. |

## VI. Reactivity Data

| STABILITY | CONDITIONS TO AVOID |
|---|---|
| STABLE X<br>UNSTABLE ___ | Avoid contact with strong oxidizers and reducing agents. Avoid contact with strong oxidizers, heat or flames. |
| INCOMPATIBILITY | Keep dry and tightly sealed. Contact with strong oxidizers (e.g., sulfuric acide, perchlorates, chlorosulfonic acid, etc.) may cause fire or explosions. |
| HAZARDOUS COMBUSTION OR DECOMPOSITION | Burning may release toxic vapors and gases, including carbon monoxide, carbon dioxide, nitrogen oxides, sulfer oxides and hydrogen cyanide. |
| HAZARDOUS POLYMERIZATION:<br>MAY OCCUR ___<br>WILL NOT OCCUR X | CONDITIONS TO AVOID |

## VII. Spill or Leak Procedures

STEPS TO BE TAKEN

Avoid inhalation and skin contact. Wear protective clothing. Remove all ignition sources. Small spills and leaks may be collected with absorbent towels or pads and removed to a well ventilated area, such as a fume hood.

WASTE DISPOSAL METHOD

This reagent should be disposed of as a regulated hazardous waste by a permitted hazardous waste management facility in accordance with federal, state, and local regulations.

## VIII. Special Protective Equipment

RESPIRATORY PROTECTION (Specify Type)

An MSHA- or NIOSH- approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for emergencies and high concentrations.

| | |
|---|---|
| VENTILATION | Handle within a well-ventilated area. Minimize open exposure to air. |
| PROTECTIVE GLOVES | Butyl rubber, polyvinyl alcohol or similar type glove resistant to organic solvents. |
| EYE PROTECTION | Safety glasses with side shields, monogoggles or face shield. |
| OTHER PROTECTIVE EQUIPMENT | As necessary to prevent skin contact. |

## IX. Special Precautions

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE

Handle as a flammable liquid. Use with adequate ventilation. Do not breathe vapor. Store in a secure, well-ventilated location suitable for flammable materials. Keep away from heat, sparks and flame.

OTHER PRECAUTIONS

Eye wash, safety shower and fire extinguisher should be in area of use. This reagent will attack many forms of rubber, plastics, coatings and finishes.

## X. Additional Information

Note 1: Ingestion of alcoholic beverages after ingestion of TETD causes intense vasodilatation of face and neck, tachycardia and tachypnea followed by nausea, vomiting, pallor, hypotension. Occasionally convulsions, cardiac arrhythmias, myocardial infarction may occur. High doses of alcohol may cause dizziness, headache, dyspnea, unconsciousness and death.

Note 2: Chemical Structures:

$Et_2NCSSCNEt_2$

TETD

tetraethylthiuram disulfide

$CH_3CN$ acetonitrile

Note 3: Odor Threshold: Ether-like smell - 40 ppm

Note 4: Toxicity Data:

Acetonitrile

$TD_{L0}$ - Human, Oral - 570 mg/kg
$LD_{50}$ - Rats, Oral - 3800 mg/kg
$LD_{50}$ - Rat Intraperitoneal - 850 mg/kg
$LD_{50}$ - Rat Subcutaneous - 500 mg/kg
$LD_{50}$ - Intravenous - 1680 mg/kg
$LC_{L0}$ - Rats, Inhalation - 8000 ppm/4 hr.
$LD_{50}$ - Mice Intraperitoneal - 175 mg/kg
$LD_{L0}$ - Mice Subcutaneous - 700 mg/kg

TETD

$LD_{50}$ - Rats, Oral - 8600 g/k

Note 5: Nation Fire Protection Association Labeling (NFPA 704M)

Health - 2, Flammability - 3, Reactivity - 0

**CAUTION!**

**THIS PRODUCT IS STRICTLY INTENDED FOR LABORATORY RESEARCH PURPOSES ONLY. DISPOSE OF EMPTY BOTTLES PROPERLY.**

**PREPARED BY:** Alex Andrus **DATE ISSUED:** 12/14/90

**The opinions expressed herein are those of qualified experts within Applied Biosystems, Inc., information from our suppliers, and data presented in various technical publications. We believe that the information contained herein is current as of the date of this Material Safety Data Sheet. It is the user's responsibility to determine the suitability of this information for the adoption of safety precautions as may be necessary. We reserve the right to revise Material Safety Data Sheets from time to time as new technical information becomes available.**

ABI 392/394 INSTRUMENT WASTE PROFILE

Emergency Phone Numbers: (USA) 415-570-6667 Ext. 3333 (UK) 0925-825650

## I. IDENTIFICATION

All liquid waste from the 392/394 instruments is collected in a 10-Liter bottle located below the instrument. This waste is a complex mixture of reagents and solvents which may have properties of greater hazard than the individual waste components by themselves. HANDLE THIS MATERIAL WITH EXTREME CAUTION! DO NOT DISPOSE OF THIS WASTE IN SINKS OR DRAINS! THIS MATERIAL SHOULD BE DISPOSED OF AS A REGULATED HAZARDOUS WASTE!

## II. APPROXIMATE COMPOSITION (Standard Cycle)

| MATERIAL | % | TLV | PEL | CAS# |
|---|---|---|---|---|
| 1-Methylimidazole | ≤ 1 | N/A | N/A | 616-47-7 |
| Acetonitrile | 80% | 40 ppm | 40 ppm | 75-05-8 |
| Dichloromethane | 11 | 100 ppm | 500 ppm | 75-09-2 |
| Tetrahydrofuran | 5 | 200 ppm | 200 ppm | 109-99-9 |
| Phosphoramidites | ≤ 1 | N/A | N/A | N/A |
| Acetic Anhydride | ≤ 1 | N/A | N/A | N/A |
| Iodine | ≤ 1 | 0.1 ppm | 0.1 ppm | 7553-56-2 |
| 2,6-Lutidine | ≤ 1 | N/A | N/A | 108-48-5 |
| Trichloroacetic Acid | ≤ 1 | 1 ppm | 1 ppm | 76-03-9 |
| Pyridine | ≤ 1 | 5 ppm | 5 ppm | 110-86-1 |
| Tetraethylthiuram Disulfide | 1 | 2 mg/m$^3$ | 2 mg/m$^3$ | 97-77-3 |

## III. PHYSICAL DATA

| | | | |
|---|---|---|---|
| BOILING POINT, 760 mm: | N/A | FREEZING POINT: | N/A |
| SPECIFIC GRAVITY ($H_2O$ = 1) | 0.94 | pH RANGE: | 4-6 |
| VOLATILITY (vol %): | 96-98% | SOLUBILITY in $H_2O$: | Soluble |
| APPEARANCE AND ODOR: | Yellow to reddish-brown liquid with an acrid, unpleasant odor. | | |

## IV. FIRE AND EXPLOSION HAZARD DATA (Acetonitrile data only!)

FLASH POINT (Closed Cup): 5.6°C (42°F)

FLAMMABLE LIMITS: 4.4% LEL 16% UEL

FIRE EXTINGUISHING MEDIA: Dry chemical, alcohol foam, carbon dioxide or Halon.

SPECIAL FIRE FIGHTING PROCEDURES: Use self-contained breathing apparatus and protective clothing to prevent skin and eye contact.

## V. HEALTH HAZARD DATA

EXPOSURE LIMITS: See Section III. For acetonitrile, the STEL is 60 ppm and the IDLH level is 4,000. For Dichloromethane, the STEL is 1,000 ppm and the IDLH level is 5,000 ppm.

### EFFECTS OF ACUTE OVEREXPOSURE

SWALLOWING: Harmful is swallowed! Causes severe irritation of eyes, nose and throat. Higher concentrations may cause nausea, vomiting, convulsions, liver and kidney damage, unconsciousness and death.

SKIN: May cause severe irritation and burns. Allergic skin sensitization may also occur.

INHALATION: May cause severe irritation of eyes, nose, throat and lungs. Higher concentrations may cause pulmonary edema, unconsciousness and death.

EYES: May cause severe irritation or burns.

### EMERGENCY AND FIRST AID PROCEDURES

SWALLOWING: If conscious, give large quantities of water immediately and induce vomiting. Get medical attention immediately. Do not induce vomiting if unconscious.

SKIN: Remove contaminated clothing. Flush the contaminated area with water and wash with mild soap or detergent. Get medical attention.

INHALATION: Provide fresh air and rest. If breathing is difficult, provide oxygen and get medical attention immediately.

EYES: Flush eyes immediately with large amounts of water for at least 15 minutes. Get medical attention.

## VI. REACTIVITY DATA

STABILITY: Stable

INCOMPATIBILITY: Contact with strong oxidizing agents or concentrated acids or bases may cause fire or explosion.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS: Burning may release toxic vapors and gasses including phosgene, hydrogen cyanide, hydrogen chloride, carbon monoxide and oxides of nitrogen.

HAZARDOUS POLYMERIZATION: Will not occur.

## VII. SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN: Avoid inhalation and skin contact. Wear protective clothing. Ventilate area of spill or leak. Remove all ignition sources. Small quantities may be collected with absorbent towels or pads and removed to a well-ventilated area away from ignition sources. Larger amounts (1 liter or more) may be collected with an inert absorbent (kitty litter or similar material) or commercially available spill pillows designed for solvent collection. This waste material must not be allowed to enter confined spaces (such as a sewer!) because of the possibility of an explosion.

WASTE DISPOSAL: The instrument waste solution should be disposed of as a regulated hazardous waste by a properly permitted hazardous waste management facility in accordance with federal, state and local regulations. Recommended disposal methods include high temperature incineration or solidification for secure chemical landfill disposal.

## VIII. SPECIAL PROTECTIVE EQUIPMENT

RESPIRATORY PROTECTION: An MSHA- or HIOSH- approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for high vapor concentrations and emergency situations.

VENTILATION: Handle within a well-ventilated area. Minimize open exposure to air.

PROTECTIVE GLOVES: Butyl rubber to similar type resistant to strong solvents.

EYE PROTECTION: Safely glasses with side shields, monogoggles or face shield.

OTHER PROTECTIVE EQUIPMENT: As necessary to prevent skin contact.

## IX. SPECIAL PRECAUTIONS

PRECAUTIONS TO BE TAKEN: Handle as a flammable, poisonous liquid. Maintain adequate ventilation at all times. Do not breathe vapor. Do not get in eyes, on skin, or on clothing. Accidental contact should be washed away immediately. Keep away from heat, sparks and flame. Keep containers tightly closed. Eye bath, safety shower and spill collection materials should be in area of use.

OTHER: This waste solution has strong solvent properties and will attack many forms of rubber, plastics, coatings and finishes.

## X. ADDITIONAL INFORMATION

When not directly attached to the instrument, this waste material should be stored in a secure, well-ventilated location suitable for flammable materials. Store away from light, heat or potential ignition sources. Contact the appropriate state hazardous waste regulatory agency for proper disposal procedures and lists of registered service companies.

THIS WASTE MATERIAL IS HAZARDOUS AND SHOULD ONLY BE HANDLED BY PERSONS THOROUGHLY TRAINED IN HAZARDOUS MATERIALS HANDLING PROCEDURES!

### ACRONYMS USED

ACGIH: American Conference of Government Industrial Hygienists.

CAS#: Chemical Abstracts Service compound reference number.

IDLH: Immediately Dangerous to Life and Health.

LEL: Lower Explosion Limit.

MSHA: Mine Safety and Health Administration.

NIOSH: National Institute for Occupational Safety and Health.

OSHA: Occupational Safety and Health Administration.

PEL: Permissible Exposure Limit. The federal OSHA limit, usually expressed as a TWA for an 8 hour work shift.

PPM: Parts Per Million.

SCBA: Self-Contained Breathing Apparatus.

TLV: Threshold Limit Value. The ACGIH recommended TWA, usually for an 8 hour work shift.

TWA: Time Weighted Average.

UEL: Upper Explosive Limit.

# 1

# General Introduction

This section provides information on:

- How to use this manual
- How to get help
- How the instrument works


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User Bulletins ..... 1–2
Special Text Usage ..... 1–2
Attention Users ..... 1–2
Commands ..... 1–3
Safety Information/Material Safety Data Sheets ..... 1–4
How to Get Help ..... 1–5
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General Description of the Model 392/394 ..... 1–7

# About This Manual

The first part of this manual, Sections 1 through 4, gives you the information that you will need to get started. Step-by-step operating procedures are provided in Section 2, the software is described in detail in Section 3, and then a complete listing of functions, cycles, and procedures is presented in Section 4.

Sections 5 and 6 provide more detailed principles of operation (system description for hardware) and a detailed description of the chemistry required for automated DNA//RNA synthesis. More specialized information is included in the appendices, such as the instrument warranty; instrument schematics and plumbing diagrams; a list of functions, cycles, and procedures; detailed descriptions of flow and pressure tests; an illustrated instrument parts list; a preinstallation manual; procedures for manual preparation, cleavage, and deprotection of phosphoramidites; and relevant user bulletins. A safety section containing material safety data sheets (MSDSs) and an instrument waste profile is also included in this manual.

For an overview and a general understanding of the synthesizer, read the General Description in this section and the introductions to Sections 3 through 6. Be sure to read the rest of this first section for other important information such as how to get help if you have questions or problems.

This manual is designed to allow for the addition of new User Bulletins. New User Bulletins will be sent to you as they become available.

*NOTE This manual is intended for use with both Model 392 and 394 instruments. Displays show information needed for Model 394 instruments configured with 8 base positions and may show information not needed for instruments equipped with 2 columns or 5 base positions.*

## User Bulletins

User Bulletins (UBs) contain technical information that is essential to the Model 392/394 operation and related laboratory techniques. UBs are the quickest way to ensure that you have current information. They are produced periodically and will be mailed to you as they become available. Please read the UBs before operating your Model 392/394. Current User Bulletins are found under their own tab at the end of the manual.

## Special Text Usage

### Attention Users

Four *User Attention* words appear in the text of this manual. They are designed to draw your attention to safety issues or to issues relevant to proper operation of the instrument. Each one requires a certain level of observation or action as follows:

*NOTE* *Used to call attention to information.*

***IMPORTANT*** *Indicates information that is necessary for proper instrument operation.*

---

***CAUTION*** *Damage to the instrument could result if you do not comply with this information.*

---

---

***WARNING*** ***Physical injury to you or other people could result if these required precautions are not taken.***

---

## Commands

In this manual, commands are presented in the text as words in bold or italics. To execute a command, press the key indicated in bold or italics. Bold is used to indicate that a particular labeled keyboard key is to be pressed. Italics are used to indicate that a particular "soft" key is to be pressed. (Soft keys are discussed after Figure 1-2.)

An example of the bold convention is **Main Menu**, which is pressed when the initial instrument display is present to proceed to Page 1 of the Main Menu. A related convention, which is presented on the initial display (shown in Figure 1-1), is the use of single apostrophe marks around "Main Menu."

```
          Applied Biosystems, Inc.
394 DNA/RNA Synthesizer  (version 1.01)
4 Columns, 8 Base Positions
Press 'Main Menu' to begin.
```

Figure 1-1. Initial Instrument Display

Pressing **Main Menu** as prompted displays page 1 of the Main Menu, which like many other system menus uses "soft keys. "

```
–Main Menu–                    Edit Sequence>
                               Start Synthesis>
                             Monitor Synthesis>
Page 1 of 5                        Next Page>
```

Figure 1-2. Main Menu, page 1

Soft keys are the row of four unlabeled keys to the right of the display. These keys are called "soft keys" because their purpose changes with the menu or display presented. For exam-

ple, to execute the *Edit Sequence* command, press the key to the right of this prompt on the display shown in Figure 1-2. In a procedure or detailed task description, whenever you find *Edit Sequence* or another command name in italics, press the corresponding soft key to execute the command.

## Safety Information/Material Safety Data Sheets

Safety warnings are presented in the format shown below. This type of warning addresses chemical safety and is supplemented by the Material Safety Data Sheets (MSDS) and the 392/394 Waste Profile under the "Safety Data" tab in the back of the manual. Each time you see a message labeled **WARNING**, be aware that information relevant to your personal safety is presented.

---

***WARNING*** ***Some chemicals used with this instrument are considered hazardous. Hazard warnings are prominently displayed on the bottle labels of all hazardous chemicals. In addition, Material Safety Data Sheets (MSDSs) provide information about physical characteristics, hazards, precautions, first aid, spill cleanup, and disposal procedures. The MSDSs are included in the Safety Data section of this manual. Please be sure to familiarize yourself with the information in these documents before attempting to operate the instrument or use the reagents. Additional copies of the MSDS are available from Applied Biosystems at no extra cost.***

---

# How to Get Help

Applied Biosystems and its representatives maintain a large staff of fully trained service and technical specialists strategically located throughout the world. They can answer specific questions concerning instrument operation as well as more general questions about DNA/RNA synthesis, analysis, and purification.

## In the United States

The Applied Biosystems Technical Support Group in Foster City provides you with both service and applications support. You will receive prompt attention to your questions and service needs by directly calling Technical Support.

The Technical Support Group is made up of service specialists who can help you solve instrument problems quickly and effectively. They have operated ABI instruments in either research and development or quality assurance laboratories and are thoroughly familiar with problems commonly encountered in instrument operation.

If you call with a question concerning a chemical application, your call may be forwarded to a specialist within the U.S. Field Marketing Department. These specialists spend a good deal of their time in the laboratory and so can be especially helpful in addressing chemistry and applications questions.

When you request service for your synthesizer, the Technical Support Group will immediately notify the service engineer in your territory. Your request and a brief description of the problem will be entered into a computer database. This information allows the Field Service Department to maintain a history of your instrument, track its performance, and take precautionary measures to ensure its reliability.

If you have a technical question or a request for service, just call the Technical Support Group. Please have the serial number of your instrument available. The Technical Support Center operates from 6:30 a.m. to 5:30 p.m. Pacific Standard Time, with the exception of weekends and holidays.

Applied Biosystems, Inc., 850 Lincoln Centre Drive, Foster City, California 94404

| | |
|---|---|
| Telephone: | (415) 570–6667 |
| Toll Free: | (800) 831–6844 (outside California) |
| | (800) 831–3582 (in California) |
| FAX: | (415) 572–2743 |

## International

If you have questions about operating your instrument or about DNA/RNA synthesis, analysis and purification, please call the nearest office:

**Canada**
Applied Biosystems Canada, Inc.
6535 Millcreek Drive, Unit #74
Mississauga, Ontario, Canada, L5N 2M2
Telephone: (416) 821–8183, Facsimile: (416) 821–8246

**United Kingdom**
Applied Biosystems, Ltd.
Birchwood Science Park North,
Warrington, Cheshire, WA3 7PB, United Kingdom
Telephone: 0925–825650, Facsimile 0925–828196, Telex 629611 APBIO G

**Japan**
Applied Biosystems Japan, Inc.
3–3–6. Minamisuna, Koto–Ku
Tokyo, 136, Japan
Telephone (03) 699–0700, Facsimile (03) 699–0733

**West Germany**
Applied Biosystems, GmbH
Robert–Koch–Strasse 16, 6108 Weiterstadt, West Germany
Telephone 06151–87940, Telex 4197318 Z ABI D, Fax: 06151 84899

**Australia**
Applied Biosystems Pty. Ltd.
26 Harker Street, Burwood, Victoria 3125, Australia
Telephone (03) 288–7777, Facsimile (03) 877 1469

**France**
Applied Biosystems S.A.R.L.
13 Rue De La Perdrix, BP 50086
ZAC Paris Nord II, 95948 Roissy Charles de Gaulle, Cedex, France
Telephone (1) 48 63 24 44, Facsimile (1) 48 63 22 82, Telex 230458 ABIF

**Italy**
Applied Biosystems, S.r.l.
Via Carlo Darwin, 22
20143, Milano, Italy
Telephone: 02 89404561, Facsimile 02–832–1655 Telex: 353378 APBIO I

**Netherlands**
Applied Biosystems B.V.
Industrieweg 66–68, 3606 AS Maarssen, Holland
Telephone: (0)3465 74868, Facsimile: (0)3465 74904, Telex: 70896 ABI NL

# General Description of the Model 392/394

The Applied Biosystems Model 392 and Model 394 DNA Synthesizers automate all steps of single stranded oligonucleotide synthesis. When used as a system including ABI reagents and columns, these instruments produce the highest quality synthetic DNA currently attainable while minimizing synthesis time and cost.

The Model 392 and 394 use the phosphoramidite method of oligonucleotide synthesis because of its inherently high coupling efficiency and the stability of the starting materials. The synthesis begins with the 3' terminal nucleoside attached to a solid support, which is contained within a disposable column (the reaction chamber). Nucleotide bases are added one at a time to the support-bound DNA chain until the sequence is fully synthesized. Solid support synthesis allows excess reagents to be removed by filtration and eliminates the need for purification between base additions.

Applied Biosystems synthesizers use a pressure driven chemical delivery system to deliver reagents and solvents to the reaction column chamber. Reagent and solvent deliveries also rely on our patented *zero-dead volume* valves which increase reliability, eliminate cross-contamination and reduce reagent costs.

The Model 392 and 394 are controlled via menu-driven software. A four-line screen displays various options and necessary information about the syntheses or the status of the instrument. The synthesizers are fully programmable. Users can run pre-programmed protocols or can create custom cycles.

Applied Biosystems has been perfecting the science of nucleic acid synthesis on automated instruments since 1982. The Model 392 and 394 are our most advanced instruments to date. The company's philosophy, however, is to provide researchers with complete systems, not standalone instruments. Our DNA synthesis "system" includes instruments, chemicals, service and technical support.

All of our chemicals are purified and rigorously analyzed to ensure high yield synthesis. Each phosphoramidite, column reagent, and solvent manufactured at Applied Biosystems is unconditionally guaranteed. If you are not completely satisfied by the product (and it is used prior to any applicable expiration date and under the correct operating conditions), it will be replaced at no charge.

Applied Biosystems also maintains a large staff of fully trained service engineers strategically located around the world. If any questions arise while operating the synthesizer, call your service representative. In addition, technical experts are available to answer any questions about DNA synthesis, analysis, and purification. Please feel free to use these resources extensively.

# 2
# Operation

This section provides step-by-step procedures for operating the Model 392 or Model 394 DNA Synthesizer. Before beginning a synthesis, be sure to read the following subsections: Pre-synthesis Check List, Installing Phosphoramidite Bottles, Installing Reagent Bottles, Performing a Synthesis, and Post Synthesis Operations. Use the rest of this section as needed.


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Connecting an RS-232 Printer ..... 2-3
Pre–synthesis Check List ..... 2-4
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# Power On

1. Plug the instrument into a dedicated power source (refer to your pre-installation manual) and turn the power switch on. The power switch is located on the front of the instrument to the right of bottle position five or eight.

2. Press **Main Menu**, as prompted from the following display:

```
        Applied Biosystems, Inc.
394 DNA/RNA Synthesizer  (version 1.01)
4 Columns, 8 Base Positions
Press 'Main Menu' to begin.
```

*NOTE* *If necessary, adjust the intensity of the display by turning the "intensity wheel," located on the instrument just below and to the left of the display.*

*Look at this display to ascertain the model number of the instrument, the version of software, the number of columns, and the number of bases for which it is equipped. For example, a Model 392 synthesizer is equipped with two columns and may be equipped with either five or eight base positions. A Model 394 synthesizer is equipped with four columns and also may be equipped with either five or eight base positions.*

*Illustrations of the display presented in this manual show information needed for four-column/ eight-base position equipped instruments. If your instrument is a two-column instrument or a four-column instrument equipped with five base positions, please ignore the extra information presented on the example displays.*

Page 1 of the Main Menu will then be shown:

```
–Main Menu–                Edit Sequence>
                          Start Synthesis>
                        Monitor Synthesis>
Page 1 of 5                    Next Page>
```

# Connecting an RS-232 Printer

The Model 392 or 394 DNA Synthesizer requires an RS-232 printer to produce listings related to DNA synthesis. These include synthesis setup, cycle, procedure, and function listings. Any RS-232 (serial) printer that prints ASCII characters should operate with the synthesizers. The HP Thinkjet 2225D (ABI Part No. 401084) is recommended. Also, an 8-pin/25-pin, 9-foot printer cable, appropriate for connecting a printer to the synthesizer, is available from ABI (Part No. 100390).

Following is a procedure for setting up a printer and connecting it with a synthesizer:

1. Set the printer for the following protocol:
   - 8 data bits
   - 1 stop bit
   - 1 start bit
   - No parity
   - 9600 baud
   - X-On/X-Off
2. Connect the circular DIN connector of the printer cable to the RS-232 port on the synthesizer.
3. Connect the 25-pin D connector of the printer cable to the printer.
4. Connect the printer to a proper power source.

# Pre–synthesis Check List

Before beginning a synthesis, be sure to complete the following steps.

1. **Check reagent levels of all reservoirs.** Replace empty reservoirs with bottles of fresh reagents. Set interrupts for particular reservoirs at a point prior to reagent depletion.

   From the Main Menu, choose *Change Bottles* and follow the prompts. For further instructions, refer to "Installing Reagent Bottles," page 2-11.

2. **Ensure that bottles are installed correctly, with a tight seal, on every position.** Since all phosphoramidite bottles are pressurized simultaneously with a single valve, all bottles (five or eight) must be attached to the instrument (empty bottles at unused positions) in order to synthesize.

3. **Check that all alarms are set correctly.** Refer to "Main Menu Option: Bottle Alarms" in Section 3 for instructions on how to set the bottle alarms or turn them off.

4. **Prepare phosphoramidites using the auto-dilution feature.** Refer to "Using the Auto Dilution Procedure," page 2-7.

   If for any reason, you want to prepare phosphoramidites manually, refer to the procedure in Appendix VI.

5. **Check the pressure of the argon tank.** The low pressure gauge should read about 60 psi.

   Change the tank if it is depleted. Be ready to change the tank when the high pressure gauge drops below 400 psi. With average synthesizer use, an argon tank should last approximately 2 months. To change an argon tank, refer to the instructions on page 2-23.

6. **Check the waste level.** The synthesizer generates about 1 liter of hazardous, halogenated, organic liquid waste per 100 base additions (10 µmole scale generates more). The waste is collected in a 10–liter bottle.

   When the waste bottle is full, it must be emptied and the waste disposed of properly. The bottle can be changed prior to a synthesis or when a synthesis is interrupted. To change the waste bottle, refer to the instructions on page 2-24.

7. **Fill the fraction collector with 10- to 15-mL tubes to collect the trityl cation released at each detritylation step.** If you have selected the "Trityl collect = All." option, use one tube for each base in the sequence. If you have selected the "Trityl collect = 1st & last 2" option, at least four tubes per active column are required. (See the next paragraph for an explanation of how to determine the number of tubes required.) Be sure to label the tubes and align the fraction collector so tube number one collects the first trityl. The tube in position number one does not collect the first fraction. The second tube or set of tubes will collect the first fraction because the cycle advances the fraction collector first.

   Figure 2-1 shows how trityl collection works when the option "Trityl collect = 1st & last 2" is selected. The actual number of tubes required depends upon the number of bases in the

**Column 1 sequence (C1):** 5' > A G C T A <3'

**Column 2 sequence (C2):** 5'> A G C T A 5 6 T <3'

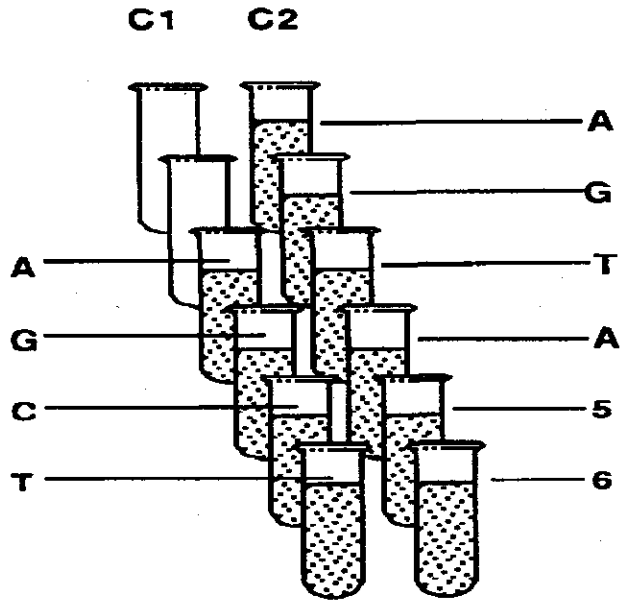



Figure 2-1. Trityl collection using the "1st & last 2" option

sequences being synthesized on the active columns. In this example, columns 1 and 2 are active. Collection always starts at the first active column (in this case, column 1). Trityl cation is not normally collected at the first base position (A on the 3' end) because this base is attached to the support and the trityl is sent to waste. The first two collections from column 1 are made at the second and third positions (T and C). Then trityl is collected at the fourth and fifth positions (G and A)—the last two positions in the sequence. On column 2, a collection is taken, as expected, at the second and third positions (6 and 5). Next, collections are made at the fourth and fifth positions (A and T) because collections were made at these positions on column 1. Finally, collections are taken at the last two positions on column 2 (G and A). Thus, once a collection is taken at a base position on one column, a collection will also be taken at that same position on subsequent active columns, even if it is not one of the first or last two positions. If all the columns were to contain sequences of the same length, then just four collections would be made per column. Otherwise, more than four will be made.

8. **Ensure that columns and collection vials have been installed.**

# Installing Phosphoramidite Bottles

The prepackaged phosphoramidites are bottled as powders and sealed under argon pressure. In this state, they are stable for at least 1 year. The Model 392/394 accommodates installation of phosphoramidites in powder form. Powdered phosphoramidites are dissolved in acetonitrile on the instrument using the Auto Dilution feature (described on page 2-7).

If desired, phosphoramidites can also be installed in liquid form (manually dissolved ahead of time). The installation procedure for bottles containing predissolved phosphoramidites can be found on page 2-9. For information on dissolving phosphoramidites manually, see Appendix VI.

Whether you use the Auto Dilution procedure or install bottles of predissolved phosphoramidites, a bottle must be present at the instrument position where you plan to install the new bottle. This is necessary because the first few steps of either procedure rinses the lines and purges the rinse into the old (or empty) bottle.

## Preparing Phosphoramidite Reservoir Positions

After the phosphoramidite positions are prepared as described below, follow either the Auto Dilution procedure or the procedure for installing predissolved phosphoramidites, depending upon whether the phosphoramidites are already in dissolved form or are still in powdered form.

*NOTE If you want to reuse dissolved phosphoramidites, see "How to Remove and Store Dissolved Phosphoramidites" in Appendix VI.*

### How to Install Bottles

1. Firmly push each bottle up around its receptacle while pressing the black button. As necessary, maneuver the bottle into place by carefully moving it side to side while pushing.

2. When the bottle is correctly engaged, release the button and it will return to its *out* position. If the button remains in, the bottle is not seated properly and must be repositioned.

### How to Remove Bottles

1. Remove a bottle by firmly pulling it straight down while pressing the black button above its receptacle. If the bottle seems to stick, carefully move it side to side while pulling it off.

2. Wipe the delivery line with a lint-free tissue.

## Using the Auto Dilution Procedure

The Auto Dilution procedure is intended for use with A, G, C, and T phosphoramidite bottles in positions one through four. On instruments equipped with eight phosphoramidite positions, this procedure is also used for A, G, C, and T phosphoramidite bottles in positions five through eight. If you wish to change the delivery volumes, you may create a custom Auto Dilution procedure. (See "Creating a User Auto Dilution Procedure" in Section 3.)

1. Press the *Change Bottle* soft key on Page 2 of the Main Menu to display the Bottle Change menu, shown in Figure 2-2.

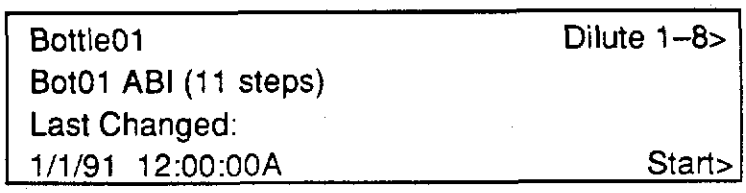



Figure 2-2. Bottle Change menu

2. To automatically dilute powdered phosphoramidites on the instrument, press *Dilute 1–8*. A warning message tells you not to remove the old phosphoramidite bottles until prompted to do so. Pressing *Okay* brings up the Auto Dilution menu, shown in Figure 2-3.

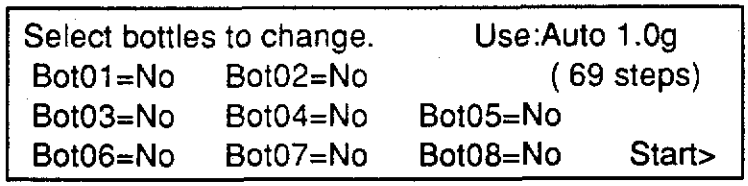



Figure 2-3. Auto Dilution menu

*NOTE* *Since the β-cyanoethyl phosphoramidite bottles provided contain either 0.25 gram, 0.5 gram, or 1.0 gram of phosphoramidite, three different procedures are available to give the proper dilutions for the three amounts. Two of the procedures, the 0.25 gram and the 0.5 gram, are used for both DNA and RNA auto-dilution. The third procedure is used for 1.0 gram DNA auto-dilution.*

*When you select multiple bottles from the Auto Dilution menu, be sure that all bottles selected contain the same amount of material. If bottles containing different amounts of phosphoramidite are installed on the instrument, you will have to use a different Auto Dilution procedure for each amount.*

3. Select the bottle positions to undergo the Auto Dilution procedure. One or all of the bottle positions listed can be selected by placing the cursor on the "No" after the desired bottle and using the **Prev** or **Next** key to switch "No" to "Yes."

4. Select the Auto Dilution procedure to be used on the bottles selected in step 3.

   This is done by placing the cursor on the procedure name (Auto 1.0g in this example) and using the **Prev** and **Next** keys to scroll through the available procedure choices, shown in Table 2-1.

## Table 2-1
## ABI Auto Dilution Procedures

| Name on Auto Dilution Menu | Purpose of Procedure |
|---|---|
| Auto 0.25g | .25 gram DNA/RNA dilution |
| Auto 0.5g | .5 gram DNA/RNA dilution |
| Auto 1.0g | 1 gram DNA dilution |
| Auto01 user to Auto 09 user | User Auto Dilution procedures |

Aut01 user through Aut09 user are names for procedures that users can create. Procedure Aut10 Mac is reserved for future use.

*NOTE* *Since the low consumption cycle uses a 0.05 M phosphoramidite solution rather than a 0.1 M solution, the bottle set to be used with this cycle should be diluted in double the normal volume of acetonitrile. In other words, Procedure Auto 0.5g should be used with 0.25 gram phosphoramidites and Procedure Auto 1.0g should be used with 0.5 gram phosphoramidites.*

5. Press the *Start* soft key when you are ready to initiate the Auto Dilution procedure. The Monitor Change Bottle screen, shown in Figure 2-4, appears. The old reagent is rinsed out of the lines and then purged into the old or empty phosphoramidite bottle(s).

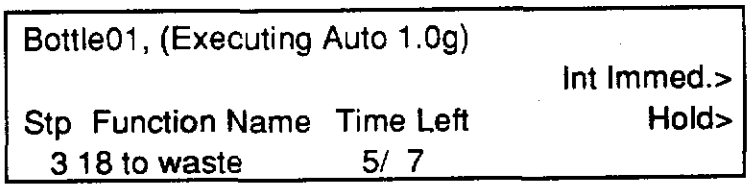



Figure 2-4. Monitor Change Bottle screen

6. After rinsing the line, the procedure will interrupt. When the interrupt occurs, do the following for each bottle to be replaced:
   a. Remove and discard the old bottle.
   b. Uncover or uncap the new phosphoramidite bottle to be used. This bottle should contain powdered phosphoramidite.
   c. Remove the rubber septum and immediately install the bottle on its position on the instrument to prevent water contamination.

7. As soon as the new bottle is (or bottles are) in position, press the *Resume* soft key to continue the Auto Dilution procedure by filling the new bottle(s) with the proper amount of acetonitrile, mixing the acetonitrile and phosphoramidite in each bottle, and filling the lines with the new phosphoramidite. When this process is finished, you will see a display indicating that the bottle change is complete. Press the *Okay* soft key to return to the menu shown in Figure 2-3.

***CAUTION*** *Do not reuse the old phosphoramidites after these steps are completed because the reservoirs now contain extra acetonitrile from Bottle 18.*

## Installing Pre-dissolved Phosphoramidites

The following procedure is used for installing bottles containing phosphoramidites in liquid form, prepared manually by the procedure provided in Appendix VI.

1. Press the *Change Bottle* soft key on Page 2 of the Main Menu to bring up the Bottle Change menu, shown in Figure 2-5.

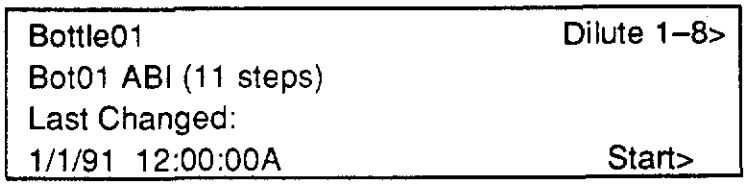



Figure 2-5. Bottle Change menu

2. Select a procedure for changing a particular bottle as follows:
   a. Place the cursor on the bottle number and then use the **Next** and/or **Prev** key to select the bottle to be changed, or enter the bottle number via the numeric key pad.
   b. Place the cursor on the change procedure name on the second line and use **Next** and/or **Prev** again to select the change procedure.

      Choices available include either a procedure provided by ABI (labeled "Botxx ABI") or a user–created procedure (Botxx user).

3. Press the *Start* soft key to initiate the change procedure for a particular bottle, and the menu shown in Figure 2-6 will appear. Acetonitrile will be delivered to the old bottle to rinse the old reagent out of the line and gas will be used to flush the line.

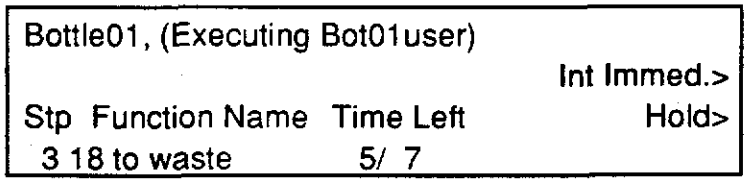



Figure 2-6. Monitor Change Bottle screen

4. After rinsing and flushing the line, the procedure will interrupt. When the interrupt occurs, do the following:
   a. Remove the old bottle.
   b. Uncover or uncap the phosphoramidite bottle to be used. The phosphoramidite in this bottle should already be dissolved. (See Appendix VI for the manual phosphoramidite preparation procedure.)

c. Remove the rubber septum and quickly install bottle on the instrument to prevent water contamination.

5. As soon as the new bottle is in position, press the *Resume* soft key to continue the bottle change procedure. The bottle will be purged with argon, and the line will be filled with the new phosphoramidite. When this process is finished, you will see a display indicating that the bottle change is complete. Press the *Okay* soft key to redisplay the menu shown in Figure 2-5. If you wish, you can now initiate changing of another bottle.

# Installing Reagent Bottles

A reagent bottle can be changed before beginning a synthesis or after an active synthesis has been stopped, either intentionally by setting an interrupt or automatically by the bottle alarm. When removing and replacing bottles, always select *Change Bottle* from the Main Menu and follow the prompts. Note one exception: if you are going to store and later reuse the phosphoramidites, refer to "How to Remove and Store Dissolved Phosphoramidites," in Appendix VI.

---

***WARNING*** ***Consider each chemical in the synthesizer potentially harmful. Do not inhale vapors. Work in a well ventilated area. Always use eye protection and wear gloves and a lab coat. Do not leave any chemicals uncapped. If any chemical is ingested or comes in contact with the eyes, immediately consult a physician. If there is any physical contact, wash immediately with ample water. Refer to the "Safety Data" appendix and the MSDS for further instructions about storing and handling each reagent.***

---

*NOTE The phosphoramidites, tetrazole, and acetonitrile, are atmosphere-sensitive. Upon opening one of these bottles, quickly place it on the instrument to prevent water contamination.*

## How to Install Reagent Bottles (Bottles 9-15)

These bottles screw directly into a threaded cap mounted on the synthesizer.

1. Remove a bottle by unscrewing it, turning counterclockwise. Remove the disposable polyethylene insert. Recap it to minimize residual vapor release.

*NOTE The disposable insert forms an airtight seal between each cap assembly and bottle. It is designed for single use and should be replaced with each bottle change. Inserts are supplied at no charge when you order the chemical reagent kits. To order inserts separately, use the following Part Numbers (PNs): P/N 400501 for 450 mL bottles, P/N 400790 for 200 mL bottles.*

2. Open the new bottle. Place an unused polyethylene insert inside the bottle neck. Screw the bottle snugly into its threaded cap on the instrument by turning it clockwise.

*NOTE Receptacles 9-15 have a ratchet cap assembly. A built-in torque-limiting feature reduces the possibility of overtightening.*

## How to Install Bottle 18

Reservoir 18, acetonitrile, is a 4-liter bottle (2.5-liter in some markets) that is placed on the left side of the instrument, inside a protective carrier. To replace the bottle, slowly unscrew the cap assembly to release pressure. Remove the cap assembly and then screw it on a fresh acetonitrile bottle. Make sure the bottle cap assembly lines are connected correctly to instrument pressure (P) with pressure on instrument and to delivery (D) with delivery on instrument.

***IMPORTANT*** *Use HPLC or UV grade acetonitrile with a specification of less than 100 ppm of water. A higher water content will lead to a significant decrease in synthesis efficiency (coupling).*

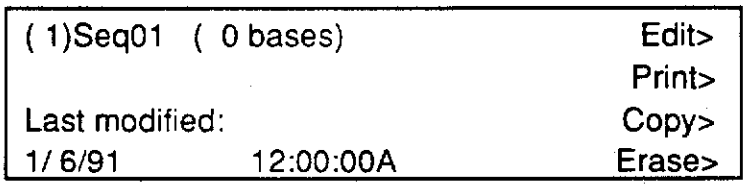
# Performing a Synthesis

## Setting Up a Synthesis

After completing the pre-synthesis check list, perform the following steps to begin a synthesis. As questions arise, refer to "Software Menu Descriptions" (Section 3) for further instructions.

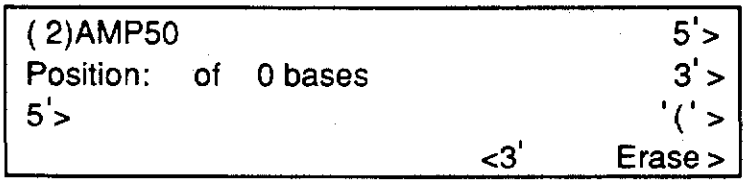
1. View Page 1 of the Main Menu:

2. From the Main Menu, select *Edit Sequence*. Then place the cursor on the number preceding the sequence name. Press **Next** or **Prev** until you find an empty sequence (one with 0 bases).




From this menu, you may give the sequence a name consisting of up to 10 alphanumeric characters. Move the cursor onto the characters of the existing name and press the **Next** or **Prev** keys to scroll through the alphanumeric characters.

3. Press the *Edit* soft key to start entering the sequence:




*NOTE If the sequence is already entered, Edit is used to view the sequence.*

4. Enter the sequence (5' to 3') using the A, G, C, T, and 5 keys on five-base position instruments. (The editor on eight-base instruments additionally allows 6, 7, and 8 base position entries.) The editor automatically adds a space after every third base to automatically separate a sequence into triplets for ease of reading. If possible, print the sequence and verify it.

   Note that mixed bases or bases from bottle positions 5, 6, 7, or 8 can be inserted in any sequence position except the 3'-terminus. To enter mixed bases, press the open parenthesis

[ '(' ] soft key. Then enter the desired bases (a maximum of four). Finally, press the close parenthesis [ ' )' ] soft key. (The close parenthesis soft key appears in place of the open parenthesis key as soon as the open parenthesis is entered.) Multiple bases are added to the support-bound oligonucleotide by simultaneous delivery of the specified phosphoramidites.

An example of a sequence is shown below:

```
( 2)AMP50                                    5' >
Position: 25  of  40 bases                   3' >
5'>AGC GCT AAC TGA G56 8GA AGT               '(' >
CCC GTA ACG A6A 7AA GC5 AG  <3'  Erase>
```

If you would like to print a sequence displayed on the screen, press **Prev Menu** and select *Print*. When finished, return to Page 1 of the Main Menu by pressing **Main Menu**.

5. From Page 1 of the Main Menu, select *Start Synthesis* (soft key):

```
Col 1 Yes ( 2)AMP50          Cycle:.2 um CE
    2 No  (  )
    3 No  (  )
    4 No  (  )               Start Columns>
```

Configure the synthesis as follows:

a. Choose the column or columns to be used in the synthesis by changing the state designator to "Yes" or "No." This is done by placing the cursor on the "Yes/No" state and pressing **Next** or **Prev** to switch between states.

b. Choose the sequence to be synthesized in each column. After selecting "Yes" for a column, the cursor will automatically move to the sequence name appearing after a selected column. **Next** or **Prev** can then be used to scroll through the available sequences. Stop when the required sequence name is showing. The above example shows that Amp50 will be synthesized in Column 1.

c. Place the cursor on the cycle name (.2 um CE, etc.) and press **Next** or **Prev** until the desired synthesis cycle is shown. See "Synthesis Cycles" in Section 4 for a list of the available cycles. Only one synthesis cycle is allowed.

d. Press the *Start Columns* soft key from the Start Synthesis menu. This will present the End Method menu:

```
Col 1  DMT Off , Auto   (End CE     )
    2  DMT Off , Manual (          )
    3  DMT On  , Auto   (End CE     )
    4  DMT On  , Manual (          )  Start>
```

*NOTE Five end procedures are available. Step 5 g describes how to choose the end procedure. See Table 4-11 in Section 4 for information on when to use a particular end procedure.*

e. Place the cursor on each DMT field to be edited and press the **Next** or **Prev** key to switch "DMT Off" to "DMT On" or vice versa.

The 5'-protecting group, dimethoxytrityl, is attached to each nucleoside phosphoramidite and is cleaved from the growing oligonucleotide chain during each cycle of base addition. Programming the 392/394 to remove the *last* DMT yields a 5' hydroxyl. When purifying by polyacrylamide gel electrophoresis (PAGE) or ion exchange HPLC, the last DMT group should be removed. When purifying by trityl-specific OPC or reverse-phase HPLC, the last DMT group should be left on.*

f. Place the cursor on "Auto" or "Manual" and press the **Next** or **Prev** key to switch between the two deprotection/cleavage methods. If "Manual" is selected, refer to "Manual Cleavage and Deprotection" in Appendix VI.

g. If you have chosen "Auto" for a column, place the cursor on the End procedure (End CE, etc.) and press **Next** or **Prev** until the desired ending method is shown for each column.

## Starting a Synthesis

When all information is displayed correctly, press the *Start* soft key. The 392/394 then checks all bottles for usage, using bottle alarm information entered during the last bottle change. If there is not enough reagent to complete the synthesis, a message like that shown below will be displayed.

| | |
|---|---|
| Bottle 9 will interrupt | Cancel Start> |
| in18 cycles. | Next Alarm> |
| | Change Bottle> |
| Total of 2 alarms | Continue Start> |

Check the reagent level of the bottle. Replace it with fresh reagent, if necessary. To replace the reagent bottle, select *Change Bottle* and follow the instructions. If the bottle is not empty, you can reenter an appropriate alarm setting on the Bottle Alarm menu (displayed by selecting *Bottle Alarms* on page 2 of the Main Menu). Check the reagent level of the other bottles and decide whether to replace them now or wait until the synthesis is interrupted by the alarm.

The other soft keys on the menu illustrated above allow you to cancel the synthesis start (*Cancel Start*) or continue the synthesis (*Continue Start*). When the total number of alarms is greater than one, the *Next Alarm* soft key will allow you to view the next bottle to interrupt.

When you are ready to continue the synthesis, press the *Continue Start* soft key. This presents the following display.

* Evaluation and purification of synthetic oligonucleotides, *Applied Biosystems User Bulletin 13* (Revised) (1987).

| | |
|---|---|
| Col 1 Install A-Column | Set Run ID> |
| 2 Not Selected | Base Source> |
| 3 Install T-Column | Print> |
| 4 Not Selected | Start> |

*NOTE The "Base Source" parameter (8–base instruments only) and the "Set Run ID" parameter are described in Section 3 under "Starting a Synthesis."*

This display tells you to install the proper column ("Install x-column," where "x" is the 3' nucleoside) or displays "Not Selected," which indicates that a column was not selected for the synthesis. Selecting the *Print* soft key prints the synthesis setup.

---

***WARNING Do not start a synthesis or initiate manual control without first installing a column or column connector. Otherwise, reagent might spray out of the lower luer and cause physical harm.***

---

Place the correct color-coded column on the instrument. This column will correspond to the base at the 3' end of your DNA sequence. Make sure it matches what is displayed on the screen and is the correct synthesis scale. Record the column's serial number to identify the synthesis.

| CPG-bound nucleoside | column color-code |
|---|---|
| A | green |
| G | yellow |
| C | red |
| T | blue |

Wide-pore CPG columns are recommended for synthesizing oligonucleotides greater than 60 bases. They are available on the 0.2 µmol scale only.

**Table 2-2.**
**Distinguishing Pore Sizes of ABI Columns**

| CPG Pore Size | Scale | Serial No. | Column Label |
|---|---|---|---|
| 1000Å wp | 0.2 µmol | 4XXXXX | broken color |
| 1000Å wp | 0.2 µmol FOD* | 5XXXXX | broken color |
| 500Å | 0.2 µmol | 2XXXXX | broken color |
| 500Å | 1.0 µmol | 3XXXXX | solid color |
| 500Å | 10 µmol | 3XXXXX | solid color, large column |

*FOD stands for fast oligonucleotide deprotection. The FOD (A, G, and C) columns and phosphoramidites have alternative base-protecting groups. For additional information,see Section 6.

## Installing a Column

1. Tap the ends of the SNAP synthesis column on a dark surface to check for leaks. If CPG falls out, *do not* use the column. Using a leaky column could damage the instrument. Any faulty columns will be replaced by Applied Biosystems without charge.
2. Firmly push either end straight down onto the lower luer fitting on the instrument. Since the column is symmetrical, it can be attached in either direction.
3. Firmly push the upper luer fitting straight down onto the top of the column. The column should fit securely. *Do not* twist the fittings!

Once the column is installed, press *Start* to continue with the synthesis. The subsequent display allows selection of a trityl collect option and a begin procedure. You can use the **Next** and **Prev** keys to change the trityl collect option from "All" to "1st & last 2" or to "None."

```
Check Waste Bottle!                    Cancel Start>
Trityl collect = All
                                                 No>
Execute ABI Begin   (14 steps)?                 Yes>
```

*NOTE* *The beginning procedure fills the lines with fresh phosphoramidite. If the instrument has been idle for more than 12 hours (6 hours in a humid environment), the beginning procedure should be run.*

Select the desired beginning procedure by placing the cursor on the beginning procedure name and pressing either the **Prev** or **Next** key to access the previous or the next beginning procedure. The soft key options give the user the ability to execute the specified beginning procedure (*Yes*), circumvent the execution of any beginning procedure (*No*), or cancel the synthesis (*Cancel Start*). If the synthesis is canceled, the display returns to Page 1 of the Main Menu. If option 1 or 2 is selected, the synthesis is started and the display proceeds to the Monitor Steps menu of the first active column.

```
Col 1  (Executing .2 um CE)          *Int Ahead>
Synthesizing base 1 of 4             Int Immed.>
Step Function Name  Time Left              Hold>
  5  B+TET to COL1    5/  3                Jump>
```

The top left corner of the display shows which column is being monitored. Selecting another column can be done by positioning the cursor on the column identifier and pressing the **Prev** and **Next** keys until the desired column is displayed.

Now, press **Prev Menu** to display the Monitor Synthesis menu, shown next:

```
Col 1  (Seq01     ,25 bases)          Steps>
     2 (Inactive)                     Sequence>
     3 (Seq04     ,40 bases)          Unlocked>
     4 (Inactive)                     Print>
```

From this menu you can determine which columns are included in the active synthesis, which sequences are being used, and the lengths of the sequences. The *Steps* soft key allows you to monitor the steps of the synthesis as they are occurring. The *Sequence* soft key allows you to view the sequence that is being synthesized on a particular column. The base currently being added will flash. The *Unlocked* soft key changes to *Locked* when pressed, protecting the synthesis from keypad intervention.

## Monitoring the Synthesis

During the synthesis, you can continue to view the Monitor Synthesis menu or return to the Main Menu to access other menus. During an active synthesis, Self Test, Manual Control, and Shut Down cannot be accessed; however, you can access the Cycle Editor, Sequence Editor, Procedure Editor, or Function Editor.

When the synthesis is finished, *Inactive* is displayed on the Monitor Synthesis menu.

# Cleavage and Deprotection

After the synthesis of an oligonucleotide on the Model 392/394, the molecule produced is not yet biologically active. The phosphate groups and exocyclic amines of the bases are protected to prevent side reactions during synthesis.

After the synthesis is complete, when auto cleavage has been selected (either DMT On or DMT Off), the oligonucleotide is cleaved from the support on the machine using concentrated ammonium hydroxide at room temperature. During cleavage, the β-cyanoethyl phosphate protecting groups are also removed. When standard phosphoramidites are used, the benzoyl (dA and dC) and isobutyryl (dG) base-protecting groups are completely removed by concentrated ammonium hydroxide in 8 to 15 hours at 55° C. When FOD phosphoramidites are used, the dimethylformamidine (dA and dG) and isobutyryl (dC) base-protecting groups are removed by concentrated ammonium hydroxide in 1 hour at 55° C or in 8 hours at room temperature. (For more information, see "Base Deprotection" in Section 6.)

# Post Synthesis Operations

After auto cleavage and deprotection, the oligonucleotide-containing ammonia solution is ready for analysis and purification.

1. Remove the collection vial containing the oligonucleotide in ammonium hydroxide.
2. Remove and dispose of the synthesis column.
3. Remove the trityl tubes and perform the following:
   a. Visually inspect the trityl solutions. Be sure there are no clear tubes and that the intensity of the orange color gradually decreases.
   b. If desired, perform the trityl cation assay to approximate the coupling efficiency of the synthesis. Refer to "Dimethoxytrityl (DMT) Cation Assay" in Appendix VI for instructions. Once the collection vial, column and trityl tubes are removed, the instrument is ready to begin another synthesis.
4. If desired, analyze the crude oligonucleotide mixture by either HPLC, capillary electrophoresis, or gel electrophoresis, described in User Bulletin 13 (Revised). These assays are the true test of synthesis success.
5. Prepare the DNA for use in experiments by removing the ammonia and then desalting. Purify the product, if necessary.

   Applied Biosystems' Oligonucleotide Purification Cartridge (OPC) provides a fast and easy way to purify and desalt in one step. An overview of OPC and other purification methods can be found in Section 6, "Chemistry for Automated DNA/RNA Synthesis." For additional information, refer to "OPC (Oligonucleotide Purification Cartridge)" in Appendix VI. Refer to User Bulletin 13 (Revised) for other detailed instructions about analysis and purification.

# Interrupting or Aborting a Synthesis

***CAUTION*** *Except for emergencies, when you want to interrupt immediately (**Int Immed.** command), program the interrupt to occur at a spot you consider safe using the interrupt ahead (Int Ahead) command. The best spots to interrupt a synthesis are between Start Detrityl and Waste - Port, corresponding to the step numbers listed in Table 2-3 for various cycles. Failure to interrupt at a safe step could result in clogged valve blocks or lines and a failed synthesis.*

1. Press the *Steps* soft key from the Monitor Synthesis menu.
2. For emergencies, press the *Int Immed* command from the Monitor Steps menu. This will interrupt synthesis on all active columns.
3. For routine programmed interrupts, decide which of two types of interrupts to use. Use a cycle interrupt to interrupt all active columns at a specific step number and after a designated number of cycles. Use a column interrupt to interrupt selected columns at a specified base number with DMT set off or on.

*NOTE* *Be sure that you use the type of interrupt that you intend. If you want to interrupt or abort synthesis on a single column, use a Column interrupt. If you want to interrupt or abort synthesis on all columns, use a Cycle interrupt.*

4. Program a cycle interrupt as follows:
   a. From the Step Monitor menu, press the *Int Ahead* soft key and move the cursor to the last line on the screen, which reads:
   "**(No) Interrupt after 000 cycles, step 001**"
   b. First set the number of cycles and the step number and then change "No" to "Yes" using the **Next** or **Prev** keys. An * will appear next to *Int Ahead* on the Monitor Steps menu. It is not necessary to change the other lines of the display.
5. Program a column interrupt as follows:
   a. From the Monitor Steps menu, press the *Int Ahead* soft key and toggle the columns to be interrupted from "No" to "Yes" using the **Next** or **Prev** keys.
   b. Set the base number at which to interrupt and choose "DMT Off" or "DMT On." It is not necessary to change the last line of the display. An * will appear next to *Int Ahead* on the Monitor Steps menu.

## Table 2-3
## Recommended Steps for an Interrupt (Immediate or Ahead)

| | 394 Step Numbers | 392 Step Numbers |
|---|---|---|
| Primer | 72-78 | 54-60 |
| 0.2 um CE | 64-70 | 44-50 |
| 1.0 um CE | 64-70 | 44-50 |
| 10 um CE | 53-59 | 41-47 |
| 0.2 um RNA | 64-70 | 44-50 |
| 1.0 um RNA | 64-70 | 44-50 |
| 10 um RNA | 53-59 | 41-47 |
| 0.2 um sulfur | 69-75 | 49-55 |
| 1 .0 um sulfur | 75-81 | 56-62 |
| 10 um sulfur | 61-67 | 49-55 |

6. When the synthesis interrupts, the screen will display the Interrupted Status. If you wish to abort the synthesis, select *Abort*. You will then see a display that allows you to confirm or cancel the *Abort* command.

   If you abort synthesis at a step other than Cycle Entry or Cycle End, the lines and valve blocks must be rinsed and dried. To do this, select Manual Control and activate the following functions for 10 seconds each:

   - Function 64, "18 to Waste"
   - Function 42, "18 to Column"
   - Function 1, "Block Flush "
   - Function 2, "Reverse Flush "

# Changing an Argon Tank

You can replace an empty argon tank before beginning a synthesis or when a synthesis has been interrupted. Replace the empty cylinder as follows:

1. Shut off the cylinder both at the tank and at the needle valve on the tank regulator.
2. Remove the regulator from the empty tank, clean the threads on the fittings, and install the regulator on a full tank. For maximum gas lifetime, wrap the threads with teflon tape before installing.
3. Turn the tank on and check for leaks at the connection of the tank to the regulator.
4. Open the needle valve.

   A size 1A tank of 2500 psi argon should last about two months.

*NOTE You may hear a hissing sound while the new argon supply is recharging the vacuum assist.*

---

***WARNING*** ***Compressed gas cylinders must be safely attached to the wall or bench by means of approved brackets or chains. FAILURE TO DO SO COULD CAUSE THE CYLINDERS TO FALL OVER AND EXPLODE, WHICH COULD RESULT IN PHYSICAL HARM.. The cylinder should always be capped when not in use. Never transport a cylinder with a regulator attached!***

---

# Changing the Waste Bottle

The synthesizer generates about 1 liter of hazardous, halogenated, organic liquid waste per 100 base additions. The waste is collected in a 10-liter polyethylene bottle that is placed on the floor or on a nearby bench *lower* than the instrument. When the bottle is full, it should be emptied as follows:

1. When a synthesis is not in progress, unscrew the cap assembly and immediately recap the bottle to prevent the release of vapors.
2. Discard the waste. Place the liquid in a sealed container labeled "WASTE FLAMMABLE," "NOS (Halogenated)." Dispose of the waste following applicable government regulations.

---

***WARNING*** ***Synthesizer waste must be disposed of properly and carefully. When handling the waste for disposal, wear gloves and eye protection, and avoid inhalation and skin contact. Refer to the Waste Profile and MSDSs in the Safety Data Appendix for details. All waste must be disposed of as a regulated hazardous waste in accordance with applicable federal, state, and local government regulations.***

---

3. After disposal, securely screw the cap assembly supplied by Applied Biosystems onto the emptied waste bottle.

***IMPORTANT*** *The waste bottle is the low pressure side of the delivery system and must always be kept vented to atmosphere. Be sure the vent line is properly routed to a fume hood. If the vent line is blocked, back pressure will be generated and will inhibit the deliveries of reagents and solvents. See the ventilation drawing in the Model 392 and 394 DNA/RNA Synthesizer Pre-Installation Manual, included in Appendix V, to verify proper ventilation requirements.*

# 3

# *Software Menu Descriptions*

This section explains how to use each menu option shown on the display screen. A summary of Main Menu options appears on page 3-7. Prior to your initial synthesis, be sure to read about the following Main Menu options: Sequence Editor, Start Synthesis, Monitor Synthesis and Change Bottles. Read about the remaining Main Menu options as you need to use them.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# Introduction

The Model 392/394 software is "menu-driven." Menus and pages of menus are shown on a 4 line by 40 character liquid crystal display (LCD). The menus present various options and necessary information about the synthesis or status of the instrument. In response, you select an option and give instructions by pressing the appropriate key on the keyboard.

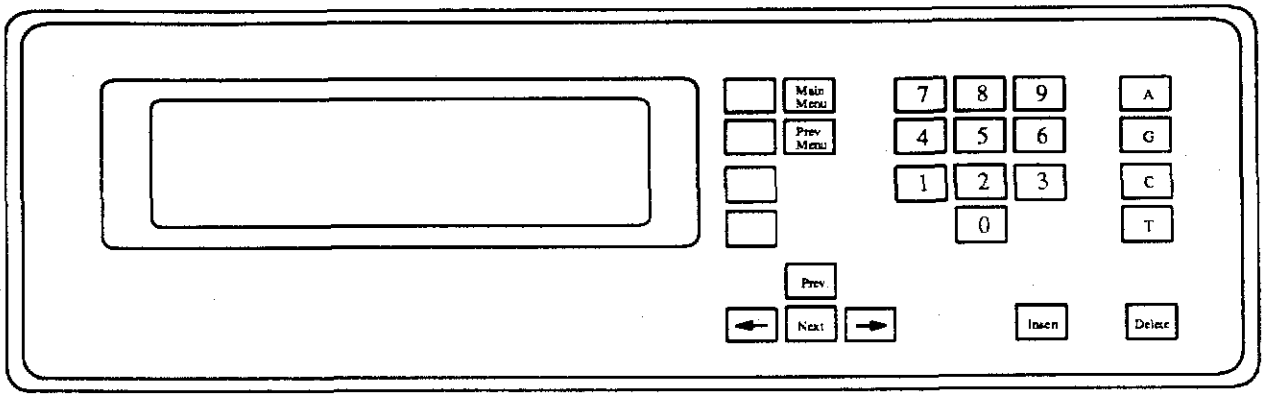
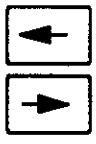



Figure 3-1. The keyboard

The keyboard has several labeled keys: **0-9**; **A, G, C, T**; ◄ ► (left and right cursor keys); **Main Menu**; **Prev Menu**; **Prev**; **Next**; **Insert**; **Delete**; and four "soft keys" (unlabeled keys to the right of the display). The labels and actions of the soft keys change depending on the information displayed to the left of them.

Pressing one of the keys labeled 0 through 9, enters a numeric value at the cursor position.

These keys, the Base keys, are found to the right of the 0-9 keypad. The Base keys are only active when inserting or modifying a base in a DNA sequence. Alternatively, numeric keys 1-4 can also be used to enter, respectively, bases A, G, C, and T in a sequence.



To make entries or deletions, the cursor must be in the correct position and often must be moved. Pressing the left (◄) or right (►) arrow key moves the cursor one position in the arrow's direction. Continuously pressing an arrow repeats the action.

Main Menu

Pressing **Main Menu** from another menu always returns the display to Page 1 of the Main Menu. Pressing **Main Menu** while a page of the Main Menu is on display leads to the next page of the Main Menu.

Prev Menu

Pressing the **Prev Menu** key returns the display to the menu previously shown. Pressing this key repeatedly will return the display to the Main Menu.

Prev.

Next

The **Prev** (Previous) and **Next** keys are used to scroll through the steps in a procedure or through all the choices available at the current cursor position (e.g., the DMT Off/On option on the End Method menu is changed using these keys).

Insert

**Insert** is used in the cycle and procedure editor to insert a new step. Pressing the **Insert** key adds a step and prompts you to enter the function number for the new step.

Delete

Pressing the **Delete** key erases an entry at the cursor position. Pressing the **Delete** key when the cursor is on a step number (within a cycle or procedure) deletes the entire step. Continuously pressing **Delete** will repeatedly erase entries.

(soft keys)

The soft keys are the vertical row of four keys to the right of the display. A soft key is active when the line to the left on the display ends with a command, a word or phrase followed by a ">" symbol.

# The Power-up Menu

Once the instrument is connected to a power source and the power is turned on, the following display appears:

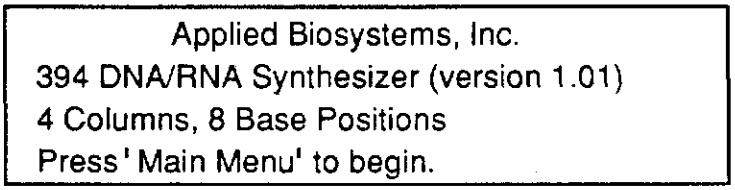



Figure 3-2. Power-up menu

*NOTE* *In this manual, displays will be representative of instruments configured with four columns and eight base positions and may show information not needed for other instruments equipped with two columns or five base positions.*

# The Main Menu

## Accessing Main Menu Options

Press **Main Menu,** from the Boot Menu, and the screen will display Page 1 of the Main Menu, presenting four options:

| | |
|---|---|
| -Main Menu- | Edit Sequence> |
| | Start Synthesis> |
| | Monitor Synthesis> |
| Page 1 of 5 | Next Page> |

*NOTE The* Next Page *soft key is used to proceed to the next set of Main Menu options*

| | |
|---|---|
| - Main Menu- | Change Bottle> |
| | Bottle Alarms> |
| | Manual Control> |
| Page 2 of 5 | Next Page> |

Each press of the *Next Page* soft key displays a new page of the Main Menu, showing additional options:

| | |
|---|---|
| - Main Menu- | Self Test> |
| | Edit Cycle> |
| | Edit Procedure> |
| Page 3 of 5 | Next Page> |

| | |
|---|---|
| - Main Menu- | Edit Function> |
| | Power Fail> |
| | Set Time/Date> |
| Page 4 of 5 | Next Page> |

| | |
|---|---|
| - Main Menu- | Relay Configuration> |
| | Shut Down> |
| Page 5 of 5 | Next Page> |

Selecting the *Next Page* soft key again returns the display to Page 1 of the Main Menu.

All other menus or menu pages are accessed by selecting the appropriate soft key from Page 1, 2, 3, 4, or 5 of the Main Menu. To move from one menu (e.g., Edit Sequence) to another

(e.g., Start Synthesis) requires returning to the first page of the Main Menu by pressing the **Main Menu** key. An alternate way of returning to Page 1 of the Main Menu (or of returning to the previous page) is pressing the **Prev Menu** key. Each press of **Prev Menu** will return the display to the previous menu. Continue to press **Prev Menu** until Page 1 of the Main Menu is displayed.

## Summary of Main Menu Options

| | |
|---|---|
| Edit Sequence | Used to create, edit, print, copy, and erase up to 30 DNA sequences. |
| Start Synthesis | Initiates the steps to begin a synthesis. From this menu, you choose the desired columns, DNA sequences, cycle, beginning procedure, and ending method. This option is also used to print out a synthesis setup. |
| Monitor Synthesis | Displays the status of the instrument during a synthesis. Use it to identify which base is being added and which step is being performed. |
| Change Bottle | Provides a procedure for removing empty bottles and replacing them with new bottles of phosphoramidites or fresh reagents. |
| Bottle Alarms | Shows bottle usage and allows you to set a bottle alarm. |
| Manual Control | Used to manually activate functions and to open and close individual valves. |
| Self Test | Verifies the correct operation of the electronic components of the instrument. Accesses the Flow Test menu. |
| Edit Cycle | Used to create, edit, erase, copy, or print synthesis cycles. |
| Edit Procedure | Used to create, edit, print, copy, or erase the Bottle Change, Shut Down, or Begin and End procedures. |
| Edit Function | Used to create and edit user functions and to print the standard and user functions. |
| Power Fail | Used to view list of power failures and program the instrument for selective restarts upon power failure. |
| Set Time/Date | Used to enter the current time and date. |
| Relay Config. | Used to set the relay operation pulse time (to advance the fraction collector). |

Shut Down

Prepares the instrument for long-term storage by washing and drying all chemical pathways.

# Main Menu Option: Sequence Editor

The Sequence Editor is used to create, edit, print, copy, or erase up to thirty DNA sequences. The sequences or strands are named Seq01, Seq02, Seq03, ..., Seq30. The Sequence Editor is also used to view Seq31 through Seq34, which are reserved for future use and cannot be edited or modified directly by a user.

When you press the *Edit Sequence* soft key on Page 1 of the Main Menu, the screen displays the sequence last modified and shows the number of bases in that sequence. For example, the following menu shows Seq01, which currently has forty bases:

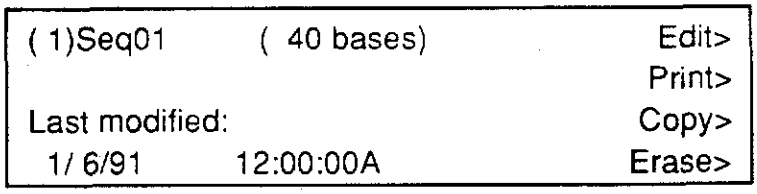



Figure 3-3. Select Sequence menu

The cursor will initially be positioned on the number in parentheses preceding the sequence name to allow selection of another sequence. An alternative to making a selection by scrolling with the **Prev** or **Next** keys is to enter the number of the desired sequence through the numeric keypad. This is done without moving the cursor.

The sequence name may be changed to any name containing up to 10 alphanumeric characters. To change the name, place the cursor under the first character ("S" in Figure 3-3) and use the **Prev** and **Next** keys to scroll through the alphanumeric characters (0-9 and A-Z), stopping at the desired character. Then move the cursor to the next character and repeat the process. Each character in the name can be changed by scrolling through the alphanumeric characters.

To use the Sequence Editor on the selected sequence, select one of the four commands listed below by pressing the soft key.

| | |
|---|---|
| Edit | Select to create or edit the DNA sequence designated in the menu. |
| Print | When a printer is connected to the instrument, select this option to print the designated sequence. |
| Copy | Select to copy the designated sequence or its complement into any other position. |
| Erase | Select to erase the designated sequence or erase all sequences. |

## Editing Sequences

When you press the *Edit* soft key on the Select Sequence menu, a display like that in Figure 3-3 apppears:

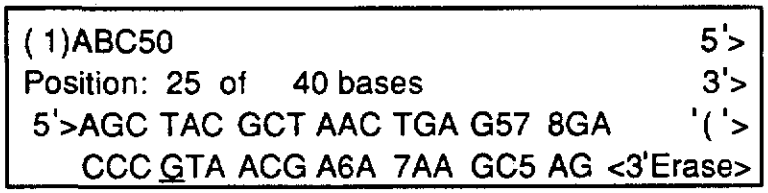



Figure 3-4. Edit Sequence menu

### General Rules for Editing

The number and name at the top left corner of the screen represent the current sequence. To select another sequence from this menu, move the cursor to the sequence number (in parentheses) and use the **Prev** or **Next** key to scroll to the desired sequence.

The information after "Position:" indicates the current base position of the cursor and the number of bases contained in the sequence. In Figure 3-4, notice that the cursor is located at the 25th base position.

*IMPORTANT* *Following convention, a DNA sequence is entered 5' to 3'. Likewise, the DNA Editor Menu displays the sequence in a 5' to 3' orientation. However, the DNA sequence is actually synthesized 3' to 5'.*

You can enter up to 999 bases, using a combination of bases and parentheses, to create a single sequence. Since the bottom two lines of the display can show a maximum of 54 characters at any time (including the spaces which are automatically added for codons), an ellipsis (...) replaces either 5'> or <3', or both when the sequence grows beyond the 54-character screen limit.

When additional characters are entered, the display shifts to the left and the base(s) at the 5' position can no longer be seen. To view parts of a sequence not displayed, press the left- or right-arrow key (◄— or —►). One of these keys will move the cursor toward the area you want to see and scroll the unseen bases onto the screen. When the end of the sequence is displayed, the cursor moves to the sequence name. Also, the 5' and 3' soft keys can be used to move the cursor to either end. This circumvents having to scroll through the entire sequence.

The *Erase* soft key is used to erase the sequence being shown. A confirmation screen will appear to prevent accidental deletion.

### Entering a New Sequence

Press the *Edit* soft key on the Select Sequence menu (Figure 3-3). With the cursor on the sequence number (shown in parentheses), press **Next** or **Prev** to scroll to an empty sequence

(one with 0 bases). Then press the right-arrow key to move the cursor to the starting position for entering a sequence, immediately after the "5'>" symbol. For each entry, press one of the four base keys or press the key corresponding to a specific bottle position (5, 6, 7, 8). The keys 1, 2, 3, and 4 may also be used to enter A, G, C, and T, respectively. The base (or the designated position) then appears on the display, the cursor moves one position to the right, and the base position number increases by one. After entry of the initial character, the display appears as shown in Figure 3-5:

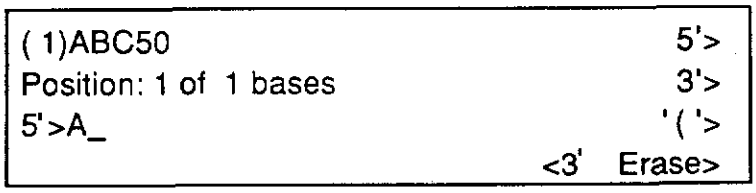



Figure 3-5. Entering bases in a sequence

If a base other than an A, G, C, or T is present at the 3' end of the sequence, the following error message appears when you attempt to leave the editor:

"To exit the Sequence Editor, the last base must be changed to an A, G, C, or T!"

| ( ) | Use parentheses to insert multiple bases in one position for synthesizing mixed sequence probes. |
|---|---|

This is done by using the *Parenthesis* soft key to produce the left or "open" parenthesis, "(" , typing any combination of bases A, G, C, T, 5, 6, 7, 8, and then using the *Parenthesis* soft key again to produce the right or "close" parenthesis, ")." A maximum of four bases may be placed inside a single set of parentheses.

For example, to insert AGCT in the third position of the sequence 5'>AA_<3':

| | |
|---|---|
| | 5'>AA_ <3' ; |
| press *Parenthesis* soft key; | 5'>AA(_ <3'; |
| press **A, G, C, T**; | 5'>AA(AGCT_ <3'; |
| *press* Parenthesis soft key again; | 5'>AA(AGCT) _<3'; |

Multiple bases are added to the support-bound nucleotide by simultaneous delivery of the specified phosphoramidites. They can be placed in any position except the 3', support-bound terminus. Notice that a space is added after the last parenthesis to maintain the codon format.

## Inserting a Base in a Sequence

Insertions of a new base are made to the left of the present cursor position. For example, to insert T in the third position in the sequence 5'>AAA <3':

| | |
|---|---|
| (use the cursor keys to position the cursor on the third position) | 5'>AAA <3'; |
| select **T** | 5'>AAT A <3' |

Notice that T is inserted to the left of the cursor position, a blank is automatically inserted to preserve the codon format, and the original A is now in the first position of a new codon with the cursor at that position. To make an entry at the 3' position, move the cursor one space to the right of the current base at the 3' position and type in your new base.

Continuously pressing the button corresponding to a base repeatedly inserts that base.

## Deleting a Base in a Sequence

Most deletions are made at the cursor position. For example, to delete C from the sequence 5'AGCT 3':

| | |
|---|---|
| (move the cursor to the C position) | 5'>AGC T <3' |
| *press* **Delete** | 5'>AGT <3' |

When the cursor is at the 3'-end of the sequence, the deletion is made to the left of the cursor. For example, to delete T from the sequence 5'AGC T 3':

| | | |
|---|---|---|
| (move the cursor one space to the right of the T position) | 5'>AGC T <3' | 5'>AGC T_<3' |
| press **Delete** | 5'>AGC _ <3' | |

Multiple bases within parentheses are deleted when the cursor is on either parenthesis. For example, to delete AGCT from the third position in the sequence 5'AA(AGCT) TTT 3':

| | |
|---|---|
| (move the cursor to either parenthesis position) | 5'>AA(AGCT) TTT <3' |
| press **Delete** | 5'>AAT TT <3' |

To erase a single base within parentheses, place the cursor on the unwanted base and press delete. For example, to delete C from the sequence 5'AA(AGCT) TTT 3':

| | |
|---|---|
| (move the cursor to the C position) | 5'>AA(AGCT) TTT <3' |
| press **Delete** | 5'>AA(AGT) TTT <3' |

Continuously pressing **Delete** will repeatedly erase entries.

*NOTE Once a synthesis has begun, the sequence that is currently being synthesized cannot be changed.*

The Sequence Editor menu can be accessed during an active synthesis and new sequences can be created for use in subsequent syntheses. Any sequence not being used in the synthesis may be modified.

## Printing Sequences

When a printer is connected to the instrument, this option is used to print out a hard copy of the designated sequence. After selecting a sequence from the Select Sequence menu (Figure 3-3), press the *Print* soft key to print out the sequence listing. Printing can be cancelled at any time by pressing the *Cancel Print* soft key (which is active during printing). When printing is completed, *Cancel Print* is replaced by the *Print* soft key on the Select Sequence menu.

The printout from the Sequence Editor not only includes the sequence, but also lists the total number of bases, the base composition, the molecular weight expressed in Daltons, and the time and date. When bases are added from bottle positions 5, 6, 7, or 8 (or mixed-base sites are used), the molecular weight is not calculated. Figure 3-6 shows a Sequence Editor printout.

```
                              - 394 Sequence Listing -                    (Version 1.01)

Name: ABC50                                   2:18:53A,  1/ 3/90

Total bases =  50
        A=  10,  G=  13,  C=  17,  T=  10,  5=   0,  7=   0,  8=   0
        (mixed bases=  0)

MW:  16357.0

      5'>  AGG GCC GAG CGC AGA AGT GGT CCT GCA ACT TTA TCC GCC
           TCC ATC CAG TC      <3'
```

Figure 3-6. Sequence Editor printout

## Copying Sequences

The Sequence Copy menu allows you to copy one sequence (e.g., Seq01) into another sequence (e.g., Seq05). To reach this menu (shown below) press the *Copy* soft key on the Select Sequence Menu (Figure 3-3).

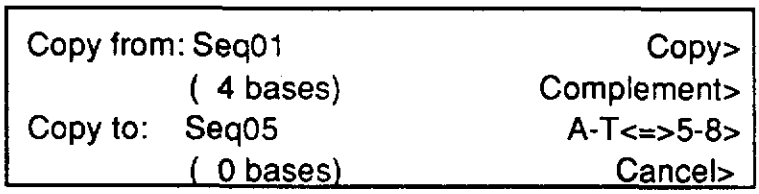



Figure 3-7. Sequence Copy menu

*NOTE The "A-T<->5-8>" soft key is only available on 8-base position instruments.*

The Sequence Copy menu allows you to select a source sequence and a destination sequence for the copy. To select a source sequence, place the cursor on the name and then use the **Prev** and **Next** keys to scroll through the available sequences. Selecting a destination sequence is done in the same manner. Beneath the source and destination sequences is listed the number of bases they contain.

Once you select source and destination files, you have four choices:

Press the *Copy* soft key to make a straightforward copy of the source sequence. It is copied into the destination sequence.

Press the *Complement* soft key to copy the complement of the source sequence into the destination sequence. For example:

```
( 1)SEQ01
Position: 1 of  4 bases
5'>AAG G
                              <3'
```

would become:

```
( 5)SEQ05
Position: 1 of  4 bases
5'>CCT T
                              <3'
```

Press the *A-T<->5-8* soft key to copy the source sequence into the destination sequence and, at the same time, transcribe the base letters to numbers. Each A in the sequence is copied

as "5," each G as "6," each C as "7," and each T as "8." The last base at the 3' end will not be transcribed into a number; it will remain an A, G, C, or T. This capability is useful because it allows you to enter a sequence using the A, G, C, and T keys and then translate into 5 to 8 to use phosphoramidite positions 5 to 8. Alternatively, sequences containing numbers can be transcribed into letters. Each 5 is copied as A, each 6 as G, each 7 as C, and each 8 as T. Transcription from letters to numbers and vice versa is available only on 8-base position instruments.

Press the *Cancel* soft key to cancel the copy procedure and return to the Select Sequence menu (Figure 3-3).

## Erasing Sequences

Another soft key function accessible from the Edit Sequence menu is *Erase*. When you press the *Erase* soft key on the Select Sequence menu (Figure 3-3), a menu like the following is displayed:

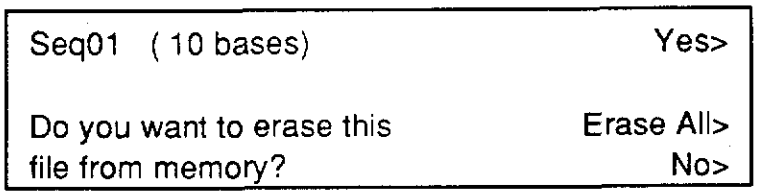



Figure 3-8. Sequence Erase menu

The upper left hand corner of the menu indicates which sequence will be erased. By placing the cursor on this name and using the **Prev** and **Next** keys, you can select any of the 30 editable sequences. To the right of the sequence name, the number of bases in the sequence is shown. Three choices can be made by pressing a soft key on this menu. When you press *Yes*, the designated sequence is erased. When you press *No*, the erase request is canceled. When you press *Erase All*, the menu shown in Figure 3-9 is displayed to enable all sequences stored in the 392/394 to be erased:

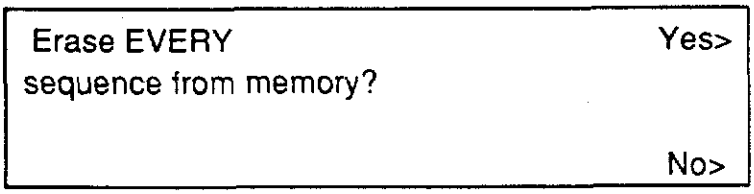



Figure 3-9. All Sequence Erase menu

Pressing the *Yes* soft key on the above menu erases *every* sequence in the 392/394 memory. Pressing the *No* soft key leaves sequences in memory and returns the display to the previous menu.

# Main Menu Option: Start Synthesis

This section describes the software of the Start Synthesis menu. For more details on how to begin a synthesis refer to Section 2.

The *Start Synthesis* soft key is available on Page 1 of the Main Menu. When you press this key, a Start Synthesis Menu like that shown below is displayed. It allows you to choose the column(s) to be used, the DNA sequences to be made, and the synthesis cycle to be used.

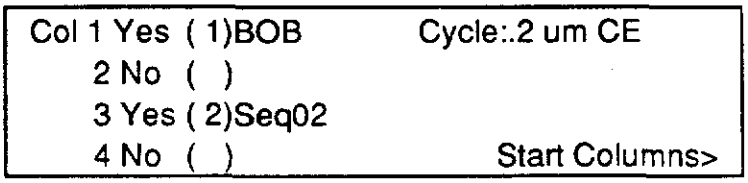



Figure 3-10. Start Synthesis menu

*NOTE Two columns are available on the 392 synthesizer.*

## Columns, Sequences, and Cycles

The first task in setting up a synthesis is to select the columns and the sequences to be included in the synthesis. To choose the active columns, place the cursor on the "Yes/No" state displayed for a particular column and then use the **Prev** and **Next** keys to switch between the two states. As soon as "Yes" is selected for a particular column, the cursor moves to the sequence number, which precedes the sequence name. If you wish to change the sequence assigned to a particular column, use the **Prev** or **Next** keys to scroll through available sequences. An alternate way of selecting a sequence is to use the numeric keys.

The name of the cycle to be used in the synthesis appears on the first line of the Start Synthesis menu. When more than one column is selected, the same cycle is used on all columns. To change the cycle, place the cursor on the cycle name and scroll through the available cycles using the **Prev** and **Next** keys. Any of the cycles listed may be selected.

When you display the Start Synthesis menu while a synthesis is active, the menu reflects this active state. In the following example, Columns 1 and 3 have a synthesis in progress. Columns 2 and 4 have yet to be started.

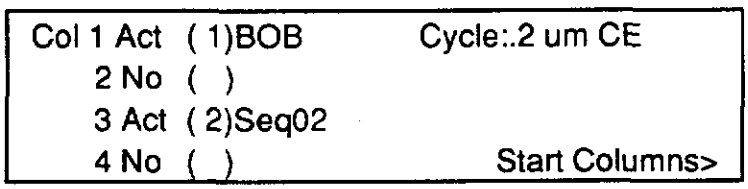



Figure 3-11. Active Start Synthesis menu

The active columns are indicated by the label "Act." You will not be able to move the cursor to this label or to the sequence indicated on the right. If a "No" label appears after a column,

you can change "No" to "Yes," select a sequence, and press the *Start Columns* soft key to include the column in an active synthesis. The new sequence will be synthesized with the cycle that is currently active. It is not possible to select a different cycle.

## Specifying the Ending Method

The next task required to configure a synthesis is to specify the end method. When you press the *Start Columns* soft key on the Start Synthesis menu, the following menu appears:

```
Col 1: DMT  On , Auto    (End CE    )
    2: DMT  On , Manual  (          )
    3: DMT  Off , Manual (          )
    4: DMT  Off , Auto   (End CE    ) Start>
```

Figure 3-12. End Method menu

*NOTE Five end procedures are available. The third paragraph below describes how to choose the End Method. See Table 4-11 in Section 4 for information on when to use a particular end procedure.*

From the End Method menu, you can select the DMT state, end method, and end procedure for each column. To switch between the "DMT Off" (dimethoxytrityl off) state and the "DMT On" state, place the cursor on the state for a particular column and use the **Prev** or **Next** keys.

The 5' terminus of the fully synthesized DNA chain can either remain protected by a dimethoxytrityl group (DMT On), or can be detritylated to yield a 5'-hydroxyl (DMT Off). DMT On is usually selected when purifying by trityl-specific OPC or reverse phase HPLC. DMT Off is usually selected when purifying by gel electrophoresis or ion exchange HPLC.

To choose an end method, place the cursor on the end method (Auto or Manual) and use the **Prev** or **Next** keys to switch between the two selections. If you select "Auto," you must also select an end procedure. To do so, place the cursor on the end procedure name and use the **Prev** or **Next** keys to scroll to the desired end procedure. During the execution of an end procedure, a synthesis may be configured and started on any inactive column. If you start another synthesis be careful not to change the end procedure of the active column.

To continue configuring the synthesis, press the *Start* soft key.

*NOTE If DMT On is specified, the DNA can be manually detritylated by a 15-minute treatment with 80% acetic acid at room temperature. This is done following synthesis and base deprotection.*

## Starting a Synthesis

When all synthesis information is displayed correctly, initiate the synthesis by pressing the *Start Columns* soft key on the Start Synthesis menu. When a synthesis is started, the 392/394 checks the theoretical volume of liquid in each reagent bottle. If there is not enough reagent to complete the synthesis (based on the alarm you set via the Bottle Change menu), a message like the one shown below will be displayed.

| | |
|---|---|
| Bottle 9 will interrupt | Cancel Start> |
| in 40 cycles. | Next Alarm> |
| | Change Bottle> |
| Total of 2 alarms | Continue Start> |

Figure 3-13. Insufficient reagent warning screen

Check the reagent level of the bottle. If necessary, replace it with fresh reagent. To replace the reagent bottle, select *Change Bottle* and follow the instructions. If the bottle is not empty, you can reenter an appropriate alarm setting on the Bottle Change menu. You should set appropriate alarm settings for other bottles if you have not already done so.

The other soft keys on this menu allow you to cancel the synthesis start (*Cancel Start*) or continue the synthesis (*Continue Start*). When the number of alarms is greater than one, the *Next Alarm* soft key allows you to view the next bottle to interrupt.

When you are ready to continue the synthesis, select *Continue Start*. The following display appears:

| | |
|---|---|
| Col 1 Install A-Column | Set Run ID> |
| 2 Not Selected | Base Source> |
| 3 Install T-Column | Print> |
| 4 Not Selected | Start> |

Figure 3-14. Column Installation Prompt screen

*NOTE An asterisk adjacent to "Base Source" indicates that one or more bases have source changes programmed. Another possible message for a column is "Empty Sequence."*

After each column represented in this display, you will see one of the following: "Install *x*-column," where *x* is the 3' base, or "Not Selected," which indicates that a column was not selected for the synthesis.

### Entering Run IDs for Sequences

Pressing the *Set Run ID* soft key on the Column Installation Prompt screen (Figure 3-14) allows you to give each sequence being synthesized a 10-character, alphanumeric run ID (see

Figure 3-15). Use the left- or right-arrow keys (◄ or ►) to move from chararacter to character within the IDs and from one ID to another. Use the **Prev** or **Next** keys to scroll through the alphanumeric characters. The run ID will appear on the "Synthesis Setup" print-out (for example, Figure 3-19) and will be cleared once the synthesis is complete. Use **Prev Menu** to return to the Column Installation Prompt screen .

```
Col 1 : ( 2 25 91  RM          )
    2 : (                      )
    3 : (05 M PHOS             )
    4 : (                      )
```

Figure 3-15. Run ID menu

## Changing the Base Source

When you press the *Base Source* soft key on the Column Installation prompt screen (Figure 3-14), the Base Source menu appears, for example, as in Figure 3-16.

```
base=src:A=1,G=2,C=3,T=4,5=5,6=6,7=7,8=8
 A=1(in  0)     G=6(in 10)   C=3(in  0)
 T=8(in  5)      5=5(in  0)   6=6(in  0)
 7=7(in  0)      8=8(in  0)   in couplings
```

Figure 3-16. Base Source menu

The Base Source menu allows you to choose the bottle from which a particular base will be drawn. The settings shown on the first line are the defaults. They indicate the source of each base (unless lines 2, 3, or 4 are changed). "A=1" means that base A will be drawn from bottle #1. A given base may be drawn from any of the phosphoramidite bottles (#1 through #8) by changing these settings.

The next three lines of the Base Source menu are used to change the base source during the course of a synthesis. For example, Figure 3-17 shows that the source of base G will switch from bottle #2 to bottle #6 after 10 G additions (not after 10 cycles). The number of G additions from bottle #2 will count down from 10. When the number of additions remaining equals 0, the first line of the menu will change to G=6, and the remaining G's will be drawn from bottle #6.

```
base=src:A=1,G=2,C=3,T=4,5=5,6=6,7=7,8=8
 A=1(in  0)     G=6(in 10)   C=3(in  0)
 T=8(in  5)      5=5(in  0)   6=6(in  0)
 7=7(in  0)      8=8(in  0)   in couplings
```

Figure 3-17. Base-source changes programmed to occur during synthesis

To program a change in the defaults on line 1, place the cursor on the bottle number to be changed, for example on "1" in A=1. Then press a numeric key such as "5" to change the default

source for A from bottle #1 to bottle #5. The source for A on line 2 will automatically change to match the new default source. This bottle number will remain in effect until the source is programmed again.

To program a base-source change to occur during the synthesis, place the cursor on a bottle number on line 2, 3, or 4, for example on "3" in C=3, and press a numeric key to specify the bottle that will become the new source. Then place the cursor on the number within the adjacent parentheses. Use the numeric keys to enter the number of additions of the base to be made before the bottle change.

When base sources have been changed from the defaults, an asterisk (*) will appear next to "Base Source" on the Column Installation Prompt screen (Figure 3-14).

## Printing a Synthesis Setup

When a printer is connected to the instrument, you can print a hard copy of the synthesis setup. To do this, press the *Print* soft key on the Column Installation Prompt screen (Figure 3-14). The Print Synthesis Setup menu will appear. For example, see Figure 3-18.

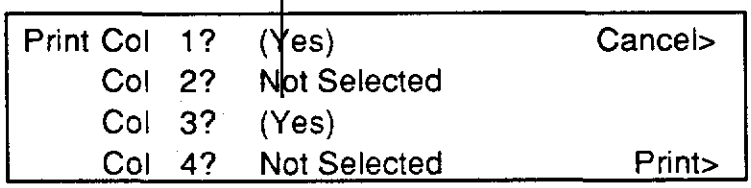



Figure 3-18. Print Synthesis Setup menu

By default, active columns are labeled "Yes." Inactive columns are labeled "Not Selected." If you do not want to print the synthesis setup for an active column, change "Yes" to "No" by using the **Prev** or **Next** key. To cancel the print-out, press the *Cancel* soft key.

For each column, the "Synthesis Setup" print-out lists the run ID, cycle, end procedure, DMT On/Off, run ID, sequence name, the bases in the sequence, and its molecular weight. The information for each column is printed on a separate page. Figure 3-19 is an example of the information that would appear on several pages.

```
                    - 394 Synthesis Setup Listing -     (Version 1.01)

Column 1                                                3:01:22A ,  1/ 6/91

  Run ID     : RM 132
  Cycle      : Primer
  End Proc   : End CESS          (DMT = On)
  Sequence   : PCR 1
  Total bases =  18
            A=  0, G=  0,  C=  0, T=  1, 5=  6, 6=  3, 7=  4, 8=  4
            (mixed bases=  0)
  MW:
    5'>   858  776  585  675  785  65T  <3'

Column 2

  Run ID     : RM 134
  Cycle      : Primer
  End Proc   : Manual            (DMT = On)
  Sequence   : PCR 2
  Total bases =  18
            A=  4, G=  4,  C=  5, T=  5, 5=  0, 6=  0, 7=  0, 8=  0
            (mixed bases=  0)
  MW:  5897.6
    5'>   AGC TCT GAA GCT TCG ACT <3'

Column 3

  Run ID     : 05 M
  Cycle      : Primer
  End Proc   : Manual            (DMT = Off)
  Sequence   : Seq01
  Total bases =  40
            A= 10, G= 10,  C= 10, T= 10, 5=  0, 6=  0, 7=  0, 8=  0
            (mixed bases=  0)
  MW:  13178.0
    5'>   AGT CAG TCA GTC AGT CAG TCA GTC AGT CAG TCA GTC AGT
          C    <3'

Column 4

  Run ID     : DRZ 1216
  Cycle      : Primer
  End Proc   : End CESS          (DMT = Off)
  Sequence   : BOB
  Total bases =  30
            A=  6, G=  8,  C= 10, T=  6, 5=  0, 6=  0, 7=  0, 8=  0
            (mixed bases=  0)
  MW:  9822.0
    5'>   AGC CCG AGC TTC ACT GAG CTA GCT GCT AGC <3'
```

Figure 3-19. Synthesis setup print-out

## Selecting a Trityl Collection Option and a Begin Procedure

When you are ready to continue the synthesis initiation, press the *Start* soft key on the Column Installation Prompt screen (Figure 3-14). You will see a display like that in Figure 3-20.

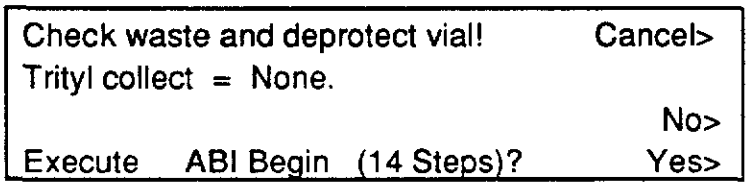



Figure 3-20. Select Begin Procedure menu

This display allows you to select two items: a trityl-collection option and a begin procedure. These are the final steps in preparing for the synthesis, which is started from this menu.

To select a trityl-collection option, move the cursor to the label following "Trityl collect =." Then use the **Prev** or **Next** keys to scroll through the options. The available options are:

None
1st & last 2
All

If you select "1st & last 2," the second, third, and last two trityls on all active columns will be collected. Additional trityls will be collected if oligos of different lengths are synthesized simultaneously. For more information on this option, see "Pre-synthesis Check List" in Section 2.

A begin procedure fills the phosphoramidite and tetrazole lines with fresh reagent. To select a begin procedure, move the cursor to the label following "Execute." Then use the **Prev** or **Next** keys to scroll through those available.

The soft keys on the Select Begin Procedure menu, allow you to start the synthesis or cancel it. Press *Yes* to start the synthesis, using the current begin procedure. Press *No* to start the synthesis, circumventing any begin procedure. In either case, the Monitor Steps menu for the first active column will be displayed. To cancel the synthesis, press *Cancel*, and the Main Menu will return.

*NOTE If the instrument has been idle for more than 12 hours (6 hours in a humid environment), you should run a begin procedure.*

## After a Synthesis is Under Way

Once you start a synthesis, you cannot choose an alternative cycle or sequence. Also, you cannot edit an active sequence (one being used for the synthesis); however, you can return to the Main Menu and press *Start Synthesis* to set up and start another synthesis on any inactive column. You can change a function number or step time in an active synthesis cycle, but steps may not be inserted or deleted. The end procedure may be changed during a synthesis, provided the change is made before the final base addition of the shortest sequence is completed.

# Main Menu Option: Monitor Synthesis

The *Monitor Synthesis* soft key is available on page 1 of the Main Menu. When you press this key, the Monitor Synthesis Menu (Figure 3-21) is shown. It is used for monitoring the progress of the synthesis on all columns (two for the 392 and four for the 394). It allows you to monitor each column individually by viewing either the steps in the cycle or the sequence for that column. You can also lock the synthesis. (The third soft key on this menu specifies either *Locked* or *Unlocked*, depending on the current state of the synthesis.) Locking a synthesis disables the soft keys on the Monitor Steps menu (Figure 3-22), preventing inadvertent interrupts, holds, or jumps.

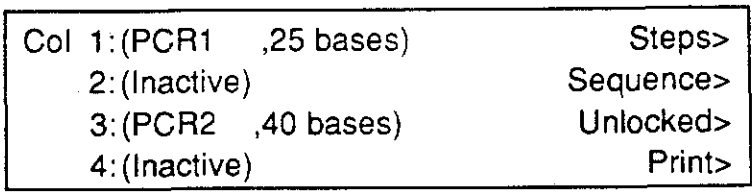



Figure 3-21. Monitor Synthesis menu

From this menu you can determine which columns are included in the active synthesis, which sequences are being synthesized, and,the total number of bases in each sequence.

## Monitor Steps

If you press the *Steps* soft key while the Monitor Synthesis menu is active, the menu shown in Figure 3-22 is displayed. This menu always shows the column last viewed using Monitor Steps.

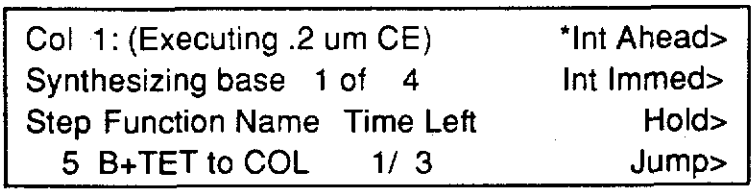



Figure 3-22. Monitor Steps menu

*NOTE The asterisk * indicates that an interrupt is set. See "Interrupting Syntheses" for explanations of Interrupt, Hold, and Jump.*

The top left corner of the display shows which column is being monitored. You can select another column by positioning the cursor on the column identifier and pressing the **Prev** and **Next** keys until the desired column is displayed.

To the right of the column identifier, the message "Executing .2 um CE" indicates which cycle is being executed. Two other messages that may appear in this field are:

**Executing ABI Begin** (the name of the Begin procedure in progress)
**Executing End CE** (the name of the End procedure in progress)

The base currently being synthesized is displayed on the second line. In Figure 3-22, the message "Synthesizing base 1 of 4" indicates that Base 1 (from the 3' end) of a total of 4 bases is currently being added.

The last two lines show the step that is currently being executed. Included are the step number, the function name, the amount of time left, and the total active time for that step.

## Time Augmentation

A "Time Left" value of "1/ 3," for example, indicates that the step shown in Figure 3-22 has 1 second remaining out of a total of 3 seconds.

If the step is in process on two or more columns at the same time, the time left increases to a value greater than the total time. This change is due to the system's compensation for the reduced flow that occurs during multiple column delivery. The system increases the time of the step so that the total flow volume remains the same. The time for the step will increase with each additional active column during the step.

## Interrupting, Holding or Jumping

The following soft keys on the Monitor Steps menu enable additional capabilities: *Int Ahead* (Interrupt Ahead), *Int Immed.* (Interrupt Immediate), *Hold,* and *Jump.* Pressing *Interrupt Ahead* allows you to stop a synthesis at a desired step mid-synthesis. Pressing *Interrupt Immed.* executes an interrupt immediately. (See "Interrupting Syntheses" on page 3-25.) Pressing the *Hold* soft key continues a cycle step indefinitely. (See "Holding Syntheses" on page 3-27.) Pressing the *Jump* soft key allows any step in the cycle to be executed after the current step, even steps listed earlier in the cycle. (See "Jumping to a New Step" on page 3-28.)

## Monitor Sequence

When you press the *Sequence* soft key on the Monitor Synthesis menu, the menu shown in Figure 3-23 is displayed.

```
Col 2: PCR 2                                    5'>
Synthesizing base  2 of  18                     3'>
5'>  AGC TCT GAA GCT TCG ACT
                                              <3'
```

Figure 3-23. Monitor Sequence menu

The top left corner of the display shows which column is being monitored. Use the **Prev** or **Next** key to select another column. The soft keys allow you to view the 5' and 3' ends of the sequence. The left- and right-arrow keys scroll the bases to the left or to the right. The base that is currently being added (underlined in the example above) flashes.

## Interrupting Syntheses

Two types of interrupts can be set: column and cycle. A column interrupt is used to interrupt one or more columns at a specified base with DMT set on or off. Columns not selected will not be interrupted. A cycle interrupt is used to interrupt all active columns after a specified number of cycles and at a specific step.

***IMPORTANT*** *Interrupt synthesis at a safe step, such as Start Detrityl or Cycle End, so that the DNA is left chemically stable.*

### Interrupt Ahead

When you press the *Int Ahead* soft key (on the Monitor Steps menu, Figure 3-22), the following menu is displayed:

```
Col: Int Base    DMT   Col:  Int Base    DMT
  1: Yes  3      On      2:   No
  3: Yes  5      Off     4:   Yes  7     On
(No) Interrupt after 000 cycles, step 000
```

Figure 3-24. Interrupt Ahead menu

This menu allows you to program an interrupt to occur at a future step any time during the synthesis. It can be specified as either a column interrupt (lines 1-3) or a cycle interrupt (line 4). A column interrupt, for a single column or multiple columns, is set or canceled by placing the cursor on the interrupt state and pressing the **Prev** or **Next** key. When the state is set to "Yes," you are allowed to specify a base number and a deprotection state. To set the base number, place

the cursor on the base number field for the desired column, for example "3" for Col 1 and type in the new number. To set the deprotection state for a column, place the cursor on the desired "On" or "Off" field and use **Prev** or **Next** to switch to the other state.

A cycle interrupt is programmed in the same way on line 4 of the display shown in Figure 3-24. Use a cycle interrupt when you want to interrupt all columns at a specified step during the cycle defined by the "000 cycles" field. Make entries by placing the cursor on the cycle and step fields, typing in the desired cycle and step values, and then changing "No" to "Yes" using the **Next** or **Prev** keys. Note that your entry in the cycle field should be a number representing cycles, not bases. For example, if you enter "005" in the cycle field and "002" in the step field, the synthesis will be interrupted at step 2 in the fifth cycle beyond the current cycle.

Use the **Prev Menu** key to return to the Monitor Steps menu (Figure 3-25).

## Interrupt Immediate

This type of interrupt can be considered a cycle interrupt in that it interrupts all active columns. When you press the *Int Immed.* soft key on the Monitor Steps menu (Figure 3-22), it changes as shown in Figure 3-25. Notice that the soft key options are now *Int Ahead*, *Resume*, *Abort*, and *Jump*.

```
Col 1: (Executing ABI Begin)          Int Ahead>
                                        Resume>
Step Name          CYC INTERRUPT        Abort>
  3 A to Waste      1/ 2                Jump>
```

Figure 3-25. Monitor Steps menu during Cycle Interrupt

This menu indicates whether a column or cycle interrupt is in effect and allows several actions to be taken. When "CYC INTERRUPT" appears on the third line (replacing the "Time Left" header), a cycle interrupt is in effect. When "COL INTERRUPT" appears on the third line, a column interrupt is in effect.

When the synthesis is interrupted, the *Int Ahead*, *Resume*, and *Abort* actions are possible. If desired, the synthesis can be resumed at the next step by pressing the *Resume* soft key, or the synthesis can be aborted by pressing the *Abort* soft key. When you press *Abort*, the display returns to that shown in Figure 3-22. The synthesis on the affected column will be shown as "inactive."

To see which columns have interrupts in progress or interrupts ahead, place the cursor on the column name and scroll using the **Prev** or **Next** keys. If an interrupt is in progress, the display will appear as in Figure 3-25. If an interrupt ahead is set, an * will appear next to *Int Ahead*, as shown in Figure 3-22.

The third choice available from the interrupted Monitor Steps menu is jumping to a new step in the cycle. This is discussed in "Jumping to a New Step," page 3-28.

## Holding Syntheses

When you press the *Hold* soft key on the Monitor Steps menu (Figure 3-22), the menu below is displayed. The active step continues to be active indefinitely, the *Hold* soft key changes to *Resume*, and the Time Left header changes to "HOLDING."

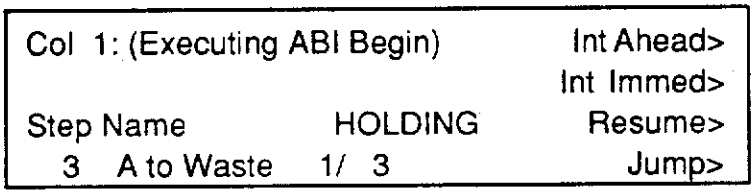



Figure 3-26. Monitor Steps menu - Hold active

While a synthesis is being held, the step currently being executed will continue indefinitely. Notice that *Hold* can be used to increase the delivery time of a reagent. During a hold, all activated valves remain open.

***IMPORTANT*** *When a chemical delivery step is on hold, the extra reagent consumption is not recorded in bottle usage and is not included in the alarm tabulation.*

During a hold, the time left continues to decrease until the value zero is reached. When zero is reached, the total active time of the step (3 in the example above) is then incremented as long as the hold is active. When the *Resume* soft key is pressed, the synthesis resumes with the next step (or the step is completed if there is still time remaining).

## Jumping to a New Step

When you press the *Jump* soft key on the Monitor Steps menu, the following menu is displayed:

```
Col  4:          .2 um CE                          Jump >
                                                   Next Step >
Step   Function Name    Num  Time AGCT5678 S
  10   B+TET to COL     33   3               Y
```

Figure 3-27. Jump menu

This menu allows any step in the cycle to be executed after the current step, even steps listed earlier in the cycle. Jumping to out-of-sequence steps enables you to repeat or skip parts of the chemical cycle. The step to which you jump is determined by the step number on line 4. You can change the step number by placing the cursor on it and using the **Prev** and **Next** keys. Alternatively, you can enter the step number by using the numeric keypad. To jump to the selected step, press the *Jump* soft key. To jump to the next step, press the *Next Step* soft key. Jumping is allowed only within a synthesis cycle. It is not possible to jump within a begin or end procedure. Jumping affects every active column.

# Main Menu Option: Change Bottle

The *Change Bottle* soft key appears on page 2 of the Main Menu. This option leads to two menus for removing empty reservoirs and replacing them with bottles of fresh reagents. The first, the Bottle Change menu, allows selection of both a bottle and a procedure, and also provides access to the second menu, the Auto Dilution menu.

## Purpose of the Bottle Change Procedure

The bottle change procedure is used to remove empty reservoirs and replace them with bottles of fresh reagents. It can be done before beginning a synthesis or when an active synthesis has been interrupted either manually or by an alarm. This procedure is especially important for phosphoramidites and tetrazole bottles because they are the most sensitive to atmospheric oxygen and water.

In summary, the procedure for bottles 1-9 begins by removing old reagent from the delivery line and forcing it back into the reservoir by an acetonitrile wash and argon flush. The bottles are then removed and replaced. In all bottle change procedures (bottles 1-18), argon purges the reservoir's headspace to eliminate air and the delivery line is then refilled with fresh reagent. For a complete description of the bottle change procedures, see Section 4.

When the procedure is finished, a "Bottle change complete" message appears. Press *Okay* to return to the Bottle Change menu shown in Figure 3-28. To return to the Main Menu, press the **Main Menu** key. Now you can begin the synthesis by selecting the *Start Synthesis* option or continue the synthesis by selecting the *Monitor Synthesis* option. An interrupted synthesis is continued from *Monitor Synthesis* by releasing the interrupt (i.e., display the Monitor Steps menu and press *Resume*).

## Using the Bottle Change Menu

When you press the *Change Bottle* soft key, the Bottle Change menu is displayed:

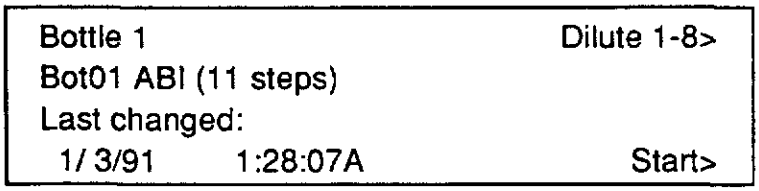



Figure 3-28. Bottle Change menu

You can use the bottle change procedures provided by this menu or you can select a second menu, which provides procedures for automatically diluting the phosphoramidites (bottles 1-8). If you want to use the auto dilution procedures, press the *Dilute 1-8* soft key and proceed to "Using the Auto Dilution Menu," page 3-31.

In the top left corner of the display shown in Figure 3-28, the bottle is identified. Other bottles can be selected by positioning the cursor on the bottle identifier and using the **Prev** and **Next** keys. Bottle identifiers include the range from "1" to "12" as well as identifiers "14," "15," and "18."

The second line of the Bottle Change menu identifies the procedure to be used for the bottle change and the number of steps in the procedure. One of two procedures can be used for the bottle change, Botxx ABI or Botxx user. The number listed after Bot, "xx," identifies the bottle to be used in the procedure (see previous paragraph). Procedure Botxx ABI is a procedure supplied with the synthesizer and Procedure Botxx user is a user-created procedure.

The third and fourth lines of the Bottle Change menu show the date and time when the bottle was last changed. The date and time are automatically updated after a bottle change.

To change a bottle, select the bottle change procedure to be used and press the *Start* soft key. A prompt will tell you when to install the new bottle. After installing the new bottle, press *Resume*. You can interrupt a bottle change by pressing *Int. Immed*, or you can extend a step by pressing *Hold*.

## Using the Auto Dilution Menu

The *Dilute 1-8* soft key on the Bottle Change menu provides access to the Auto Dilution procedures. When you press this key, the following warning message appears:

```
                    – WARNING –          Okay>
  Leave old bottles on the instrument when
  starting the auto dilution. New bottles
  should be attached when prompted.
```

When you press *Okay*, the Auto Dilution menu shown in Figure 3-29 appears:

```
Select bottles to change.   Use:Aut01 user
Bot01=No    Bot02=No          (  2 steps)
Bot03=No    Bot04=No    Bot05=No
Bot06=No    Bot07=No    Bot08=No        Start>
```

Figure 3-29. Auto Dilution menu

One or all of the bottle positions listed can be selected for dilution by using the **Prev** or **Next** key.

To select the Auto Dilution change procedure, place the cursor on the procedure name ("Aut01 user" in this example) and use the **Prev** and **Next** keys to scroll through the available choices, shown in Table 3-1. The procedures listed in the first column are those supplied by ABI and may be used for DNA or RNA monomers. The procedure editor (see "Main Menu Option: Procedure Editor," page 3-48) may be used to create custom Auto Dilution procedures (Aut01 user through Aut09 user).

### Table 3-1
### Auto-dilution Procedures

| Uneditable Procedures | Editable Procedures |
|---|---|
| Auto 0.25g | Aut01 user through Aut09 user |
| Auto 0.5g | |
| Auto 1.0g | |

*NOTE To ensure that the instrument delivers exactly 0.25, 0.5, or 1.0 gram of acetonitrile during an auto-dilution procedure, you can enter actual flow rate values through the Auto Dilute Calibration menu (Figure 3-41). The instrument uses these values to calculate the length of time to maintain acetonitrile delivery. For more information, see page 3-41.*

## Starting a Dilution Procedure

When you press the *Start* soft key on the Auto Dilution menu (Figure 3-29), the dilution procedure is initiated and the display shown in Figure 3-30 appears.

```
Bottle 1, (Executing Auto 1.0g)
                                    Int Immed>
Stp  Function Name  Time Left       Hold>
 5 18 to waste          5/ 10
```

Figure 3-30. Monitor Change Bottle screen

This menu monitors the progress of the auto dilution procedure. If, for any reason, you want to interrupt the dilution, press the *Int Immed* soft key. You can select *Hold* to extend the length of a step.

Unless you press *Int Immed* or *Hold*, the dilution procedure continues until the interrupt function is reached and you are prompted to change the bottle.

```
Change Bottle  1 3 5 8
                                    Resume>
Press 'Resume' when the new         Abort>
bottle is installed.
```

Figure 3-31. Change Bottle prompt screen

Change the bottles that are listed, installing the correct phosphoramidites (0.25 gram, 0.5 gram, or 1.0 gram ) in powdered form. Pressing the *Resume* soft key changes the display to that shown in Figure 3-30 and the remainder of the auto dilution procedure is executed for the selected bottles. When the dilution is complete, the Bottle Change menu, Figure 3-28, returns. While a bottle change procedure is in progress, the **Main Menu** and **Prev Menu** keys are inactive.

Pressing the *Abort* soft key presents this warning message:

```
                  -WARNING-          Yes>

Do you really want to abort
this procedure?                      No>
```

*NOTE The auto dilution procedures provided by ABI assume that position 5 = A, position 6 = G, position 7 = C, and position 8 = T.*

# Main Menu Option: Bottle Alarms

The *Bottle Alarms* soft key appears on page 2 of the Main Menu. This option leads to a menu (Figure 3-32) that allows you to set alarms that will be triggered when a bottle has been used for a specified number of addition cycles. When the alarm is triggered, the instrument interrupts the synthesis and displays a bottle alarm message. To operate effectively, the alarm must be set correctly. If set too high, the reservoir could run dry, causing the synthesis to fail.

| Bottle # | 1 | 2 | 3 | 4 | 5 | |
|---|---|---|---|---|---|---|
| | On | Off | On | Off | Off | |
| Cycles | 12 | 15 | 20 | 25 | 0 | |
| Alarm at | 25 | 0 | 30 | 0 | 0 | More> |

Figure 3-32. Bottle Alarm menu, page 1

Bottles 1 through 5 are listed on the first line of the first page of the Bottle Alarm menu (Figure 3-32). Pressing the *More* soft key displays page 2, showing bottles 6 through 10. Pressing *More* a second time displays page 3 with bottles 11 through 18.

The second line of the Bottle Alarm menu allows you to turn a particular bottle alarm on or off. Use the **Prev** or **Next** key to switch between "On" and "Off."

The third line of this menu, beginning with "Cycles," lists the number of times that a bottle has been accessed. This number increases by 1 each time the bottle is used. The number of cycles is automatically set to 0 when a bottle is changed.

The last line allows you to set a bottle alarm after a given number of cycles. To set an alarm for a bottle, move the cursor to the "Alarm at" value under that bottle.Then use the **Prev** or **Next** key or the numeric keypad to enter the number of cycles after which the alarm should be triggered. Use Table 3-2, which contains bottle usage data, as a guide to setting alarms. These data represent the average number of uses (or cycles) obtainable per bottle. When initially setting an alarm, use values that are 5 to 10% less than these averages and adjust as necessary. Chemical consumption varies with each instrument because of slightly different pressure regulator settings and the use of different cycle scales.

## Table 3-2
## Bottle Usage Data
(cycles per bottle)

| Reagent | | Synthesis Scale: Primer (cycles) | 0.2 µmol (cycles) | 1.0 µmol (cycles) | 10.0 µmol (cycles) |
|---|---|---|---|---|---|
| Phosphoramidites | | | | | |
| 0.25 gram | | | | | |
| | A | 52 | 23 | 17 | 5 |
| | G | 72 | 26 | 17 | 5 |
| | C | 66 | 26 | 19 | 6 |
| | T | 75 | 31 | 20 | 6 |
| 0.5 gram | | | | | |
| | A | 104 | 46 | 35 | 9 |
| | G | 145 | 53 | 35 | 9 |
| | C | 133 | 52 | 38 | 11 |
| | T | 151 | 62 | 40 | 13 |
| 1.0 gram | | | | | |
| | A | | 107 | 71 | 19 |
| | G | | 107 | 71 | 20 |
| | C | | 104 | 76 | 22 |
| | T | | 124 | 81 | 26 |
| Tetrazole (180 ml) | | 750 | 446 | 417 | 266 |
| Acetic anhydride (180 ml) | | 621 | 621 | 621 | 153 |
| NMI (180 ml) | | 692 | 692 | 692 | 165 |
| TCA (450 ml) | | 385 | 385 | 385 | 106 |
| Iodine (200 ml) | | 488 | 488 | 488 | 115 |
| Acetonitrile (4 liters) | | 570 | 500 | 500 | 130 |

***IMPORTANT*** *Since consumption rates vary from instrument to instrument, it is important to visually monitor reagent consumption for the first several syntheses. Once you determine the usage, the alarms can be set more effectively.*

Note that the number of uses obtainable per bottle will change depending on which cycle is being performed (e.g., Primer, .2 um CE, 1 um CE, 10 um CE). Be aware that 10 µmol-scale syntheses consume large volumes of reagents per cycle.

Phosphoramidites used during the phosphoramidite purge procedure are not tabulated in the cycle counter. This process uses one-fifth the reagent that is used in a coupling step of a one-micromole synthesis. In addition, manual deliveries (using the Manual Control menu), deliveries during a hold step, and acetonitrile used during the bottle change procedure are not tabulated in the cycle counter. When synthesizing mixed-sequence probes, each phosphoramidite delivered is tabulated in the cycle counter.

When the alarm is triggered, the instrument interrupts the synthesis and displays a bottle alarm message (Figure 3-33).

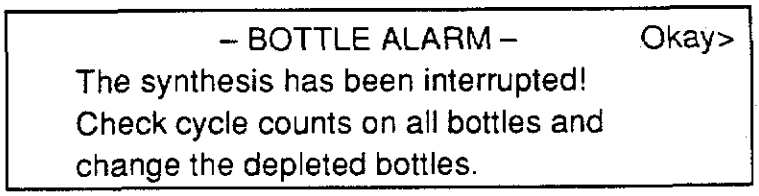



Figure 3-33. Bottle Alarm message

Press *Okay* to return to the Bottle Alarm menu (Figure 3-32), which allows you to examine the bottle data to determine which bottle has triggered the alarm. If an alarm is triggered but a bottle is not yet empty, you can continue the synthesis by clearing the interrupt. This is done by pressing *Resume*. If this situation occurs, it is important to reset the alarm to an appropriate number.

If the reservoir needs to be changed now, see "Installing Phosphoramidite Bottles" or "Installing Reagent Bottles" in Section 2.

# Main Menu Option: Manual Control

Use the Manual Control Menu to manually activate functions and to open and close individual valves.

***IMPORTANT*** *Before opening any valves, refer to the DNA synthesizer schematic or the plumbing diagram (Appendix II) and the function list (Appendix I) to be sure the correct flow path will be formed. Also refer to "Functions" in Section 4 and Figure 4-1. Manual control will not operate during a synthesis unless you interrupt the run.*

## About the Menu

The *Manual Control* soft key is available on Page 2 of the Main Menu. When you press this key, a menu like the following is displayed:

| | | |
|---|---|---|
| Manual Control | (0.06 psi) | Function> |
| Valves On: | (valve: 20) | Valve On> |
| 42 10 30 | | Valve Off> |
| | | All Off> |

Figure 3-34. Manual Control menu

The first line of the Manual Control menu shows the pressure reading from the transducer. This reading will be accurate only when a pressure test is run (see page 3-40). On the second line of this menu, you can switch the valve status from "Valves On" to "Valves Off" or vice versa by pressing the **Prev** or **Next** key. The on or off status applies to all the valves listed on the third line (valves 42, 10, and 30 in the example of Figure 3-34). If the status is "Valves On," these valves are turned on. Likewise, if the status is "Valves Off," these valves are turned off.

To add a valve to the list, place the cursor on the number in parentheses on line 2 (20 in the example above). Enter the valve number by using the numeric keypad. Then press the *Valve On* soft key. To remove a valve from the list, enter the valve number and press the *Valve Off* soft key. To turn on several valves simultaneously, set the valve status to "Valves Off" and enter the valve numbers. Then change the valve status to "Valves On."

Pressing the *All Off* soft key turns off all active valves and deletes the entire valve list. Also, all valves are turned off when you exit the Manual Control menu.

## Activating Functions

When you press the *Function* soft key on the Manual Control menu, the following menu appears:

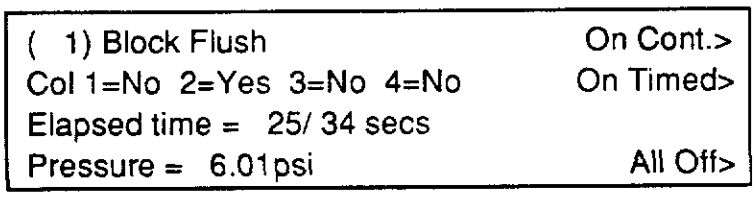



Figure 3-35. Function menu

To select a function, place the cursor on the number listed before the function name ("Block Flush" in the example above) and use the numeric keypad to enter the desired function number. Alternatively, use the **Prev** or **Next** key to scroll through the available functions. A complete list of functions is presented in Appendix I.

The columns that are active (Col x=Yes) or inactive (Col x =No) for a particular function can be changed by placing the cursor on "Yes" or "No" and pressing the **Prev** or **Next** keys.

Either the *On Cont.* or the *On Timed* soft key can be used to turn on the selected function. *On Cont.* turns the function on continuously. *On Timed* turns the function on for a period of time that you specify. To specify the length of time, move the cursor to the total time value on line 3 (34 seconds in Figure 3-35) and use the numeric keypad to enter a number of seconds. Then press *On Timed.*

The *All Off* soft key turns off the current function. A function that has been turned on continuously will remain on until *All Off* is pressed. A function that has been turned on for a specified period will remain on until that period has elapsed or until *All Off* is pressed.

To return to the Manual Control menu, use the **Prev Menu** key. All valves turned on manually through the Manual Control menu remain on regardless of which functions are activated or deactivated.

# Main Menu Option: Self Test

The Self Test Menu is used to verify the correct operation of the electronic components of the instrument and to access the flow test procedure. These tests should be run if you suspect instrument malfunctioning.

To display the Self Test menu, press the *Self Test* soft key on page 3 of the Main Menu. Page 1 of the Self Test menu, shown below, will appear:

| | |
|---|---|
| Select a test: | Display> |
| | Keypad> |
| | Memory> |
| Page 1 of 3 | Next Page> |

Figure 3-36. Self Test menu, page 1

You reach the Display, Keypad, and Memory tests from page 1. To see the other pages of the Self Test menu, shown below, use the *Next Page* soft key. On page 3, the *Next Page* soft key wraps the display back to page 1.

| | |
|---|---|
| Select a test: | Valves> |
| | Pressure Test> |
| | Flow Rate Test> |
| Page 2 of 3 | Next Page> |

| | |
|---|---|
| Select a test: | System Reset> |
| | System Configuration> |
| | Edit Test> |
| Page 3 of 3 | Next Page> |

Figure 3-37. Self Test menu, pages 2 and 3

You select the desired self test by pressing the appropriate soft key. A display like that shown in Figure 3-38 reports the test results:

| | | | |
|---|---|---|---|
| Testing Memory . . . | | | Use Main Menu |
| | Pass | Fail | or Prev. Menu |
| RAM | 017 | 000 | key to exit |
| ROM | 017 | 000 | this test. |

Figure 3-38. Memory Self Test report

The memory test runs until you press the **Prev Menu** or **Main Menu** key.

*NOTE If nothing happens when the **PREV MENU** key is pressed, try pressing the key several times in succession.*

## Summary of Self Test Functions

| | |
|---|---|
| Display | Allows you to verify proper operation of the display visually. While depressed, the *Display* soft key turns on all display pixels. A pixel that does not turn on is faulty. |
| Keypad | Allows you to verify proper operation of each key visually. |
| Memory | Tests for the proper operation of RAM and ROM. Continually tests until stopped. |
| Valves | Tests for the proper operation of all valves. |
| Pressure Test | Performs 13 different pressure tests. Use when installing the system or if a leak is suspected. |
| Flow Rate Test | Tests flow rates through essential delivery lines. |
| System Reset | Allows the system to be initialized using either a cold or a warm reset. A cold reset causes loss of all user-entered programs. A warm reset retains user-entered programs. |
| System Config. | Displays DIP switch settings and software version number. Provides access to the valve-trace feature. |
| Edit Test | Allows you to edit flow tests and create user flow tests. |

## Details of Pressure Test

When you press the *Pressure Test* soft key on page 2 the Self Test menu, the menu shown in Figure 3-39 is displayed. Pressure testing consists of a series of tests designed to check the plumbing for leaks before chemicals are installed on the machine. This type of testing is not necessary except as part of installation or if a leak is suspected. A detailed description of pressure tests is included in Appendix III.

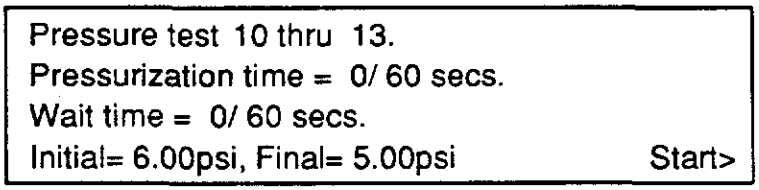



Figure 3-39. Pressure Test menu

---

***CAUTION*** *Do not run the pressure test with chemicals on the machine. Damage to the pressure transducer may result.*

---

Each pressure test consists of a pressurization time and a waiting period. After the pressurization time expires, the initial pressure is measured. After the waiting period, the final pressure is measured. The results of the test are determined by the difference between these two readings.

Thirteen pressure tests are available. On the first line of the display, you can specify any one test or a range of tests by using the cursor and the numeric keypad. For each test or range of tests, you can also set the pressurization time on the second line and the wait time on the third line.

When you press the *Start* soft key, the tests specified on the menu are executed. The bottom line of the menu displays, for each test, the initial pressure (recorded after the pressurization time has elapsed) and the final pressure (recorded after the wait time has elapsed). Once a pressure test has begun, the *Start* soft key is replaced by *Cancel* and the cursor is not available.

If a leak is detected during running of a pressure test, testing is stopped and the word FAIL appears in the upper right corner of the display. If more than one test is specified, the instrument automatically proceeds to the next test. When no error results during the running of the last test specified, the word PASS appears in the same position.

## Details of Flow Rate Tests

When you press the *Flow Rate* soft key on the Self Test menu, the menu shown in Figure 3-40 appears. Flow rate testing consists of 32 tests designed to check the flow in various parts of the instrument. (Examples of these tests are "Flow to A," "Flow to G," etc.) Flow rate tests do not need to be run except as part of installation or if an improper flow rate is suspected. For a detailed description of these tests and a table of flow rate specifications, see Section 4 and Appendix III.

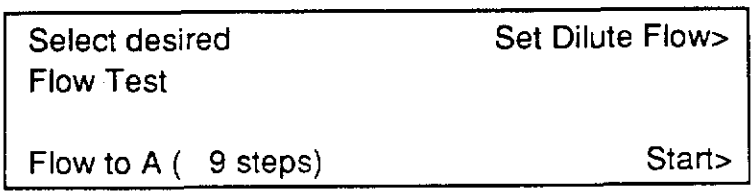



Figure 3-40. Flow Rate Test menu

---

***WARNING Do not perform a flow rate test without first installing a measuring line. Otherwise, reagent is liable to spray out of the lower luer and cause physical harm.***

---

The *Set Dilute Flow* soft key displays the Auto Dilute Calibration menu shown in Figure 3-41. You use this menu only after performing flow rate tests to measure the actual flow rate of acetonitrile to the phosphoramidite bottle positions. The menu allows auto-dilution to be calibrated. This calibration is usually done during installation.

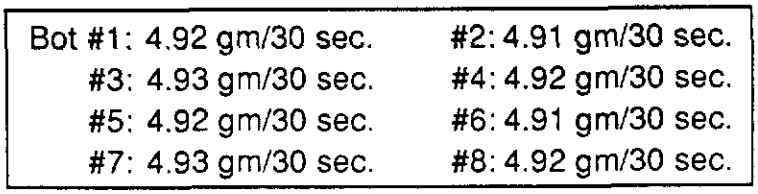



Figure 3-41. Auto Dilute Calibration menu

The first step in calibrating auto-dilution, is to perform the "Flow to A" flow test shown in Figure 3-40. This test measures the flow rate of acetonitrile to bottle #1. When you have obtained a flow rate, enter this value expressed as grams/30 seconds in the bottle#1 position of the Auto Dilute Calibration menu (Figure 3-41). Repeat this procedure for the other phosphoramidite bottles. The software uses the information in this table to determine the correct acetonitrile delivery times for the ABI Auto Dilute procedures.

# Main Menu Option: Edit Cycle

## Introduction

Thirty-two synthesis cycle locations are available on the 392/394. Ten of these locations contain permanent cycles provided with the instrument. (See the list of permanent cycles in Table 3-3.) Ten locations (Cyc01 user - Cyc10 user) are intended for user programming. The final twelve cycles (Cyc11 Mac- Cyc22 Mac) are reserved for future use and cannot be edited. The Cycle editor is used to view, copy, and print out the contents of all cycles. Actual editing and erasing of the contents of synthesis cycles is allowed only for the ten user-programmable cycles.

The ten permanent cycles provided with the synthesizer are optimized at a number of scales for the synthesis of β-cyanoethyl DNA and RNA , and phosphorothioate DNA. Permanent cycles are provided for 0.2-μmol, 1-μmol, and 10-μmol scales (labeled as ".2 um," "1 um," and "10 um," respectively) for the three types of synthesis. For β-cyanoethyl DNA synthesis, a low reagent-consumption scale, "Primer," is provided. The low-consumption cycle is used with 0.05 molar phosphoramidites. It may be used with a 40 nanomole column for the synthesis of oligos of any length or with a 0.2-μmol column for synthesis of oligos of 50 bases or less.Cycles provided by Applied Biosystems are listed in Table 3–3, and a complete listing of these cycles is presented in Appendix I.

The programmable cycles can be created anew or by first copying an existing programmable cycle or one of the permanent nonprogrammable cycles that Applied Biosystems provides with the synthesizer (for example, .2 um CE). After permanent cycles are transferred to programmable cycle locations (by using the *Copy* soft key on the Edit Cycle menu), they will be stored there indefinitely until they are changed or deleted. Once transferred, they can be edited as desired. Once you edit a cycle, the edited version will be stored and will appear on the Start Synthesis menu. The edited version will be maintained in memory even if the main power is turned off.

## Table 3-3
## Synthesis Cycles Provided by Applied Biosystems

| | |
|---|---|
| Primer | 0.2 µmol β-cyanoethyl DNA of 50 bases or less or 40 nm DNA of any length |
| .2 um CE | 0.2 µmol β-cyanoethyl DNA |
| 1.0 um CE | 1 µmol β-cyanoethyl DNA |
| 10 um CE | 10 µmol β-cyanoethyl DNA |
| .2 um RNA | 0.2 µmol RNA |
| 1.0 um RNA | 1 µmol RNA |
| 10 um RNA | 10 µmol RNA |
| .2 um sulfur | 0.2 µmol phosphorothioate DNA |
| 1 um sulfur | 1 µmol phosphorothioate DNA |
| 10 um sulfur | 10 µmol phosphorothioate DNA |

## The Edit Cycle Menu

When you press the *Edit Cycle* soft key on page 3 of the Main Menu, the menu shown below appears:

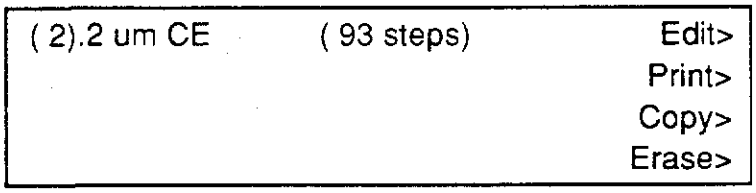



Figure 3-42. Edit Cycle menu

The first step in cycle editing is to choose the desired cycle. This is done by scrolling through the available cycles by using the **Prev** or **Next** keys. Although a cycle can be created by choosing a blank location and making entries, it is easier to copy an existing cycle into a blank location and then edit it.

The actions performed by the soft keys on the Edit Cycle menu apply to the designated cycle (.2 um CE in the example above).

Although the names of the ABI cycles cannot be changed, a user cycle (Cyc01 to Cyc10) may be given a name consisting of up to 10 alphanumeric characters. To rename a user cycle, place the cursor over the existing name and use the **Prev or Next** keys to scroll through and choose the alphanumeric characters. Directly to the right of the name, the number of steps contained in the cycle is displayed.

Edit

Select to create, modify, or review the steps of the designated cycle. The nonprogrammable cycles can be viewed but not edited directly. However, you can use the *Copy* key to transfer a nonprogrammable cycle to a user cycle location and then edit it.

| | |
|---|---|
| Print | Select to print the cycle. |
| Copy | Select to copy the cycle into a specified file. |
| Erase | Select to erase or clear the contents of the cycle. |

## Editing Cycles

You enter the Cycle Editor by pressing the *Edit* soft key on the Edit Cycle menu. The menu shown below is displayed:

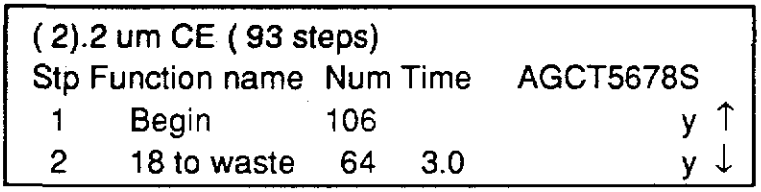



Figure 3-43. Cycle Editor menu

The number and name of the current synthesis cycle are displayed in the top left corner. You can display another cycle by placing the cursor on the cycle number and using the **Prev** and **Next** keys to scroll through the cycles.

The second line of the display is a header line, which describes different fields for each step displayed. The fields are step number (Stp), function name, function number (Num), the total time a function will be active (Time), designation of active bases (AGCT5678), and Safe Step denotation (S). Below the header line, two consecutive steps of the cycle are displayed simultaneously.

To view steps not shown on the screen, scroll up and down by using the arrow (↓ and ↑) soft keys. Pressing either of these keys continuously allows rapid scrolling through the entire cycle; however, the cycle does not wrap around when you reach the end.

To display a step immediately with no scrolling, place the cursor on either of the two step numbers and enter the number of the desired step by using the numeric keypad.

The "Num" field indicates the function number. You can change the function associated with a step by changing this number. First, place the cursor on the function number to be changed. Then enter the new function number by using the numeric keypad. Alternatively, use the **Prev** and **Next** keys to specify the previous or next function number. When you enter a new function number, the function name changes accordingly. A default of 0.0 is entered for the "Time" value. An invalid function number causes the message INVALID FNCTION to be displayed in the name field.

*NOTE You cannot change the Begin and End functions of a cycle.*

To change the duration of a function, place the cursor on the active time value (Time) and enter the new value via the numeric keypad. The units for this value are seconds with a maximum value of 999.9 seconds.

To create a new cycle, you insert steps into an empty cycle. The first entry is made by placing the cursor anywhere on the first line and pressing the **Insert** key.

To insert a new step into an existing cycle, place the cursor on the step to precede the new step, using the left (◄—) or right arrow (—►) key. Then press the **Insert** key. This will display a blank line and reset the step numbers to accommodate the new line. The cursor is left on the empty function number (Num) field, ready for input. Enter the number of the desired function. The cursor can not be moved off this field until a function number is entered.

To delete a step, place the cursor on the step number of the step to be deleted and press the **Delete** key. The cursor is left on the line that followed the deleted line.

Following the time field on line 2 of the Edit Cycle menu is a field showing the eight base positions. For each position, you can program a "Yes" or a "No" for the steps in a cycle. Each step provides the instructions for executing a function. A yes, entered as "y," signifies that the step is active for that base: thus the "y" list for a given step can be considered a list of active bases. A "-" signifies that the function is not active during the addition of the base.

On each step of a cycle, you can edit the active base field by placing the cursor under a listed base and using the **Prev** or **Next** key. Each time either of these keys is pressed, the value for the cursor position is changed; a value of "y" is changed to "-" and a value of "-" is changed to "y."

The uppercase "S" found at the end of the active base field is the Safe Step denotation. When a step is denoted as a "Safe Step" (accomplished by putting a "y" in the "S" column), the synthesis will continue after a power failure occurring during that step. If a step is not a Safe Step (denoted by a "-" in the "S" column), the synthesis continues after a power failure only if the maximum power fail time (see the Power Fail Menu, page 3-60) was not exceeded.

## Printing Cycles

To print a hard copy of a cycle, select the desired cycle on the Edit Cycle menu (Figure 3-42) and press the *Print* soft key. The menu will appear as follows while the copy is printing:

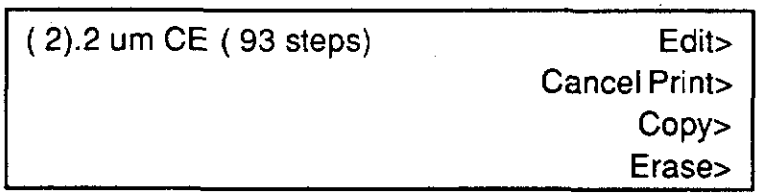



Figure 3-44. Edit Cycle menu during printing

## Copying Cycles

To copy a cycle, press the *Copy* soft key on the Edit Cycle menu. You will see a Copy Cycle menu like that shown below:

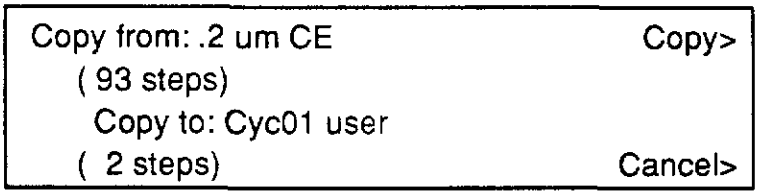



Figure 3-45. Copy Cycle menu

---

***CAUTION*** *Be aware that when you copy a source file into a destination file, the contents of the destination file are overwritten by the source file.*

---

The Copy Cycle menu allows you to select a source file and a destination file for copying. To select the source file, place the cursor on the cycle name located to the right of the "Copy From:" statement and using the **Prev** and **Next** keys to scroll through the available cycles. Select the destination file in the same way. The number of steps in each file selected is displayed below the source and destination file names.

Once the source and destination files have been selected, copying is initiated by pressing the *Copy* soft key. No cancellation is possible.

## Erasing Cycles

To erase a cycle from the instrument's memory, press the *Erase* soft key on the Edit Cycle menu. You will see a menu like that shown below:

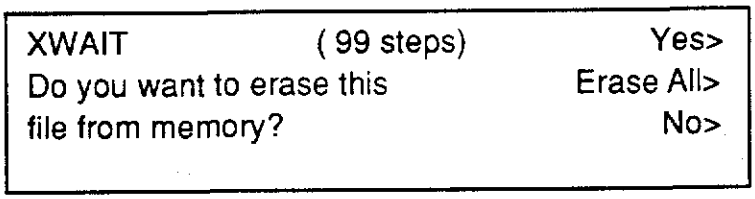



Figure 3-46. Erase Cycle menu

The name in the upper lefthand corner of this display indicates which cycle will be erased. To select a different cycle, place the cursor on the current name and use the **Prev** and **Next** keys. The number of steps in the cycle appears to the right of the cycle name.

After selecting a cycle, the soft key options available are *Yes* (erase the designated cycle), *No* (cancel the request to erase), and *Erase All* (erase all the cycles). If you press the *Erase All* soft key, the menu shown in Figure 3-47 appears:

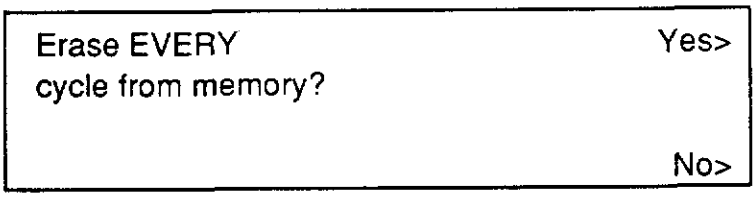



Figure 3-47. Erase All Cycle menu

If you press the *Yes* soft key, *every* programmable cycle in memory (Cyc01 user – Cyc13 user) will be erased. If *No* is pressed, the previous menu returns.

# Main Menu Option: Procedure Editor

Use the Procedure Editor to create, edit, copy, and erase any of the four procedure types (begin, end, shut-down, and bottle change). ABI-supplied procedures, further described in Section 4, are always available for use during instrument operation. You can either edit the supplied procedures or create new ones. To edit a supplied procedure, you must first copy it into a user-editable procedure. There is one user begin procedure, one user end procedure, and one user shut-down procedure. Also, there are nine user auto dilution procedures, and one user bottle change procedure per bottle.

The *Edit Procedure* soft key appears on the page 3 of the Main Menu. When you press *Edit Procedure*, the following menu appears:

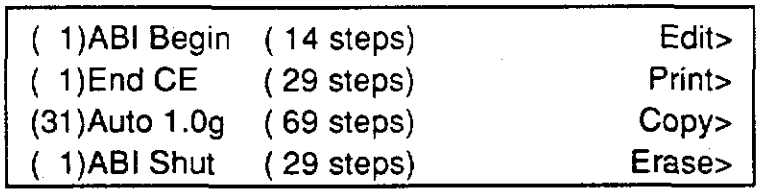



Figure 3-48. Select Procedure menu

From this menu any procedure in the system can be edited, printed, copied, or erased. To select a procedure, place the cursor on the desired procedure type by using the left (◄) or right arrow (►) key, and then scroll to the desired procedure by using the **Prev** and **Next** keys. Once the desired procedure name is displayed, the Edit, Print, Copy, and Erase functions can be carried out.

Although the names of ABI procedures *can not be changed*, you can change the names of user procedures. A user procedure may be given a name consisting of up to 10 alphanumeric characters. To rename a user procedure, place the cursor on the current name and use the **Prev** or **Next** keys to scroll through the alphanumeric characters.

## Editing Procedures

When you press the *Edit* soft key after a procedure (such as a begin procedure) has been selected, the menu shown below appears:

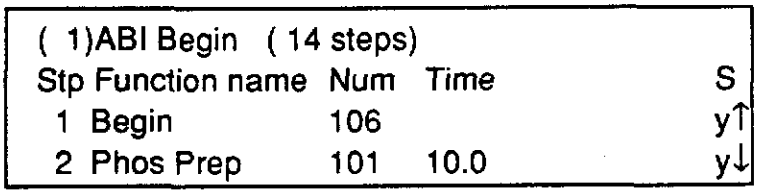



Figure 3-49. Cycle Edit menu

From this menu you can view any of the three begin procedures in the 392/394 memory. They are named ABI Begin, Beg01 User, and Beg02 Mac. Only Beg01 User can be edited. (Beg02 Mac is reserved for future use.) The name of the current begin procedure is displayed in the top left corner of the menu. To select another procedure, place the cursor on the procedure name and use the **Prev** or **Next** keys to scroll to the desired procedure.

Directly to the right of the procedure name, the number of steps in the procedure is shown.

The second line of the display is a header line that describes several fields for each step displayed. The fields are step number (Stp), the function name, the function number (Num), the total time a function will be active (Time), and Safe Step denotation (S).

Below the header line, two consecutive steps of the procedure are displayed simultaneously. The information in the step fields lines up appropriately under the header line.

To view steps not shown on the screen, use the arrow (↑ and ↓) soft keys to scroll up and down. (Wrap-around is not provided.) To display a desired step immediately (without scrolling), place the cursor on either of the two step numbers and use the numeric keypad to enter the number of the desired step.

The "Num" field indicates the function number. You can change the function associated with a step by changing this number. First, place the cursor on the function number to be changed. Then enter the new function number by using the numeric keypad. Alternatively, use the **Prev** and **Next** keys to specify the previous or next function number. When you enter a new function number, the function name changes accordingly. A default of 0.0 is entered for the "Time" value. An invalid function number causes the message INVALID FNCTION to be displayed in the name field.

To change the duration of a function, place the cursor on the active time value (Time) and enter the new value via the numeric keypad. The units for this value are seconds.

To create a new procedure, you insert steps into an empty procedure. The first entry is made by placing the cursor anywhere on the first line and pressing the **Insert** key.

To insert a new step into an existing procedure, place the cursor on the step to precede the new step, using the left (◄—) or right arrow (—►) key. Then press the **Insert** key. This will display a blank line, reset the step numbers to accommodate the new line, and renumber the following steps. The cursor is left on the empty function number (Num) field, ready for input. Enter the number of the desired function. The cursor can not be moved off this field until a function number is entered.

To delete a step, place the cursor on the step number of the step to be deleted and press the **Delete** key. The cursor is left on the line that would have followed the deleted line.

## Printing Procedures

When a printer is connected to the instrument, the *Print* soft key is used to print the designated procedure. Printing can be canceled at any time by pressing the *Cancel* soft key (which is active during printing). When printing is completed, the display will return to the Select Procedure menu.

## Editing Auto Dilution Procedures

### About the ABI Auto Dilution Procedures

If you print an ABI auto dilution procedure such as Auto 1.0g (see Table 3-4), you will notice that the functions that dilute A, G, C, T, 5, 6, 7, and 8 do not have step times. To determine the duration of acetonitrile delivery to A, the instrument checks the "Set Dilute" flow chart, which lists the flow rate of acetonitrile to bottle #1 (A). (This flow chart can be viewed and edited by selecting *Self Test* on page 3 of the Main Menu, followed by *Flow Rate* and *Set Dilute Flow*.) From this information, the instrument calculates the delivery time necessary to reach the standard volume (11.2 ml for a 1-gram A). The "Dilute 'A'" function will be present once in a 0.25-gram auto dilution, twice in a 0.5-gram auto dilution, and four times in a 1.0-gram auto dilution. Even though the "Dilute 'A'" function may appear multiple times in a print-out, dilution is carried out in one continuous delivery of acetonitrile.

# Table 3-4
# ABI Auto Dilution Procedure Auto 1.0g

– 394 Bottle Change Listing – (Version 1.01)

Name : Auto 1.0g 12:34:12A, 1/ 3/91
Steps : 69

| Step Number | Function # | Name | Step Time | Step Active for Bases | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 64 | 18 to Waste | 5.0 | | Yes |
| 3 | 1 | Block Flush | 4.0 | | Yes |
| 4 | 14 | Flush to Bases | 10.0 | | Yes |
| 5 | 86 | 18 to Bases | 5.0 | | Yes |
| 6 | 104 | Interrupt | | | Yes |
| 7 | 170 | Dilute 'A' | | | Yes |
| 8 | 170 | Dilute 'A' | | | Yes |
| 9 | 170 | Dilute 'A' | | | Yes |
| 10 | 170 | Dilute 'A' | | | Yes |
| 11 | 171 | Dilute 'G' | | | Yes |
| 12 | 171 | Dilute 'G' | | | Yes |
| 13 | 171 | Dilute 'G' | | | Yes |
| 14 | 171 | Dilute 'G' | | | Yes |
| 15 | 172 | Dilute 'C' | | | Yes |
| 16 | 172 | Dilute 'C' | | | Yes |
| 17 | 172 | Dilute 'C' | | | Yes |
| 18 | 172 | Dilute 'C' | | | Yes |
| 19 | 173 | Dilute 'T' | | | Yes |
| 20 | 173 | Dilute 'T' | | | Yes |
| 21 | 173 | Dilute 'T' | | | Yes |
| 22 | 173 | Dilute 'T' | | | Yes |
| 23 | 174 | Dilute '5' | | | Yes |
| 24 | 174 | Dilute '5' | | | Yes |
| 25 | 174 | Dilute '5' | | | Yes |
| 26 | 174 | Dilute '5' | | | Yes |
| 27 | 175 | Dilute '6' | | | Yes |
| 28 | 175 | Dilute '6' | | | Yes |
| 29 | 175 | Dilute '6' | | | Yes |
| 30 | 175 | Dilute '6' | | | Yes |
| 31 | 176 | Dilute '7' | | | Yes |
| 32 | 176 | Dilute '7' | | | Yes |
| 33 | 176 | Dilute '7' | | | Yes |
| 34 | 176 | Dilute '7' | | | Yes |

## Table 3-4 (continued)

| | | | | |
|---|---|---|---|---|
| 35 | 177 | Dilute '8' | | Yes |
| 36 | 177 | Dilute '8' | | Yes |
| 37 | 177 | Dilute '8' | | Yes |
| 38 | 177 | Dilute '8' | | Yes |
| 39 | 1 | Block Flush | 4.0 | Yes |
| 40 | 14 | Flush to Bases | 120.0 | Yes |
| 41 | 114 | Vent Bases | 3.0 | Yes |
| 42 | 101 | Phos Prep | 5.0 | Yes |
| 43 | 150 | Bottle01 On | | Yes |
| 44 | 50 | A to Waste | 2.0 | Yes |
| 45 | 151 | Bottle01 Off | | Yes |
| 46 | 152 | Bottle02 On | | Yes |
| 47 | 51 | G to Waste | 2.0 | Yes |
| 48 | 153 | Bottle02 Off | | Yes |
| 49 | 154 | Bottle03 On | | Yes |
| 50 | 52 | C to Waste | 2.0 | Yes |
| 51 | 155 | Bottle03 Off | | Yes |
| 52 | 156 | Bottle04 On | | Yes |
| 53 | 53 | T to Waste | 2.0 | Yes |
| 54 | 157 | Bottle04 Off | | Yes |
| 55 | 158 | Bottle05 On | | Yes |
| 56 | 54 | 5 to Waste | 2.0 | Yes |
| 57 | 159 | Bottle05 Off | | Yes |
| 58 | 160 | Bottle06 On | | Yes |
| 59 | 55 | 6 to Waste | 2.0 | Yes |
| 60 | 161 | Bottle06 Off | | Yes |
| 61 | 162 | Bottle07 On | | Yes |
| 62 | 56 | 7 to Waste | 2.0 | Yes |
| 63 | 163 | Bottle07 Off | | Yes |
| 64 | 164 | Bottle08 On | | Yes |
| 65 | 57 | 8 to Waste | 2.0 | Yes |
| 66 | 165 | Bottle08 Off | | Yes |
| 67 | 64 | 18 to Waste | 7.0 | Yes |
| 68 | 1 | Block Flush | 5.0 | Yes |
| 69 | 107 | End | | Yes |

## Creating a User Auto Dilution Procedure

This section illustrates the creation of a user auto dilution procedure by editing the ABI procedure Auto 1.0g (in Table 3-4). To customize this procedure, you must replace Steps 7 through 38 with Steps 7 through 30 of the user procedure shown in Table 3-5.

For each bottle that will be diluted, the following functions must be included:

| | | |
|---|---|---|
| Bottle*x* On | | (*x* = bottle number) |
| 18 to *x* | <time> | (*x* = base letter , e.g., A, or bottle number) |
| Bottle*x* Off | | (*x* = bottle number) |

Use the following equation to calculate <time>, the duration of the delivery of acetonitrile (bottle #18):

$$T = \frac{V \times 0.786\ \text{g/ml}}{R} \times 30$$

where $V$ = the desired volume in milliliters,
$R$ = the flow rate of #18 to the bottle in grams/30 seconds, and
$T$ = the delivery time in seconds.

Although the user auto dilution procedure shown in Table 3-5 contains functions for diluting all eight bases, only the bottles that are selected on the Auto Dilution menu (Figure 3-29) will actually be diluted.

# Table 3-5
# User Auto Dilution Procedure

– 394 Bottle Change Listing – (Version 1.01)

Name : Auto01 user 12:31:51A, 1/ 3/91
Steps : 61

| Step Number | Function # | Name | Step Time | Step Active for Bases | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 64 | 18 to Waste | 5.0 | | Yes |
| 3 | 1 | Block Flush | 4.0 | | Yes |
| 4 | 14 | Flush to Bases | 10.0 | | Yes |
| 5 | 86 | 18 to Bases | 5.0 | | Yes |
| 6 | 104 | Interrupt | | | Yes |
| 7 | 150 | Bottle01 On | | | Yes |
| 8 | 70 | 18 to A | 53.7 | | Yes |
| 9 | 151 | Bottle01 Off | | | Yes |
| 10 | 152 | Bottle02 On | | | Yes |
| 11 | 71 | 18 to G | 55.7 | | Yes |
| 12 | 153 | Bottle02 Off | | | Yes |
| 13 | 154 | Bottle03 On | | | Yes |
| 14 | 72 | 18 to C | 56.4 | | Yes |
| 15 | 155 | Bottle03 Off | | | Yes |
| 16 | 156 | Bottle04 On | | | Yes |
| 17 | 73 | 18 to T | 63.3 | | Yes |
| 18 | 157 | Bottle04 Off | | | Yes |
| 19 | 158 | Bottle05 On | | | Yes |
| 20 | 74 | 18 to 5 | 53.7 | | Yes |
| 21 | 159 | Bottle05 Off | | | Yes |
| 22 | 160 | Bottle06 On | | | Yes |
| 23 | 75 | 18 to 6 | 55.7 | | Yes |
| 24 | 161 | Bottle 06 Off | | | Yes |
| 25 | 162 | Bottle07 On | | | Yes |
| 26 | 76 | 18 to 7 | 56.4 | | Yes |
| 27 | 163 | Bottle07 Off | | | Yes |
| 28 | 164 | Bottle08 On | | | Yes |
| 29 | 77 | 18 to 8 | 63.3 | | Yes |
| 30 | 165 | Bottle 08 Off | | | Yes |
| 31 | 1 | Block Flush | 4.0 | | Yes |
| 32 | 14 | Flush to Bases | 120.0 | | Yes |
| 33 | 114 | Vent Bases | 3.0 | | Yes |
| 34 | 101 | Phos Prep | 5.0 | | Yes |

## Table 3-5 (continued)

| | | | | |
|---|---|---|---|---|
| 35 | 150 | Bottle01 On | | Yes |
| 36 | 50 | A to Waste | 2.0 | Yes |
| 37 | 151 | Bottle01 Off | | Yes |
| 38 | 152 | Bottle02 On | | Yes |
| 39 | 51 | G to Waste | 2.0 | Yes |
| 40 | 153 | Bottle02 Off | | Yes |
| 41 | 154 | Bottle03 On | | Yes |
| 42 | 52 | C to Waste | 2.0 | Yes |
| 43 | 155 | Bottle03 Off | | Yes |
| 44 | 156 | Bottle04 On | | Yes |
| 45 | 53 | T to Waste | 2.0 | Yes |
| 46 | 157 | Bottle04 Off | | Yes |
| 47 | 158 | Bottle05 On | | Yes |
| 48 | 54 | 5 to Waste | 2.0 | Yes |
| 49 | 159 | Bottle05 Off | | Yes |
| 50 | 160 | Bottle06 On | | Yes |
| 51 | 55 | 6 to Waste | 2.0 | Yes |
| 52 | 161 | Bottle06 Off | | Yes |
| 53 | 162 | Bottle07 On | | Yes |
| 54 | 56 | 7 to Waste | 2.0 | Yes |
| 55 | 163 | Bottle07 Off | | Yes |
| 56 | 164 | Bottle08 On | | Yes |
| 57 | 57 | 8 to Waste | 2.0 | Yes |
| 58 | 165 | Bottle08 Off | | Yes |
| 59 | 64 | 18 to Waste | 7.0 | Yes |
| 60 | 1 | Block Flush | 5.0 | Yes |
| 61 | 107 | End | | Yes |

## Copying Procedures

To copy a procedure, press the *Copy* soft key on the Select Procedure menu. The Copy Procedure menu (Figure 3-50) appears:

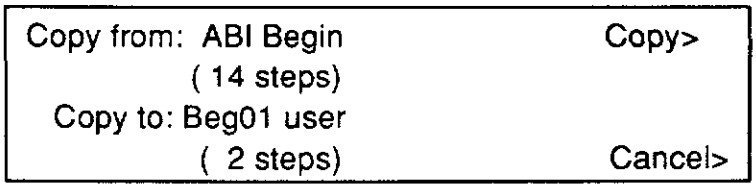



Figure 3-50. Copy Procedure menu

This menu allows you to select a source file and a destination file. The source file is copied into the destination file. To select a source file, place the cursor on the procedure name, located to the right of the "Copy from:" statement. Then use the **Prev** and **Next** keys to scroll through the available procedures. Use the same procedure to select a destination file ("Copy to:").

Once the source and destination files have been selected, you execute the copy by pressing the *Copy* soft key. Pressing the *Cancel* soft key redisplays the previous menu.

## Erasing Procedures

To clear or erase a procedure, press the *Erase* soft key on the Select Procedure menu. The Erase Procedure menu (Figure 3-51) appears:

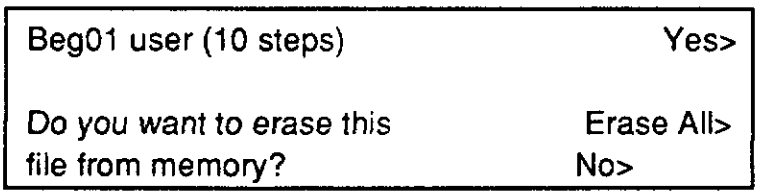



Figure 3-51. Erase Procedure menu

The upper left hand corner displays the name of the procedure to be erased. Select a procedure by using the **Prev** and **Next** keys to scroll through those available. Then erase the designated procedure by pressing *Yes*.

You also have the option to erase all user-defined begin procedures. When you press the *Erase All* soft key, the menu shown in Figure 3-52 appears:

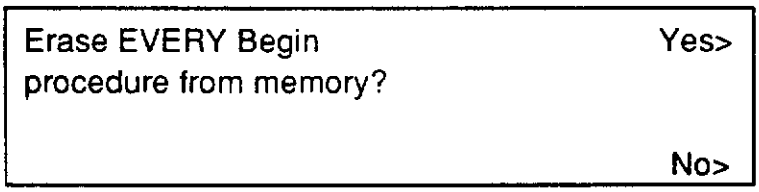



Figure 3-52. Erase Begin Procedures menu

Pressing the *Yes* soft key erases all user-defined begin procedures in the 392/394 memory.

# Main Menu Option: Edit Function

## Introduction

Use Edit Function to create and edit user functions or to print a list of functions. Applied Biosystems defines two types of functions, system functions and user functions. System functions are those provided with the instrument. They are permanent and cannot be edited. User functions, functions 200-224, can be both viewed and edited.

User functions are defined by specifying a set of valves to be opened when the function is activated. Like system functions, they can be activated from Manual Control, inserted into any synthesis cycle via the Cycle Editor, or inserted into any procedure via the Procedure Editor.

*NOTE Refer to the synthesizer schematic (Appendix II) when creating user functions and check that the desired flow path will be created.*

## Editing a User Function

The *Edit Function* soft key appears on page 4 of the Main Menu. When you press this key, the Edit Function menu appears:

| | |
|---|---|
| ( 1) Block Flush | Print All> |
| Valves. (Valve: 0) | Valve On> |
| 40 35 25 0 3 4 7 8 | Valve Off> |
| 11 12 15 | All Off> |

Figure 3-53. Edit Function menu

The display above shows Function 1, Block Flush. You can view but not edit this function because it is a system function. To select another function, place the cursor on the function number by using the left (◄—) or right arrow (—►) key. Then use the **Prev** or **Next** keys to scroll to the desired function. Alternatively, after placing the cursor on the function number, use the numeric keypad to enter the desired function number.

Although the name of a system function cannot be changed, a user function may be given a name consisting of up to 15 alphanumeric characters. To name a user function, place the cursor over the existing name and use the **Prev** or **Next** keys to scroll through the alphanumeric characters.

The last two lines are a list of the valves that are turned on when this function is active. There is a maximum of 15 valves per function.

You can insert a new valve number into a user function. First, enter the desired valve number within the parentheses following "Valve:". Then press the *Valve On* soft key. To remove a

valve from a user function, enter the valve number and press the *Valve Off* soft key. To remove all valves from a user function valve list, press the *All Off* soft key.

## Printing Functions

To print out all functions, both system and user, press the *Print All* soft key on the Edit Function menu.

# Main Menu Option: Power Fail

## General Information

The Model 392/394 automatically resumes synthesis after a power failure unless the power failure occurred on a non-safe step and the power down time exceeds that set using the Power Fail menu. (A synthesis always continues for steps denoted as safe steps.) Besides allowing you to control the maximum power failure time, the Power Fail menu is also used after a power failure to determine when the power went down and for how long.

When a power failure occurs during an active synthesis, the synthesis is stopped immediately. The instrument cannot operate until the main power returns. During the outage, all synthesis parameters are retained in memory. These parameters include the DNA sequences; user defined cycles, procedures and functions; bottle usage information; and the time and date.

A *maximum power fail* time can be set so that if a failure occurs for less than the specified time during a non-safe step, the synthesis will automatically resume when the main power returns. A synthesis is interrupted when a failure occurs during a non-safe step and lasts longer than the specified time. The synthesis may then be resumed or aborted.

If you wish to set the *maximum power fail* time, display the Power Fail menu as described below and follow the instructions under "Setting the Maximum Power Fail Time," page 3-60.

## After a Power Failure

When the main power returns, an appropriate message will appear, depending on what the instrument was doing at the time of the power failure. The following message appears if the instrument was inactive.

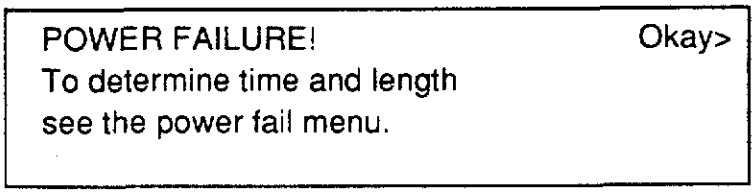



Figure 3-46. Power Fail message

At this point, you will need to display the Power Fail menu to determine the duration of the power failure. To do so, display page 4 of the Main Menu and press the *Power Fail* soft key. The Power Fail menu appears as in Figure 3-54.

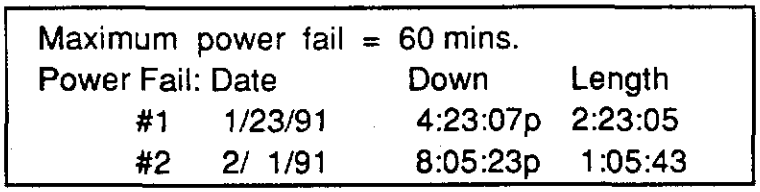



Figure 3-54. Power Fail menu

If a power failure occurs on a non-safe step for a period of time longer than that set for the maximum power fail time, the synthesis in progress is interrupted. Such a synthesis may be resumed or aborted. (A synthesis automatically resumes when power failures on non-safe steps occur for less than the power fail time.) If a power failure occurs during a safe step, the down time has no meaning since the synthesis always resumes.

## Setting the Maximum Power Fail Time

On the top line of the Power Fail menu is the maximum power failure time for a function designated as a non-safe step (60 minutes in the example above). You can change this value via the numeric keyboard. Lines 3 and 4 display information about the last two power failures experienced by the system. The system keeps track of a maximum of five power failures. To display those hidden from view, place the cursor on a Power Fail number (e.g., #1) and use the **Prev** or **Next** keys to scroll up or down.

*NOTE If you enter 999 as the maximum power fail time, the synthesis will always continue when the main power returns regardless of the duration of the outage.*

The "Down" time is the time at which the power failed. "Length" is the period of time the main power was down. Down and Length time are specified in a 24-hour format; the maximum value that can be shown is one day or 23:59:59 (23 hours, 59 minutes, and 59 seconds).

*NOTE If a synthesis is interrupted at a step other than Cycle Entry or Cycle End, the valve blocks and delivery lines must be rinsed. For details, refer to "Interrupting or Aborting a Synthesis," in Section 2.*

# Main Menu Option: Set Time and Date

Use the Set Time and Date Menu to enter the current time and date. The time is used to record when a power failure occurred and to provide dates on printouts. To display this menu, press the *Set Time/Date* soft key on page 4 of the Main Menu.

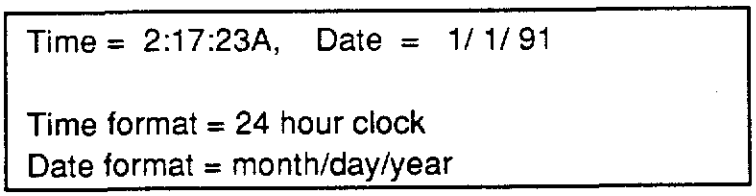



Figure 3-55. Set Time and Date menu

The time and date appear on the first line of this display. To change the hours, place the cursor on the "hours" part of the time. Then use the numeric keypad to type the new hours. The new characters overstrike the existing ones. Use the same procedure to change the "minutes" part of the time or the month, day, or year of the date.

Notice that the letter "A" is used after the time value to indicate a.m., and the letter "P" is used to indicate p.m. To change A to P or P to A, place the cursor on this character and use the **Prev** or **Next** key.

To change the time format from a 24-hour clock to a 12-clock, place the cursor on the time format field and use the **Prev** or **Next** keys.

The bottom line contains the date format. You have a choice of two, either "month/day/year" or "day/month/year." To change the date format, place the cursor on the format field and press the **Prev** or **Next** key.

# Main Menu Option: Relay Configuration

The Relay Configuration menu is used to set the pulse duration for the advance fraction collector and relay pulse functions. These functions can be placed in a cycle or procedure to drive the fraction collector and perform other external functions. To display this menu, press the *Relay Configuration* soft key on page 5 of the Main Menu.

| | | | | |
|---|---|---|---|---|
| Advance FC | : Closed = | 0.5, | Open = | 1.5 |
| Relay 2 Pulse | : Closed = | 0.5, | Open = | 1.5 |
| Relay 3 Pulse | : Closed = | 0.5, | Open = | 1.5 |
| Relay 4 Pulse | : Closed = | 0.5, | Open = | 1.5 |

Figure 3-56. Relay Configuration menu

To change an existing pulse duration, move the cursor to the appropriate "Closed" or "Open" field and enter the new time in seconds. Times as long as 999.9 seconds are allowed.

To specify the pulse width for each relay listed above, set both closed and open times in seconds. During the closed time, the synthesizer presents a contact closure to an external device. An open time is also specified for each function.

# Main Menu Option: Shut Down

The Shut Down procedure menu is used to prepare the instrument for long-term storage. The procedure removes all reagents in the delivery lines and washes and dries all chemical pathways. It takes about 20 minutes and is further described in Section 4.

When you press the *Shut Down* soft key on page 5 of the Main Menu, the following menu appears:

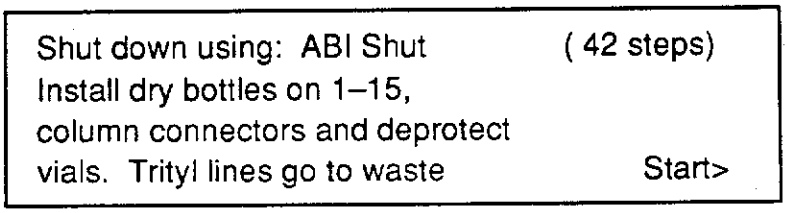



Figure 3-57. Shut Down menu

The Shut Down menu (shown above with the "ABI Shut" procedure), allows you to choose the shut down procedure. To change the procedure, place the cursor on the procedure name and use the **Prev** and **Next** keys.

*NOTE Before initiating any shut down procedure, be sure to follow the instructions presented on the display (such as those in Figure 3-57) to avoid contaminating reservoirs!*

For the example in Figure 3-57, you would remove bottles 1-15 and replace them with clean, empty bottles. Removing the bottles prevents acetonitrile from diluting reagents that could be reused at a later date. It also serves to vent the ammonia reservoir, which is at a higher pressure than acetonitrile. Next, you would install column connectors and deprotect vials. Then you would direct the trityl lines to a waste vessel. When the preparatory steps are finished, you press the *Start* soft key to initiate the shut down procedure. The following menu will appear:

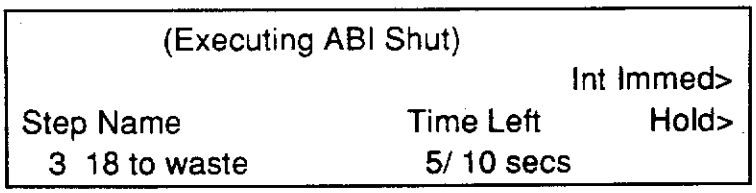



Figure 3-58. Shut Down monitor screen

This menu allows you to monitor the progress of the shut down process. If necessary, you can maintain the current step (*Hold* soft key) or interrupt or abort the process (*Int Immed* soft key). The *Hold* soft key continues the current step indefinitely. When the *Int Immed* soft key is pressed, the *Resume* and *Abort* soft keys appear, allowing access to these functions. *Resume* allows the interrupted sequence to continue at the next step. *Abort* cancels the shut down and returns the display to the Shut Down menu.

# 4

# Functions, Cycles and Procedures

This section contains information essential to understanding functions, synthesis cycles and procedures. Be sure to read the introductory material, the material on valves, the initial material on functions (pages 4–7 to 4–11) and the material on synthesis cycles (pages 4–23 to 4–28). The rest of the section can be used as needed.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# Introduction

Automated DNA synthesis requires chemical deliveries to specified destinations on the instrument, such as the column. These deliveries are controlled by electrically activating solenoid valves to open and close, creating various pathways through the Model 392/394.

Each solenoid valve is assigned a number that can be used to open or close it. A valve or set of valves opened to perform a specific delivery or task is a *function*. For example, Function 42, 18 to column, delivers acetonitrile to the column. On eight-base instruments with four columns, Function 42 opens valves 54, 42, 35, 16, 3, 17, 7, 18, 11, 19, and 15 simultaneously.

A function that is programmed to occur for a specified amount of time is a *step*. (e.g., Function 42 can be activated for 10 seconds.) A series of steps programmed to perform a chemical process repeatedly is a *cycle*. Synthesis cycles contain all the steps necessary for the efficient addition of one base. The cycle is repeated until every base in the sequence is added. *Procedures* are a series of steps programmed to complete a task, such as changing a reagent bottle. Upon command, the steps are performed once and are not repeated. There are four types of operating procedures: begin procedures, end procedures, bottle change procedures and shut down. Additional procedures are supplied for testing the system (see "Main Menu Option: Self Test" in Section 3).

# Valves

The Model 392/394 has as many as 49 solenoid valves (numbered 0 through 35, 40 through 49, and 51 through 54 ), which are opened and closed electrically and are controlled through the microprocessor. Refer to Section 5, "Delivery Valve Blocks", for a physical description of the valves.

During a synthesis, valves are automatically opened to create the correct chemical pathways and allow all necessary reagent and gas deliveries. You can also manually operate individual valves by using the Manual Control menu. This is helpful when troubleshooting and can be done before synthesis or when a synthesis is interrupted.

All valves with descriptions of what they control are listed next. Refer to Figure 4-1, a simplified DNA/RNA synthesizer schematic, to view the placement of the most important valves. Detailed schematic information is provided in Appendix II.

Valves 0 to 15 control input and output flows from the four column valve blocks (Model 394). On a two-column instrument (Model 392), only valves 0 through 7 are physically present.

| Block | Valve: | Controls the flow of: |
|---|---|---|
| A–5 | 0 | argon to Column 1 |
| | 1 | DNA/RNA output to Vial 1 |
| | 2 | trityl output from Column 1 |
| | 3 | waste from Column 1 |
| B–5 | 4 | argon to Column 2 |
| | 5 | DNA/RNA output to Vial 2 |
| | 6 | trityl output from Column 2 |
| | 7 | waste from Column 2 |
| C–5 | 8 | argon to Column 3 |
| | 9 | DNA/RNA output to Vial 3 |
| | 10 | trityl output from Column 3 |
| | 11 | waste from Column 3 |
| D–5 | 12 | argon to Column 4 |
| | 13 | DNA/RNA output to Vial 4 |
| | 14 | trityl output from Column 4 |
| | 15 | waste from Column 4 |

Valves 16 through 35 (in reagent valve blocks F–11 and R–11) and Valves 40 through 43 (in a third reagent valve block, E–5) control the input of bases, reagents, and argon to the reagent valve blocks as well as output to the columns. Valves 16 through 35 are present in both five- and eight-base instruments, whereas Valves 40 through 43 are present only in eight-base instruments.

| Block | Valve | Directs output from the : |
|---|---|---|
| F–11 | 16 | Reagent valve block to Column 1 |
| | 17 | Reagent valve block to Column 2 |
| | 18 | Reagent valve block to Column 3 |
| | 19 | Reagent valve block to Column 4 |

*NOTE To identify reagent valve blocks. See the plumbing diagram for your instrument in Appendix II.*

| Block | Valve | Controls the input of: |
|---|---|---|
| F–11 | 20 | Acetic anhydride to the front reagent valve block |
| | 21 | NMI to the front reagent valve block |
| | 22 | TCA to the front reagent valve block |
| | 23 | $I_2$ or TETD to the front reagent valve block |
| | 24 | $NH_4OH$ to the front reagent valve block |

| Block | Valve | |
|---|---|---|
| F–11 | 25 | Output to waste - for both reagent valve blocks |

| Block | Valve | Controls the input of: |
|---|---|---|
| R–11 | 26 | Base A to the rear reagent valve block |
| | 27 | Base G to the rear reagent valve block |
| | 28 | Base C to the rear reagent valve block |
| | 29 | Base T to the rear reagent valve block |
| | 30 | Base from Position 5 to the reagent valve block |
| | 31 | TET (for 5-base instruments) or the Position 6 Base (for eight-base instruments) |
| | 32 | Argon (LP Gas for 5-base instruments) or the Position 7 Base (for eight-base instruments) |
| | 33 | $CH_3CN$ (for 5-base instruments) or the Position 8 Base (for eight-base instruments) |
| | 34 | Pressure transducer (for 5-base instruments) or Tetrazole (for eight-base instruments) |
| | 35 | Argon (HP Gas for 5-base instruments) or the connection between the third reagent block and the rear reagent block (for 8-base instruments) |

| Block | Valve | Controls the input of: |
|---|---|---|
| E–5 | 40 | Argon (HP Gas) for eight-base instruments |
| | 41 | Pressure transducer for eight-base instruments |
| | 42 | $CH_3CN$ for eight-base instruments |
| | 43 | Argon (LP Gas) for eight-base position instruments |

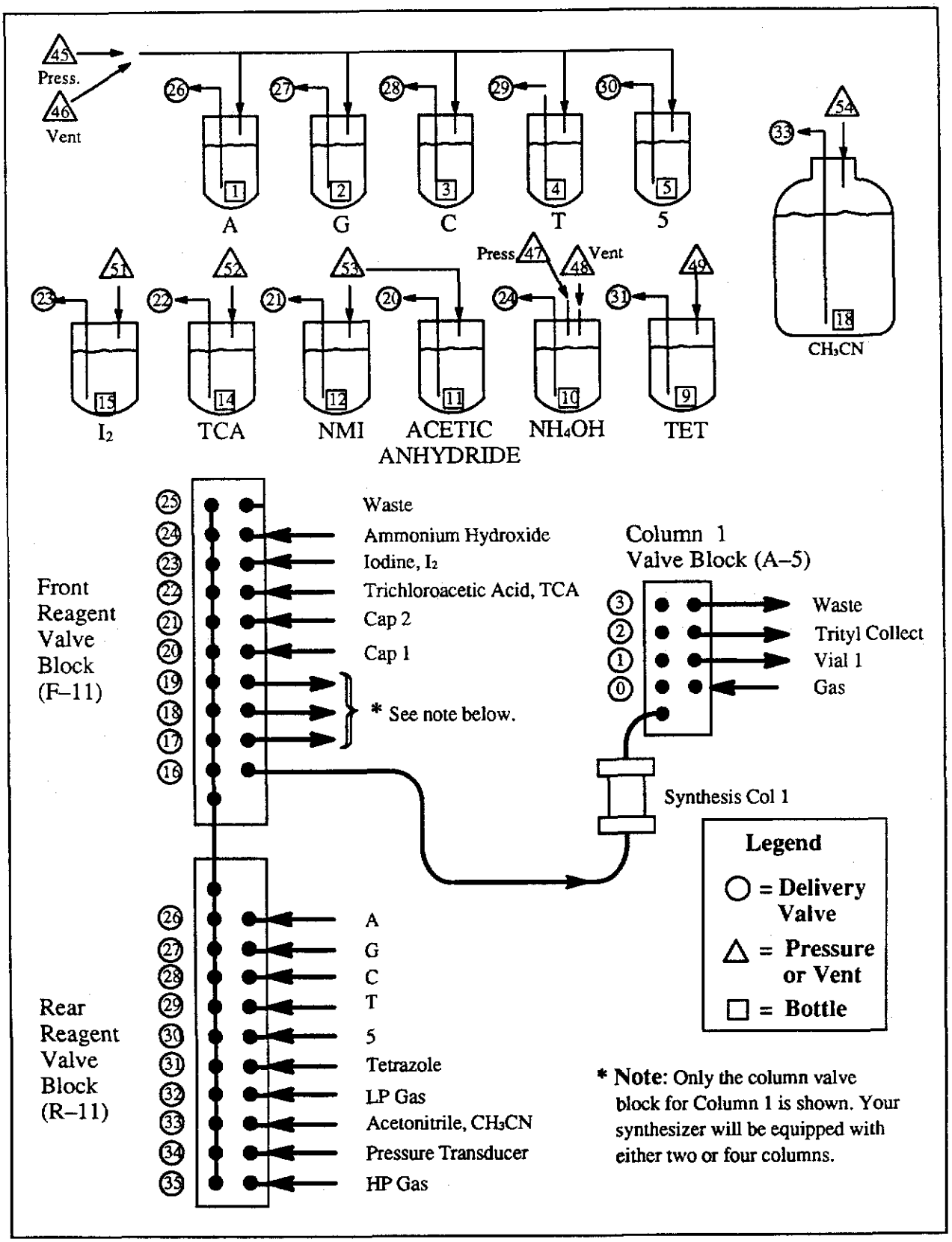


Figure 4-1. Simplified DNA synthesizer schematic (five-base instrument)

Valves 45 through 54 are pressure valves that control the flow of argon to the following manifolds (or bottles):

| **Valve:** | **Argon (or vent) to:** |
|---|---|
| 45 | Manifold A (Pressure manifold for all bases) |
| 46 | Manifold B (Vent manifold for all bases) |
| 47 | $NH_4OH$ (Pressure) |
| 48 | $NH_4OH$ (Vent) |
| 49 | Tetrazole (Pressure) |
| 51 | $I_2$ (Pressure) |
| 52 | TCA (Pressure) |
| 53 | Acetic Anhydride and NMI (Pressure) |
| 54 | $CH_3CN$ (Pressure) |

Note that for flow to actually occur, several valves must be opened simultaneously, as explained in the function descriptions in the next subsection.

# Functions

A valve or set of valves opened simultaneously to perform a specific delivery or task is a function. In order to accommodate the differing requirements of different versions of the synthesizer, each of the possible instrument configurations has a different function list (two-column/five-base, two-column/eight-base, four-column/five-base, four-column/eight-base). To describe functions in the simplest way possible, the function list (Table 4-1) and the plumbing diagram (Figure 4-2) presented in this section apply to the two-column, five-base instrument. For function information on 392 eight-base position instruments or 394 five- and eight-base position instruments, refer to the specific function descriptions in this section or the appropriate function list in Appendix I and the relevant synthesizer schematic in Appendix II.

The functions listed in Table 4-1 are used during synthesis cycles and during the following procedures: bottle change, auto-dilution, begin, end, shut down, and flow test. These functions may also be activated manually through the Manual Control menu (see Section 3). In addition, up to 25 user functions, numbered 200 to 224, can be created through the Function Editor menu (also described in Section 3).

Functions that deliver a reagent to a column or flush a column are "column-specific functions." They will ignore valves for nonactive columns. For example, if only column 2 is active, column 1 valves (16 and 3) will not activate. When a function is activated, the correct valves automatically open. After a specified time, the function is deactivated and the valves close.

Functions are given abbreviated names to describe the action they perform. For example, Function 42 is named "18 to column." It delivers the contents of bottle #18, acetonitrile, to all active columns. In Table 4-2, the functions are grouped according to their actions.

During synthesis, you can check which function is being performed by viewing the Step Monitor menu.

DNA synthesis requires various reagents to be delivered to a specified destination. A reagent can flow from its reservoir to the waste bottle, or through the column and then to either the waste bottle, the trityl collection port, or the collection vial. Functions have been defined to perform each necessary delivery. To achieve flow of a reagent, specific valves must open simultaneously so that the following occurs:

1. The reservoir is pressurized;
2. The pathway from the reservoir to the valve block is opened; and
3. An exit is provided out of the valve block (i.e., to waste, or to the column and then to either the waste port, the trityl collection port, or the collection vial).

The pathway created by activating a function can be traced for a single column using the 392 five-base position DNA synthesizer schematic, Figure 4-2. For example, Function 42 (18 to column) delivers acetonitrile to the column. Activating this function does the following:

1. Opens Valve 54 to allow argon to flow into the acetonitrile reservoir to pressurize it;
2. Opens Valve 33 to open the pathway between the reservoir and the valve block;
3. Opens Valve 16 to open the pathway from the valve block to Column 1; and
4. Opens Valve 3 to provide an exit (the waste bottle).

When all four valves are open, the argon pressure forces the acetonitrile out of the reservoir, through the reagent valve blocks, through the column, and out of the synthesizer to the waste bottle. When Function 42 is deactivated, all valves close and the flow stops.

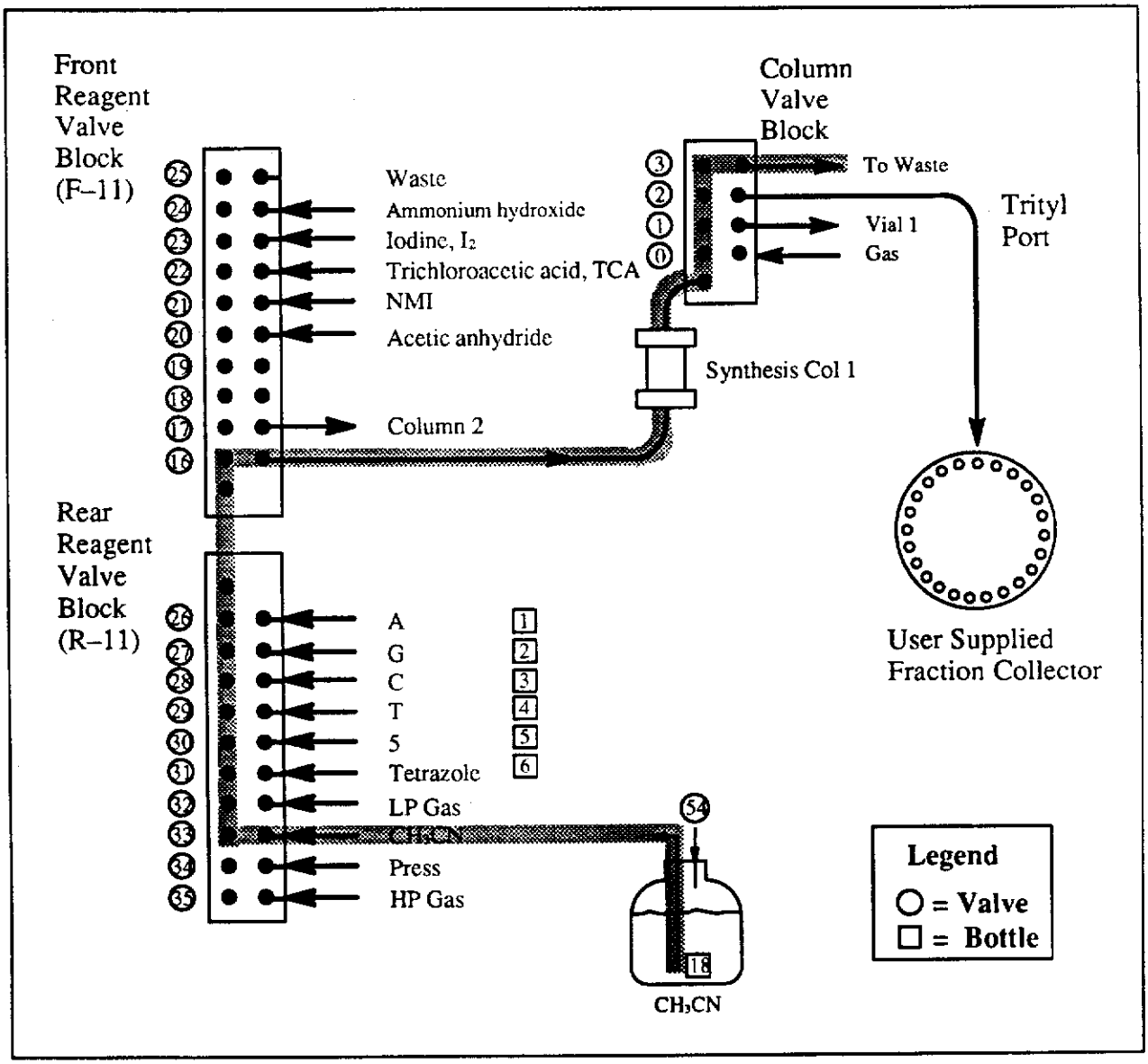


Figure 4-2. Flow path of 18 to column 1 for five-base instruments (valves 54,33,16,3)

# Table 4-1
## 392 Sequential Function List
## (two-column/five-base instrument)

| Number | Name | Valve List | Number | Name | Valve List |
|---|---|---|---|---|---|
| 1 | Block Flush | 35,25,0,3,4,7 | 58 | TET to Waste | 49,31,25 |
| 2 | Reverse Flush | 25,0,16,4,17 | 59 | 10 to Waste | 47,24,25 |
| 3 | Trityl Flush | 35,16,2,17,6 | 60 | 11 to Waste | 53,20,25 |
| 4 | Flush to Waste | 35,16,3,17,7 | 61 | 12 to Waste | 53,21,25 |
| 5 | Flush to Colect | 35,16,1,17,5 | 62 | 14 to Waste | 52,22,25 |
| 6 | Flush to A | 32,46,26 | 63 | 15 to Waste | 51,23,25 |
| 7 | Flush to G | 32,46,27 | 64 | 18 to Waste | 54,33,25 |
| 8 | Flush to C | 32,46,28 | 65-69 | INVALID FNCTION | |
| 9 | Flush to T | 32,46,29 | 70 | 18 to A | 54,46,33,26 |
| 10 | Flush to 5 | 32,46,30 | 71 | 18 to G | 54,46,33,27 |
| 11- 13 | INVALID FNCTION | | 72 | 18 to C | 54,33,46,28 |
| 14 | Flush to Bases | 32,46 | 73 | 18 to T | 54,33,46,29 |
| 15 | Flush to Tet | 35,31 | 74 | 18 to 5 | 54,33,46,30 |
| 16 | Flush to 10 | 35,24 | 75-77 | INVALID FNCTION | |
| 17 | Flush to 11 + 12 | 35,20,21 | 78 | 18 to Tet | 54,33,31 |
| 18 | Flush to 14 + 15 | 35,22,23 | 79 | 18 to 10 | 54,33,24 |
| 19 | Flush to 18 | 33,32 | 80 | 18 to 11 | 54,33,20 |
| 20- 24 | INVALID FNCTION | | 81 | 18 to 12 | 54,33,21 |
| 25 | A to Column | 45,26,16, 3,17, 7 | 82 | 18 to 11 + 12 | 54,33,20,21 |
| 26 | G to Column | 45,27,16, 3,17, 7 | 83 | 18 to 14 | 54,33,22 |
| 27 | C to Column | 45,28,16, 3,17, 7 | 84 | 18 to 15 | 54,33,23 |
| 28 | T to Column | 45,29,16, 3,17, 7 | 85 | 18 to 14 + 15 | 54,33,22,23 |
| 29 | 5 to Column | 45,30,16, 3,17, 7 | 86 | 18 To Bases | 54,33,46 |
| 30-32 | INVALID FNCTION | | 87-89 | INVALID FNCTION | |
| 33 | B+Tet to Column | 45,49,31,16, 3,17, 7 | 90 | 15 to A | 51,46,23,26 |
| 34 | Tet to Column | 49,31,16, 3,17, 7 | 91 | 15 to G | 51,46,23,27 |
| 35 | 5+Tet to Column | 45,30,49,31,16, 3,17, 7 | 92 | 15 to C | 51,46,23,28 |
| 36 | 10 to Collect | 47,24,16, 1,17, 5 | 93 | 15 to T | 51,46,23,29 |
| 37 | 11 to Column | 53,20,16, 3,17, 7 | 94 | 15 to Bases | 51,46,23 |
| 38 | 12 to Column | 53,21,16, 3,17, 7 | 95- 99 | INVALID FNCTION | |
| 39 | Cap to Column | 53,20,21,16,3,17,7 | 100 | 10 Vent | 48 |
| 40 | 14 to Column | 52,22,16,3,17,7 | 101 | Phos Prep | 45,49 |
| 41 | 15 to Column | 51,23,16,3,17,7 | 102 | Cap Prep | 53 |
| 42 | 18 to Column | 54,33,16,3,17,7 | 103 | Wait | |
| 43 | Push to Column | 54,33,16, 3,17, 7 | 104 | Interrupt | |
| 44-49 | INVALID FNCTION | | 105 | Start Detrityl | |
| 50 | A to Waste | 45,26,25 | 106 | Begin | |
| 51 | G to Waste | 45,27,25 | 107 | End | |
| 52 | C to Waste | 45,28,25 | 108 | Block to Col | 16, 3,17, 7 |
| 53 | T to Waste | 45,29,25 | 109 | Waste - Port | |
| 54 | 5 to Waste | 45,30,25 | 110 | Waste - Bottle | |
| 55-57 | INVALID FNCTION | | 111 | Block Vent | 25, 3, 7 |

## Table 4-1 (continued)

| Number | Name | Valve List |
|---|---|---|
| 112 | Trityl Advance | |
| 113 | End Advance | |
| 114 | Vent Bases | 46 |
| 115 | Prep 10 | 47 |
| 116-119 | INVALID FNCTION | |
| 120 | Advance FC | 59 |
| 121 | Relay 1 On | 59 |
| 122 | Relay 1 Off | 59 |
| 123 | Relay 1 Pulse | 59 |
| 124 | Relay 2 On | 60 |
| 125 | Relay 2 Off | 60 |
| 126 | Relay 2 Pulse | 60 |
| 127 | Relay 3 On | 61 |
| 128 | Relay 3 Off | 61 |
| 129 | Relay 3 Pulse | 61 |
| 130 | Relay 4 On | 62 |
| 131 | Relay 4 Off | 62 |
| 132 | Relay 4 Pulse | 62 |
| 133 | Relay 5 On | 63 |
| 134 | Relay 5 Off | 63 |
| 135-139 | INVALID FNCTION | |
| 140 | Column 1 On | |
| 141 | Column 1 Off | |
| 142 | Column 2 On | |
| 143 | Column 1 Off | |
| 144-149 | INVALID FNCTION | |
| 150 | Bottle 01 On | |
| 151 | Bottle 01 Off | |
| 152 | Bottle 02 On | |
| 153 | Bottle 02 Off | |
| 154 | Bottle 03 On | |
| 155 | Bottle 03 Off | |
| 156 | Bottle 04 On | |
| 157 | Bottle 04 Off | |
| 158 | Bottle 05 On | |
| 159 | Bottle 05 Off | |
| 160-169 | INVALID FNCTION | |
| 170 | Dilute 'A' | 54,46,33,26 |
| 171 | Dilute 'G' | 54,46,33,27 |
| 172 | Dilute 'C' | 54,33,46,28 |
| 173 | Dilute 'T' | 54,33,46,29 |
| 174 | Dilute '5' | 54,33,46,30 |
| 175-199 | INVALID FNCTION | |
| 200 | User01 | |
| 201 | User02 | |
| 202 | User03 | |
| 203 | User04 | |
| 204 | User05 | |
| 205 | User06 | |
| 206 | User07 | |
| 207 | User08 | |
| 208 | User09 | |
| 209 | User10 | |
| 210 | User11 | |
| 211 | User12 | |
| 212 | User13 | |
| 213 | User14 | |
| 214 | User15 | |
| 215 | User16 | |
| 216 | User17 | |
| 217 | User18 | |
| 218 | User19 | |
| 219 | User20 | |
| 220 | User21 | |
| 221 | User22 | |
| 222 | User23 | |
| 223 | User24 | |
| 224 | User25 | |

# Table 4-2. 392/394 Functions by Type

## CONTROL

103 - Wait
104 - Interrupt
105 - Start Detrityl
106 - Begin
107 - End
108 - Block to Col
109 - Waste - Port
110 - Waste - Bottle
112 - Trityl Advance
113 - End Advance
120 - Advance FC
121 - Relay 1 On
122 - Relay 1 Off
123 - Relay 1 Pulse*
124 - Relay 2 On
125 - Relay 2 Off
126 - Relay 2 Pulse*
127 - Relay 3 On
128 - Relay 3 Off
129 - Relay 3 Pulse*
130 - Relay 4 On
131 - Relay 4 Off
132 - Relay 4 Pulse*
133 - Relay 5 On
134 - Relay 5 Off

## BOTTLE CONTROL

150 - Bottle01 On
151 - Bottle01 Off
152 - Bottle02 On
153 - Bottle02 Off
154 - Bottle03 On
155 - Bottle03 Off
156 - Bottle04 On
157 - Bottle04 Off
158 - Bottle05 On
159 - Bottle05 Off
160 - Bottle06 On
161 - Bottle06 Off
162 - Bottle07 On
163 - Bottle07 Off
164 - Bottle08 On
165 - Bottle08 Off

140 - Column 1 On
141 - Column 1 Off
142 - Column 2 On

## PRIME DELIVERY LINES

50 - A to Waste
51 - G to Waste
52 - C to Waste
53 - T to Waste
54 - 5 to Waste
55 - 6 to Waste
56 - 7 to Waste
57 - 8 to Waste
58 - Tet to Waste
59 - 10 to Waste
60 - 11 to Waste
61 - 12 to Waste
62 - 14 to Waste
63 - 15 to Waste
64 - 18 to Waste
101 - Phos Prep
102 - Cap Prep
115 - Prep 10

## VENTING

100 - 10 Vent
111 - Block Vent
114 - Vent Bases

## ARGON FLUSHES

1 - Block Flush
2 - Reverse Flush
3 - Trityl Flush
4 - Flush to Waste
5 - Flush to Collect
6 - Flush to A
7 - Flush to G
8 - Flush to C
9 - Flush to T
10 - Flush to 5
11 - Flush to 6
12 - Flush to 7
13 - Flush to 8
14 - Flush to Bases
15 - Flush to Tet
16 - Flush to 10
17 - Flush to 11+12
18 - Flush to 14+15
19 - Flush to 18

## COLUMN CONTROL

143 - Column 2 Off
144 - Column 3 On
145- Column 3 Off

## REAGENTS TO RESERVOIRS

70 - 18 to A
71 - 18 to G
72 - 18 to C
73 - 18 to T
74 - 18 to 5
75 - 18 to 6
76 - 18 to 7
77 - 18 to 8
78 - 18 to Tet
79 - 18 to 10
80 - 18 to 11
81 - 18 to 12
82 - 18 to 11+12
83 - 18 to 14
84 - 18 to 15
85 - 18 to 14+15
86 - 18 to Bases
90 - 15 to A
91 - 15 to G
92 - 15 to C
93 - 15 to T
94 - 15 to bases

## REAGENTS TO COLUMN

25 - A to Column
26 - G to Column
27 - C to Column
28 - T to Column
29 - 5 to Column
30 - 6 to Column
31 - 7 to Column
32 - 8 to Column
33 - B+Tet to Column
34 - Tet to Column
35 - 5+Tet to Column
36 - 10 to Collect
37 - 11 to Column
38 - 12 to Collect
39 - Cap to Column
40 - 14 to Column
41 - 15 to Column
42 - 18 to Column
43 - Push to Column

146 - Column 4 On
147 - Column 4 Off

* Pulsed relay functions require setting pulse duration using the Main Menu Configuration Relay option.

## Synthesis Cycle Functions

This part of Section 4 categorizes the functions and explains what each function does. Note that the function lists are representative of their category, but may not be all–inclusive.

### Functions that Deliver Reagents to the Column

The following five functions provide for flow of a reagent (driven by argon pressure) from its reservoir, through the valve block, through the column, and ultimately to the waste bottle. Since the column is the site of all chemical reactions necessary for synthesis, these deliveries are critical.

**Table 4-3**
**Column Reagent Delivery**

*NOTE For two-column instruments some of the valves listed are not used. These valves are marked with an asterisk. Also, on instruments equipped with five base positions, the valve listed in parentheses is used instead of the preceding underlined valve or valves.*

| Function Number | Name | Valves | Description |
|---|---|---|---|
| F 33 | Base + Tet to Column | X,45,49,34(31),16,3,17, 7,18*,11*,19*,15* | X= 1 to 4 of the following valves: 26, 27, 28, 29, 30, 31, 32, 33 (Bases A, G, C, T, 5, 6, 7, 8) According to the sequence, the correct phosphoramidite(s) and tetrazole are simultaneously delivered to the column to perform the coupling reaction. |
| F 34 | Tet to Column | 49,34(31),16,3,17,7, 18*,11*,19*,15* | Tetrazole is delivered to the column; activates phosphoramidites for coupling reaction. |
| F 39 | Cap to Column | 53,20,21,16,3,17,7, 18*,11*,19*,15* | Both capping reagents, acetic anhydride (cap A) and NMI (cap B) are simultaneously delivered to the column; used following coupling to terminate or cap unreacted nucleotide chains. |
| F 40 | 14 to Column | 52,22,16,3,17,7,18*, 11*,19*,15* | TCA is delivered to the column; used to detritylate the support-bound oligonucleotides prior to coupling. |
| F 41 | 15 to Column | 51,23,16,3,17,7,18*, 11*,19*,15* | Iodine/TETD is delivered to the column; used to oxidize/sulfurize the DNA. |

## Table 4-3 (continued)

| Function Number | Name | Valves | Description |
|---|---|---|---|
| F 42 | 18 to Column | 54,42,35,(33),16,3,17, 7,18*,11*,19*,15* | Delivers acetonitrile to wash the column before or after reagent delivery. |
| F43 | Push to Column | 54,42,35,(33),16,3,17, 7,18*,11*,19*,15* | Delivers a specific amount of acetonitrile to the column during the coupling reaction. The instrument determines the delivery time based on which column is active and which base is being delivered. |

### Functions that Control External Events

The following functions do not activate solenoid valves, but control electrical switches. They are used to give electrical signals to a fraction collector, chart recorder, or other auxiliary equipment.

| Function Number | Name | Switch Action |
|---|---|---|
| F 120 | ADVANCE FC | PULSE |
| F 121 | RELAY 1 ON | RELAY CLOSE |
| F 122 | RELAY 1 OFF | RELAY OPEN |
| F 123 | RELAY 1 PULSE | PULSE |
| F 124 | RELAY 2 ON | RELAY CLOSE |
| F 125 | RELAY 2 OFF | RELAY OPEN |
| F 126 | RELAY 2 PULSE | PULSE |
| F 127 | RELAY 3 ON | RELAY CLOSE |
| F 128 | RELAY 3 OFF | RELAY OPEN |
| F 129 | RELAY 3 PULSE | PULSE |
| F 130 | RELAY 4 ON | RELAY CLOSE |
| F 131 | RELAY 4 OFF | RELAY OPEN |
| F 132 | RELAY 4 PULSE | PULSE |
| F 133 | RELAY 5 ON | RELAY CLOSE |
| F 134 | RELAY 5 OFF | RELAY OPEN |

Function 120, Advance FC (fraction collector), provides a pulse to Terminals 1 and 2, located on the terminal strip at the left rear of the instrument. When the terminals are connected to a fraction collector, this pulse moves the tube rack one position. The default pulse length of 500 milliseconds can be changed through the Relay Configuration menu. (For more information, see

"Main Menu Option: Relay Configuration," in Section 3.) The fraction collector is used to collect the trityl cation released during each detritylation step.

Functions 121, 122, 124, 125, 127, 128, 130, 131, 133, and 134 must be used in pairs since one function provides a contact closure (On) between a pair of terminals and a second function turns it off. See Figure 4-3 for the location of terminals for relays on the back panel relay strip. In addition to controlling a chart recorder, these functions can be used to activate and deactivate a heater. However, be sure not to run any of the heater's current through the terminals. Contact the Applied Biosystems Technical Support Department for further information.

---

***CAUTION*** *Never exceed the electrical ratings marked on the back of the instrument.*

---

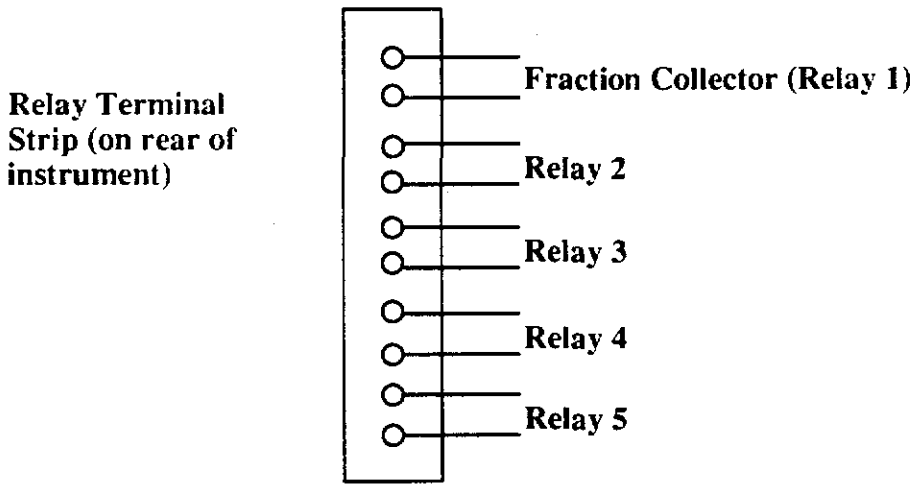


Figure 4-3. Location of Relay Terminals

A chart recorder can be connected to one of the relay terminals on the rear of the instrument so that it can be turned on and off at the proper times during a synthesis, e.g., to record a trityl assay. When recording the output of a spectrophotometer with a flow cell, a chart recorder can be used to automate trityl assays. However, these readings will not be quantitative because the trityl solution is too concentrated.

Functions 123, 126, 129, and 132 are used singly to deliver a pulse and require that the pulse duration be set using the Relay Configuration menu.

## Functions that Manipulate Chemical Pathways

The following two functions control the pathway of the column effluent:

| Function Number | Name | Valve (Block) | | Description |
|---|---|---|---|---|
| F 109 | Waste - Port | 2 (A–5) | (Column 1) | Sends the column effluent to the trityl collection port. |
| | | 6 (B–5) | (Column 2) | |
| | | 10 (C–5) | (Column 3) | |
| | | 14 (D–5) | (Column 4) | |
| F 110 | Waste - Bottle | 3 (A–5) | (Column 1) | Sends the column effluent to the waste bottle. |
| | | 7 (B–5) | (Column 2) | |
| | | 11 (C–5) | (Column 3) | |
| | | 15 (D–5) | (Column 4) | |

When Function 109 (Waste - Port) is activated, all subsequent deliveries to the column will then exit through the trityl collection port. This is done prior to TCA delivery so that the trityl cation released during detritylation can be sent to a fraction collector for subsequent analysis.

To return the flow of the column effluent to the waste bottle, Function 110 (Waste - Bottle) is activated.

## Functions that Rinse or Flush Chemical Pathways

This group of functions includes the "Argon Flushes"category listed in Table 4-2 plus functions that appear in other categories. These functions are used throughout the synthesis to clear the valve blocks, the column, and the interconnecting delivery lines, and they are performed prior to a chemical delivery to remove residual reagent from a previous delivery.

## Table 4-4
## Chemical Pathway Functions

*NOTE For two-column instruments some of the valves listed are not used. This is indicated by an asterisk. Also, on instruments equipped with five base positions, underlined values are not used. Where provided, the valve listed in parentheses is used instead of the preceding underlined valve or valves.*

| Function Number | Name | Valves | Description |
|---|---|---|---|
| F1 | Block Flush | 40,35,25,0,3,4,7,8*, 11*,12*,15* | Removes any solvent or reagent from the reagent valve blocks and the column valve blocks. Argon enters the three valve blocks simultaneously and forces all liquid to the waste bottle. |
| F 2 | Reverse Flush | 25,0,16,4,17,8*,18* 12*,19* | Removes any reagent or solvent from the column and support; argon flows from the column valve block, through the column, into the reagent valve block and then to waste. This forces the reagent out of the column in the reverse direction of normal flow (i.e., it drains the column from the top to the bottom). |
| F 3 | Trityl Flush | 40,35,16,2,17,6,18* 10*,19*,14* | Used during detritylation to flush TCA or $CH_3CN$ through the column to the trityl collection port. Produces more accurate trityl assay results. |
| F 4 | Flush to Waste | 40,35,16,3,17,7,18* 11*,19*,15* | Argon flows through the column from the bottom to the top and exits at the waste bottle. |
| F 5 | Flush to Collect | 40,35,16,1,17,5,18* 9*,19*,13* | During the end procedure, ammonia is flushed from the column into the collection vial. |

## Table 4-4 (continued)

| Function Number | Name | Valves | Description |
|---|---|---|---|
| F 42 | 18 to Column | 54,42,35,(33)16,3,17, 7,18*,11*,19*,15* | Acetonitrile is delivered to rinse the column. Argon pressure forces the acetonitrile from its reservoir, then through the reagent valve block, the column, the column valve block, and finally to the waste bottle. |
| F 64 | 18 to Waste | 54,42,35,(33),25 | Acetonitrile is delivered to rinse the reagent valve blocks. Argon pressure forces acetonitrile from its reservoir, through the reagent valve blocks to rinse them thoroughly and then to the waste bottle. |

During synthesis, certain functions listed in Table 4-4 are often performed sequentially. For example, Function 64 can be activated, followed by F 42, F 2, and F 1:

| | |
|---|---|
| Function 64 | Rinses the reagent valve blocks. |
| Function 42 | Rinses the column and the column valve block. |
| Function 2 | Removes the acetonitrile from the column. |
| Function 1 | Removes the acetonitrile from the reagent valve block and the column valve block. |

# Procedure Functions

## Functions that Prime Delivery Lines

The functions listed below are used during bottle change procedures to prime delivery lines. When a bottle of fresh reagent is placed on the instrument, the delivery line from the reservoir to the valve block must be primed or filled with the reagent. Argon pressure drives the reagent from its reservoir through the valve block to the waste bottle.

Functions 50-58 are also used during the begin procedure. Prior to synthesis, these functions fill the phosphoramidite and tetrazole lines with fresh reagent.

The following functions deliver the contents of the designated reservoir to the waste bottle. For example, Function 50, A to waste, delivers adenosine phosphoramidite to the waste bottle.

*NOTE Functions 55-57 are not available or needed on five-position instruments since bottle positions six through eight do not exist. Also, five-base position instruments use the valve listed in parentheses instead of the preceding underlined valve or valves.*

| Function Number | Name | Valves |
|---|---|---|
| F 50 | A to Waste | 45,26,25 |
| F 51 | G to Waste | 45,27,25 |
| F 52 | C to Waste | 45,28,25 |
| F 53 | T to Waste | 45,29,25 |
| F 54 | 5 to Waste | 45,30,25 |
| F 55 | 6 to Waste | 45,31,25 |
| F 56 | 7 to Waste | 45,32,25 |
| F 57 | 8 to Waste | 45,33,25 |
| F 58 | Tet to Waste | 49,34,(31)25 |
| F 60 | 11 to Waste | 53,20,25 |
| F 61 | 12 to Waste | 53,21,25 |
| F 62 | 14 to Waste | 52,22,25 |
| F 63 | 15 to Waste | 51,23,25 |
| F 64 | 18 to Waste | 54,42,35,(33),25 |

## Functions that Prepare Reagents for Delivery

The following functions pressurize reagent reservoirs to prepare them for delivery. A prep (preparation) function opens the gas valve to the reservoir allowing argon to flow to the headspace of the bottle. After several seconds, the bottle reaches the correct pressure necessary for proper delivery. These functions are used for reagents that are delivered simultaneously and in equal volumes.

| Function Number | Name | Valves | Description |
|---|---|---|---|
| F 101 | Phos Prep | 45,49 | Phosphoramidite preparation; pressurizes the tetrazole and all phosphoramidite reservoirs simultaneously. |
| F 102 | Cap Prep | 53 | Capping reagents prep (preparation): pressurizes the acetic anhydride and NMI reservoirs simultaneously. |

## Functions that Deliver Acetonitrile to a Reservoir

Before removing an empty phosphoramidite or tetrazole bottle, the old reagent in the delivery line is forced back into the reservoir and the line is rinsed. This is done during the bottle change procedure by delivering acetonitrile through the reagent valve block to the empty reservoir. In addition, functions delivering acetonitrile to bottle positions 11, 12, 14, and 15 are used during the shut down and flow test procedures to remove the reagents from the delivery lines. Functions 70-77 are used in user auto-dilution procedures and functions 170-177 are used in the ABI auto-dilution procedures to reconstitute the phosphoramidites.

The following functions deliver acetonitrile to the designated reservoir. For example, Function 70, 18 to A, delivers acetonitrile to the adenosine phosphoramidite reservoir.

*NOTE Functions 75-77 are not available or needed on five-position instruments since bottle positions six through eight do not exist. On instruments equipped with five base positions, the valve listed in parentheses is used instead of the preceding underlined valves.*

| Function Number | Name | Valve |
|---|---|---|
| F 70 | 18 to A | 54,42,46,35,(33),26 |
| F 71 | 18 to G | 54,42,46,35,(33),27 |
| F 72 | 18 to C | 54,42,46,35,(33),28 |
| F 73 | 18 to T | 54,42,46,35,(33),29 |
| F 74 | 18 to 5 | 54,42,46,35,(33),30 |
| F 75 | 18 to 6 | 54,42,46,35,31 |
| F 76 | 18 to 7 | 54,42,46,35,32 |
| F 77 | 18 to 8 | 54,42,46,35,33 |
| F 78 | 18 to Tet | 54,42,35,(31,33),34 |
| F 79 | 18 to 10 | 54,42,35,(33),24 |
| F 80 | 18 to 11 | 54,42,35,(33),20 |
| F 81 | 18 to 12 | 54,42,35,(33),21 |
| F 82 | 18 to 11 + 12 | 54,42,35,(33),20,21 |
| F 83 | 18 to 14 | 54,42,35,(33),22 |
| F 84 | 18 to 15 | 54,42,35,(33),23 |
| F 85 | 18 to 14 + 15 | 54,42,35,(33),22,23 |
| F86 | 18 to Bases | 54,42,35,(33),46 |
| F170 | Dilute 'A' | 54,42,46,35,(33),26 |
| F171 | Dilute 'G' | 54,42,46,35,(33),27 |
| F172 | Dilute 'C' | 54,42,(33),46,35,28 |
| F173 | Dilute 'T' | 54,42,(33),46,35,29 |
| F174 | Dilute '5' | 54,42,(33),46,35,30 |
| F175 | Dilute '6' | 54,42,,46,35,31 |
| F176 | Dilute '7' | 54,42,,46,35,32 |
| F177 | Dilute '8' | 54,42,,46,35,33 |

## Functions that Deliver Argon to Reservoirs

These functions are used during the bottle change procedure for the phosphoramidite, tetrazole, and acetonitrile reservoirs. After acetonitrile removes old reagent from the delivery line, the acetonitrile is removed by an argon flush. Argon travels through the reagent valve blocks (by opening Valve 43 on eight-base instruments) and into the reservoir, forcing the acetonitrile into the bottle. The phosphoramidite bottles share a single-vent valve which is open during this step. In addition, functions that deliver argon to bottle positions 11, 12, 14, and 15 are used during the shut down and flow test procedures. Function 14, Flush to Bases, is used during the auto-dilution procedures initially to flush old reagent into the reservoirs and later to mix the phosphoramidites by bubbling.

The following functions deliver argon to the designated reservoir. For example, Function 6, Flush to A, delivers argon to the adenosine phosphoramidite reservoir.

*NOTE Functions 11-13 are not available or needed on five-position instruments since bottle positions six through eight do not exist. On instruments equipped with five base positions, the valve or valves listed in parentheses are used instead of the preceding underlined valve or valves.*

| Function Number | Name | Valve |
|---|---|---|
| F 1 | Block Flush | 40,35,25, 0, 3, 4, 7, 8*,11*,12*,15* |
| F2 | Reverse Flush | 25, 0, 16, 4,17, 8*,18*,12*,19* |
| F3 | Trityl Flush | 40.35,16, 2,17, 6,18*,10*,19*,14* |
| F4 | Flush to Waste | 40,35,16, 3,17,7,18*,11*,19*,15* |
| F5 | Flush to Collect | 40,35,16, 1,17, 5,18*, 9*,19*,13* |
| F 6 | Flush to A | 43,35,(32),46,26 |
| F 7 | Flush to G | 43,35,(32),46,27 |
| F 8 | Flush to C | 43,35,(32),46,28 |
| F 9 | Flush to T | 43,35,(32),46,29 |
| F 10 | Flush to 5 | 43,35,(32),46,30 |
| F 11 | Flush to 6 | 43,35,46,31 |
| F12 | Flush to 7 | 43,35,46,32 |
| F13 | Flush to 8 | 43,35,46,33 |
| F 14 | Flush to Bases | 43,35,(32),46 |
| F 15 | Flush to Tet | 40,35,34,(31) |
| F 16 | Flush to 10 | 40,35,24 |
| F 17 | Flush to 11 + 12 | 40,35,20,21 |
| F 18 | Flush to 14 + 15 | 40,35,22,23 |
| F19 | Flush to 18 | 43,42,(33,32) |

## Test Functions

Test functions are used to perform the flow test procedure. The procedure is used during instrument manufacturing, installation, and troubleshooting. If desired, test functions can be inserted into a synthesis cycle by using the Cycle Editor or into a procedure by using the Procedure Editor. The following functions deliver the contents of the designated bottle to the column.

*NOTE Functions 30-32 are not available or needed on five-position instruments since bottle positions six through eight do not exist. Valves 18,11,19,15 are only used on four-column instruments.*

| Function Number | Name | Valves |
|---|---|---|
| F 25 | A to Column | 45,26,16,3,17,7,18*,11*,19*,15* |
| F 26 | G to Column | 45,27,16,3,17,7,18*,11*,19*,15* |
| F 27 | C to Column | 45,28,16,3,17,7,18*,11*,19*,15* |
| F 28 | T to Column | 45,29,16,3,17,7,18*,11*,19*,15* |
| F 29 | 5 to Column | 45,30,16,3,17,7,18*,11*,19*,15* |
| F 30 | 6 to Column | 45,31,16,3,17,7,18,11,19,15 |
| F 31 | 7 to Column | 45,32,16,3,17,7,18,11,19,15 |
| F 32 | 8 to Column | 45,33,16,3,17,7,18,11,19,15 |
| F 37 | 11 to Column | 53,20,16,3,17,7,18*,11*,19*,15* |
| F 38 | 12 to Column | 53,21,16,3,17,7,18*,11*,19*,15* |
| F 40 | 14 to Column | 52,22,16,3,17,7,18*,11*,19*,15* |
| F 42 | 18 to Column | 54,42,35,(33),16,3,17,7,18*,11*,19*,15* |
| F112 | Trityl Advance | |
| F113 | End Advance | |

## Miscellaneous Functions

### Function 103 Wait

Function 103 is a pause used following a chemical delivery (such as base + tetrazole to column) to allow the reagents to remain in the column so the reaction can be completed.

### Function 105 Start Detrityl

This function signals the controller to perform the first set of chemical steps necessary for base addition (detritylation). Note that this does not mean the first numerical step of the cycle.

### Function 104 Interrupt

This function is used during the bottle change and shut down procedures. It provides a pause when reagent bottles are being removed and replaced.

# Synthesis Cycles

## General

A function performed for a specified time is called a step. During synthesis, steps are arranged in a particular order to create a synthesis cycle. The cycle completes all operations necessary for one base addition and is repeated until the oligonucleotide length is fully synthesized.

The 10 cycles provided with the 392 or 394 are optimized for the synthesis of β-cyanoethyl DNA and RNA, and phosphorothioate DNA. At the 0.2 µmol, 1.0 µmol, and 10 µmol scales for β-cyanoethyl DNA, a low reagent consumption scale, "Primer" is provided. The Primer cycle is used with 0.05 molar phosphoramidites at the 40 nanomole scale for oligos of any length or at the 0.2 µmol scale for oligos of 50 bases or less.

Although the 10 permanent cycles listed in Table 4-5 can not be changed or deleted, they can be copied into one of the 10 user cycle locations and edited with the Cycle Editor. You can also create entirely new cycles in any programmable user cycle location. All changes made with the Cycle Editor will be stored in memory, even if the power is turned off. There are an additional 12 cycles that are reserved for future use and can not be edited.

### Table 4-5
### Synthesis Cycles Provided by Applied Biosystems

| | |
|---|---|
| Primer | 0.2 µmol β-cyanoethyl DNA of 50 bases or less or 40 nm DNA of any length |
| .2 um CE | 0.2 µmol β-cyanoethyl DNA |
| 1.0 um CE | 1 µmol β-cyanoethyl DNA |
| 10 um CE | 10 µmol β-cyanoethyl DNA |
| .2 um RNA | 0.2 µmol RNA |
| 1.0 um RNA | 1 µmol RNA |
| 10 um RNA | 10 µmol RNA |
| .2 um Sulfur | 0.2 µmol phosphorothioate DNA |
| 1 um Sulfur | 1 µmol phosphorothioate DNA |
| 10 um Sulfur | 10 µmol phosphorothioate DNA |

## About Cycles

The 40 nanomole or 0.2 µmol scale provides sufficient quantities of oligonucleotide for most applications. At the 40 nanomole scale, synthesis of a 20-mer with the Primer cycle typically yields 5-10 odu (or 165-330 micrograms) of crude oligonucleotide. At the 0.2 µmol scale, synthesis of a 20-mer with the .2 um CE or Primer cycle typically yields approximately 20 odu (or 660 micrograms) of crude oligonucleotide (33 µg/odu). When larger quantities of DNA are needed, the 1 µmol or 10 µmol scale cycle can be used. With the 1 µmol cycle, synthesis of a 20-mer will yield about 100 odu (or 3.3 milligrams) of crude oligonucleotide (1 µmol crude yield, odu = length × 5). Synthesizing a 20-mer with the 10 µmol cycle will yield about 800 OD (or 26.4 milligrams) of crude DNA.

Using the primer cycle results in a 40% cost savings per base addition. These savings are achieved primarily by using lower-concentration (0.05 molar) phosphoramidites. Coupling efficiency is maintained at this lower concentration by oscillating the phosphoramidite and tetrazole up and down in the column during the coupling reaction. The Primer cycle should be used at the 0.2 µmol scale for synthesizing oligonucleotides of 50 bases or less or at the 40 nanomole scale for oligos of any length.

The steps in each cycle have optimized delivery times that ensure completion of each chemical reaction while minimizing reagent consumption and preventing unwanted side reactions. The cycle times are listed in Table 4-6.

### Table 4-6
### Model 392/394 Cycle Times (minutes)

| | 1 Column | 2 Columns | 3 Columns | 4 Columns |
|---|---|---|---|---|
| **DNA** | | | | |
| Primer | 4:46 | 5:10 | 5:37 | 6:02 |
| .2 um CE | 4:52 | 5:40 | 6:11 | 6:27 |
| 1 um CE | 5:08 | 5:33 | 6:24 | 6:50 |
| 10 um CE | 18:35 | 20:09 | 21:41 | 23:20 |
| **RNA** | | | | |
| .2 um RNA | 14:28 | 15:07 | 15:39 | 16:03 |
| 1 um RNA | 14:43 | 15:21 | 16:05 | 16:25 |
| 10 um RNA | 27:56 | 28:50 | 29:23 | 30:05 |
| **Phosphorothioate DNA** | | | | |
| .2 um Sulfur | 20:12 | 20:47 | 21:05 | 21:53 |
| 1 um Sulfur | 21:27 | 22:02 | 22:39 | 23:25 |
| 10 um Sulfur | 38:54 | 40:56 | 42:51 | 44:57 |

All permanent cycles are listed in Appendix I. In addition, the standard 0.2 µmol cycle is shown in Table 4-8. Each cycle consists of the required number of steps, listed with the corresponding function number, name, and step time. To determine the purpose of each step, refer to "Synthesis Cycle Functions," page 4–12 .

## More About the Standard 0.2 µmol Cycle

The four essential chemical reactions necessary for synthesis are listed in Table 4-7 with their corresponding cycle step numbers. The cycle step numbers shown are based on the 0.2 µmol CE cycle for a four-column instrument.

### Table 4-7
### Essential Chemical Reactions for Four-Column 0.2 µmol CE Cycles

| | | |
|---|---|---|
| 1. | Detritylation | Steps 64-93 |
| 2. | Coupling | Steps 1-45 |
| 3. | Capping | Step 46-54 |
| 4. | Oxidation | Steps 55-63 |

Although each reaction requires different treatment, the following generalizations can be made:

1) Prior to the chemical reaction:
   a. The valve blocks, the column, and the interconnecting delivery lines are rinsed with acetonitrile and flushed dry with argon; and
   b. The reagent reservoir(s) are prepared for delivery.
2) Performing the chemical reaction requires reagent delivery to the column, often followed by a Wait step to complete the reaction.

## Table 4-8
## Standard 0.2 µmol Cycle for a 4-Column Instrument

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 106 | Begin | | | | | | | | | | Yes |
| 2 | 64 | 18 to Waste | 3.0 | | | | | | | | | Yes |
| 3 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 2 | Reverse Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | | | | | | | | Yes |
| 7 | 140 | Column 1 On | | | | | | | | | | Yes |
| 8 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 10 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 11 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 12 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 13 | 43 | Push to Column | | | | | | | | | | Yes |
| 14 | 141 | Column 1 Off | | | | | | | | | | Yes |
| 15 | 142 | Column 2 On | | | | | | | | | | Yes |
| 16 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 17 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 18 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 19 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 20 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 22 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 23 | 43 | Push to Column | | | | | | | | | | Yes |
| 24 | 143 | Column 2 Off | | | | | | | | | | Yes |
| 25 | 144 | Column 3 On | | | | | | | | | | Yes |
| 26 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 27 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 28 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 29 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 30 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 31 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 33 | 43 | Push to Column | | | | | | | | | | Yes |
| 34 | 145 | Column 3 Off | | | | | | | | | | Yes |
| 35 | 146 | Column 4 On | | | | | | | | | | Yes |
| 36 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 37 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

## Table 4-8 (continued)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 38 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 40 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 41 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 43 | Push to Column | | | | | | | | | | Yes |
| 44 | 147 | Column 4 Off | | | | | | | | | | Yes |
| 45 | 103 | Wait | 25.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 102 | Cap Prep | 3.0 | | | | | | | | | Yes |
| 47 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 48 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 50 | 39 | Cap to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 51 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 53 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 54 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 55 | 41 | 15 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 56 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 57 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 58 | 103 | Wait | 15.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 59 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 60 | 4 | Flush to Waste | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 61 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 62 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 63 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 64 | 105 | Start Detrityl | | | | | | | | | | Yes |
| 65 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 66 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 67 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 68 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 69 | 112 | Trityl Advance | | | | | | | | | | Yes |
| 70 | 109 | Waste - Port | | | | | | | | | | Yes |
| 71 | 120 | Advance FC | | | | | | | | | | Yes |
| 72 | 120 | Advance FC | | | | | | | | | | Yes |
| 73 | 120 | Advance FC | | | | | | | | | | Yes |
| 74 | 120 | Advance FC | | | | | | | | | | Yes |
| 75 | 113 | End Advance | | | | | | | | | | Yes |
| 76 | 40 | 14 to Column | 6.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 77 | 3 | Trityl Flush | 5.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 78 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 79 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 80 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |

## Table 4-8 (continued)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 81 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 82 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 83 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 84 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 85 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 86 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 87 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 88 | 3 | Trityl Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 89 | 110 | Waste - Bottle | | | | | | | | | | Yes |
| 90 | 42 | 18 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 91 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 92 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 93 | 107 | End | | | | | | | | | | Yes |

# Procedures

## The Begin Procedure

The begin procedure (ABI Begin) is a phosphoramidite purge procedure that fills all phosphoramidite and tetrazole delivery lines (from the reservoir to the reagent valve block) with fresh reagent. This process consumes approximately one-fifth the amount of phosphoramidite used in a coupling step of a 1-micromole synthesis. The begin procedure is used prior to beginning a synthesis when the instrument has been idle for more than 12 hours (more than 6 hours in humid environments) or if one of the phosphoramidite reservoirs has not been accessed within 12 hours. Over this time, oxygen and atmospheric water can penetrate the delivery lines and may cause failures in the first coupling. A phosphoramidite purge eliminates this problem and helps ensure a successful synthesis.

***IMPORTANT*** *Not performing a begin procedure when it is needed will cause a synthesis to fail. When in doubt, it is best to purge since it may save a synthesis.*

Before beginning a synthesis, you must choose whether to perform the procedure. Refer to "Main Menu Option: Start Synthesis" in Section 3 for further information. Note that a phosphoramidite purge is not included in the bottle cycle counter that tabulates the alarm.

As shown in Table 4-9, the begin procedure consists of fourteen steps. If you want a procedure with different steps, copy ABI Begin into "Beg01 user," the location intended for user editing and change steps through the Procedure Editor menu. A third begin procedure location, Beg02 Mac, cannot be edited and is intended for future use.

# Table 4-9
# The Begin Procedure

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 103 | Begin | | | Yes |
| 2 | 101 | Phos Prep | 10.0 | Pressurizes the phosphoramidite and tetrazole reservoirs prior to delivery. | Yes |
| 3 | 50 | A to Waste | 2.0 | Steps 3-11 fill the delivery lines with fresh reagent by delivering the phosphoramidites and tetrazole to waste. | Yes |
| 4 | 51 | G to Waste | 2.0 | | Yes |
| 5 | 52 | C to Waste | 2.0 | | Yes |
| 6 | 53 | T to Waste | 2.0 | | Yes |
| 7 | 54 | 5 to Waste | 2.0 | | Yes |
| 8 | 55 | 6 to Waste | 2.0 | | Yes |
| 9 | 56 | 7 to Waste | 2.0 | | Yes |
| 10 | 57 | 8 to Waste | 2.0 | | Yes |
| 11 | 58 | Tet to Waste | 2.0 | | Yes |
| 12 | 64 | 18 to Waste | 10.0 | Removes the phosphoramidite and tetrazole left in the reagent valve block. Rinses the valve block. | Yes |
| 13 | 1 | Block Flush | 10.0 | Removes acetonitrile from the reagent valve blocks. | Yes |
| 14 | 107 | End | | | Yes |

## The End Procedure

An end procedure is used to automatically cleave the oligonucleotide from the synthesis support at the end of a synthesis. The synthesizer uses ammonium hydroxide to cleave the oligo, delivering the oligo into the collection vial along with the ammonia. Table 4-11 lists which end procedure to use with supplied cycles and the volumes of $NH_4OH$ delivered to the collection vial. See Appendix I for listings of other standard end procedures.

### Table 4-10
### End Procedure "End CE"

| Step Numb. | Function # | Function Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 2 | Reverse Flush | 60.0 | Flushes columns and column blocks in reverse. | Yes |
| 3 | 115 | Prep 10 | 15.0 | Prepares ammonia for delivery | Yes |
| 4 | 36 | 10 to Collect | 11.0 | Fills column with ammonium hydroxide. | Yes |
| 5 | 64 | 18 to Waste | 5.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 6 | 1 | Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 7 | 103 | Wait | 900.0 | Ammonium hydroxide cleaves oligo from support. | Yes |
| 8 | 36 | 10 to Collect | 11.0 | Ammon. hydrox. delivery to col. & collection vial. | Yes |
| 9 | 64 | 18 to Waste | 5.0 | 2nd rinse with acetonitrile. | Yes |
| 10 | 1 | Block Flush | 5.0 | 2nd block flush. | Yes |
| 11 | 103 | Wait | 900.0 | Ammonium hydroxide cleaves oligo from support. | Yes |
| 12 | 36 | 10 to Collect | 9.0 | 3rd delivery of ammonium hydroxide. | Yes |
| 13 | 64 | 18 to Waste | 5.0 | 3rd rinse with acetonitrile | Yes |
| 14 | 1 | Block Flush | 5.0 | 3rd block flush. | Yes |
| 15 | 103 | Wait | 900.0 | Cleavage of oligo from support. | Yes |
| 16 | 36 | 10 to Collect | 11.0 | 4th delivery of ammonium hydroxide. | Yes |
| 17 | 64 | 18 to Waste | 5.0 | 4th rinse with acetonitrile. | Yes |
| 18 | 1 | Block Flush | 5.0 | 4th block flush. | Yes |
| 19 | 103 | Wait | 900.0 | Cleavage of oligo from support. | Yes |
| 20 | 5 | Flush to Collect | 9.0 | Flushs ammonium hydroxide into collect. vial. | Yes |
| 21 | 36 | 10 to Collect | 9.0 | 5th delivery of ammonium hydroxide. | Yes |
| 22 | 5 | Flush to Collect | 9.0 | 2nd flush to collect. | Yes |
| 23 | 2 | Reverse Flush | 60.0 | 2nd reverse flush. | Yes |
| 24 | 1 | Block Flush | 4.0 | 5th block flush. | Yes |
| 25 | 64 | 18 to Waste | 5.0 | 5th rinse with acetonitrile | Yes |
| 26 | 42 | 18 to Column | 30.0 | Washes column with acetonitrile | Yes |
| 27 | 2 | Reverse Flush | 60.0 | 3rd reverse flush | Yes |
| 28 | 1 | Block Flush | 10.0 | 6th block flush | Yes |
| 29 | 100 | 10 Vent | 30.0 | Vents ammonia bottle | Yes |
| 30 | 107 | End | | | Yes |

## Table 4-11
## Use of Each End Procedure Supplied

| EndProcedure | Volume of $NH_4OH$ Delivered to Collection Vial | Used with These Cycles |
|---|---|---|
| End CE | ~ 2.0 ml | *Primer, .2 um CE, 1 um CE, .2 um Sulfur, 1 um Sulfur |
| End CESS | ~ 1.0 ml | *Primer, .2 um CE, .2 um Sulfur |
| End CE10 | ~ 4.0 ml | 10 um CE, 10 um Sulfur |
| End RNA | ~ 2.0 ml | .2 um RNA, 1 um RNA |
| End RNA 10 | ~ 4.0 ml | 10 um RNA |

*Either the End CE or End CESS procedure may be used with Primer, .2 um CE, and .2 um Sulfur, depending upon the volume of $NH_4OH$ desired for delivery to the collection vial.

## Bottle Change Procedures

Bottle change procedures are used to remove empty reservoirs and replace them with bottles of fresh reagents. They can be performed before beginning a synthesis or when a synthesis is interrupted either manually or by the alarm. The bottle change procedure prevents oxygen and water contamination of atmosphere-sensitive phosphoramidites by rinsing and flushing the system out during the procedure. Especially important is the argon flush to the head space of the newly installed bottle to remove water and oxygen introduced by opening the bottle.

Two types of bottle change procedures are available for each of the 15 bottles, an ABI-supplied procedure and a user-specified procedure. The ABI procedure for a particular bottle is named "Botxx ABI," where "xx" specifies the bottle to be changed. A user-supplied procedure only exists if one has been created through the Procedure Editor. The name for a particular user bottle change procedure is "Botxx user."

### Phosphoramidite Change Procedures

The change procedures for phosphoramidites are identical except for the functions delivering reagents to or from particular phosphoramidite bottles and the functions flushing delivery lines to a particular phosphoramidite position. The phosphoramidite bottle change procedure

listed in Table 4-12 is for bottle position 1 (A phosphoramidite) and is representative of bottle change procedures for other positions. See Appendix I for complete listings of bottle changes.

## Table 4-12
## Bot 1 ABI Change Procedures

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 3 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 4 | 70 | 18 to A | 3.0 | Washes old reagent into reservoir. | Yes |
| 5 | 6 | Flush to A | 10.0 | Flushes acetonitrile into reservoir. | Yes |
| 6 | 104 | Interrupt | | User installs new bottle. | Yes |
| 7 | 6 | Flush to A | 5.0 | Headspace purged with argon. | Yes |
| 8 | 50 | A to Waste | 2.0 | Line filled with fresh reagent. | Yes |
| 9 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks. | Yes |
| 10 | 1 | Block Flush | 5.0 | Flushes column and reagent blocks. | Yes |
| 11 | 107 | End | | | Yes |

### Auto Dilution Procedures

Three auto-dilution procedures are provided with the synthesizer to automatically dilute powdered β-cyanoethyl and RNA phosphoramidites in 0.25 gram, 0.5 gram and 1.0 gram bottles. The procedure listed in Table 4-13 is used for auto-dilution of 1.0 gram bottles. Also, user auto-dilution procedures may be created. (For more information, see "Creating a User Auto Dilution Procedure," in Section 3.)

*IMPORTANT* *If you are going to remove the phosphoramidites and save them for later use, do not initiate this procedure. Otherwise, back-flushed phosphoramidite and excess acetonitrile will contaminate the bottles. Refer to Appendix VI for instructions on how to store dissolved phosphoramidites.*

# Table 4-13
## Phosphoramidite Auto Dilute Procedure
## (1.0 gram)

| Step Number | Function # | Function Name | Step Time | Safe Step |
|---|---|---|---|---|
| 1 | 106 | Begin | | Yes |
| 2 | 64 | 18 to Waste | 5.0 | Yes |
| 3 | 1 | Block Flush | 4.0 | Yes |
| 4 | 14 | Flush to Bases | 10.0 | Yes |
| 5 | 86 | 18 to Bases | 5.0 | Yes |
| 6 | 104 | Interrupt | | Yes |
| 7 | 170 | Dilute 'A' | | Yes |
| 8 | 170 | Dilute 'A' | | Yes |
| 9 | 170 | Dilute 'A' | | Yes |
| 10 | 170 | Dilute 'A' | | Yes |
| 11 | 171 | Dilute 'G' | | Yes |
| 12 | 171 | Dilute 'G' | | Yes |
| 13 | 171 | Dilute 'G' | | Yes |
| 14 | 171 | Dilute 'G' | | Yes |
| 15 | 172 | Dilute 'C' | | Yes |
| 16 | 172 | Dilute 'C' | | Yes |
| 17 | 172 | Dilute 'C' | | Yes |
| 18 | 172 | Dilute 'C' | | Yes |
| 19 | 173 | Dilute 'T' | | Yes |
| 20 | 173 | Dilute 'T' | | Yes |
| 21 | 173 | Dilute 'T' | | Yes |
| 22 | 173 | Dilute 'T' | | Yes |
| 23 | 174 | Dilute '5' | | Yes |
| 24 | 174 | Dilute '5' | | Yes |
| 25 | 174 | Dilute '5' | | Yes |
| 26 | 174 | Dilute '5' | | Yes |
| 27 | 175 | Dilute '6' | | Yes |
| 28 | 175 | Dilute '6' | | Yes |
| 29 | 175 | Dilute '6' | | Yes |
| 30 | 175 | Dilute '6' | | Yes |
| 31 | 176 | Dilute '7' | | Yes |
| 32 | 176 | Dilute '7' | | Yes |
| 33 | 176 | Dilute '7' | | Yes |
| 34 | 176 | Dilute '7' | | Yes |
| 35 | 177 | Dilute '8' | | Yes |
| 36 | 177 | Dilute '8' | | Yes |
| 37 | 177 | Dilute '8' | | Yes |
| 38 | 177 | Dilute '8' | | Yes |
| 39 | 1 | Block Flush | 4.0 | Yes |
| 40 | 14 | Flush to Bases | 120.0 | Yes |
| 41 | 114 | Vent Bases | 3.0 | Yes |
| 42 | 101 | Phos Prep | 5.0 | Yes |
| 43 | 150 | Bottle01 On | | Yes |
| 44 | 50 | A to Waste | 2.0 | Yes |

## Table 4-13 (continued)

| Step Number | Function # | Function Name | Step Time | Safe Step |
|---|---|---|---|---|
| 45 | 151 | Bottle01 Off | | Yes |
| 46 | 152 | Bottle02 On | | Yes |
| 47 | 51 | G to Waste | 2.0 | Yes |
| 48 | 153 | Bottle02 Off | | Yes |
| 49 | 154 | Bottle03 On | | Yes |
| 50 | 52 | C to Waste | 2.0 | Yes |
| 51 | 155 | Bottle03 Off | | Yes |
| 52 | 156 | Bottle04 On | | Yes |
| 53 | 53 | T to Waste | 2.0 | Yes |
| 54 | 157 | Bottle04 Off | | Yes |
| 55 | 158 | Bottle05 On | | Yes |
| 56 | 54 | 5 to Waste | 2.0 | Yes |
| 57 | 159 | Bottle05 Off | | Yes |
| 58 | 160 | Bottle06 On | | Yes |
| 59 | 55 | 6 to Waste | 2.0 | Yes |
| 60 | 161 | Bottle06 Off | | Yes |
| 61 | 162 | Bottle07 On | | Yes |
| 62 | 56 | 7 to Waste | 2.0 | Yes |
| 63 | 163 | Bottle07 Off | | Yes |
| 64 | 164 | Bottle08 On | | Yes |
| 65 | 57 | 8 to Waste | 2.0 | Yes |
| 66 | 165 | Bottle08 Off | | Yes |
| 67 | 64 | 18 to Waste | 7.0 | Yes |
| 68 | 1 | Block Flush | 5.0 | Yes |
| 69 | 107 | End | | Yes |

## The Shut Down Procedure

The Shut Down procedure prepares the instrument for long-term storage (storage for periods of one month or longer). It removes all reagents in the delivery lines and washes and dries all chemical pathways. To perform this procedure, select Shut Down from the Main Menu and follow the instructions in Section 3.

### Table 4-14
### The Shut Down Procedure

| Step Number | Function # | Function Name | Step Time | Safe Step |
|---|---|---|---|---|
| 1 | 106 | Begin | | Yes |
| 2 | 1 | Block Flush | 15.0 | Yes |
| 3 | 64 | 18 to Waste | 15.0 | Yes |
| 4 | 1 | Block Flush | 15.0 | Yes |
| 5 | 42 | 18 to Column | 30.0 | Yes |
| 6 | 1 | Block Flush | 30.00 | Yes |
| 7 | 5 | Flush to Colect | 15.0 | Yes |
| 8 | 109 | Waste - Port | | Yes |
| 9 | 42 | 18 to Column | 30.0 | Yes |
| 10 | 3 | Trityl Flush | 30.0 | Yes |
| 11 | 110 | Waste - Bottle | | Yes |
| 12 | 42 | 18 to Column | 60.0 | Yes |
| 13 | 2 | Reverse Flush | 60.0 | Yes |
| 14 | 42 | 18 to Column | 60.0 | Yes |
| 15 | 1 | Block Flush | 60.00 | Yes |
| 16 | 2 | Reverse Flush | 60.0 | Yes |
| 17 | 70 | 18 to A | 10.0 | Yes |
| 18 | 71 | 18 to G | 10.0 | Yes |
| 19 | 72 | 18 to C | 10.0 | Yes |
| 20 | 73 | 18 to T | 10.0 | Yes |
| 21 | 74 | 18 to 5 | 10.0 | Yes |
| 22 | 75 | 18 to 6 | 10.0 | Yes |
| 23 | 76 | 18 to 7 | 10.0 | Yes |
| 24 | 77 | 18 to 8 | 10.0 | Yes |
| 25 | 78 | 18 to Tet | 10.0 | Yes |
| 26 | 79 | 18 to 10 | 10.0 | Yes |
| 27 | 82 | 18 to 11 + 12 | 20.0 | Yes |
| 28 | 85 | 18 to 14 + 15 | 20.0 | Yes |
| 29 | 6 | Flush to A | 60.0 | Yes |
| 30 | 7 | Flush to G | 60.0 | Yes |
| 31 | 8 | Flush to C | 60.0 | Yes |
| 32 | 9 | Flush to T | 60.0 | Yes |
| 33 | 10 | Flush to 5 | 60.0 | Yes |
| 34 | 11 | Flush to 6 | 60.0 | Yes |
| 35 | 12 | Flush to 7 | 60.0 | Yes |
| 36 | 13 | Flush to 8 | 60.0 | Yes |
| 37 | 15 | Flush to Tet | 60.0 | Yes |

## Table 4-14 (continued)

| Step Number | Function # | Function Name | Step Time | Safe Step |
|---|---|---|---|---|
| 38 | 16 | Flush to 10 | 60.0 | Yes |
| 39 | 17 | Flush to 11+12 | 60.0 | Yes |
| 40 | 18 | Flush to 14+15 | 60.0 | Yes |
| 41 | 1 | Block Flush | 15.0 | Yes |
| 42 | 107 | End | | |

## Flow Test Procedures

Flow test procedures are used to measure flow rates through all essential delivery lines. They can be used for routine maintenance, troubleshooting, and recalibration of the instrument if you suspect flow-related problems.

To perform a flow test procedure, select *Self Test* on page 3 of the Main Menu. Then, press the *Next Page* soft key until the *Flow Test* soft key is presented. Further information appears under "Main Menu Option: Self Test," in Section 3, and in Appendix III. Table 4-15 contains a list of flow-rate specifications.

## Table 4-15
## 392/394 Flow Rate Specifications

| Bottle | Grams/30 sec $CH_3CN$ or Reagent | ml/min $CH_3CN$ | ml/min Reagent |
|---|---|---|---|
| 1–8 (amidite) | 1.18 – 1.22 | 3.00 – 3.10 | 2.94 – 3.04 |
| 9 (tetrazole) | 1.18 – 1.22 | 3.00 – 3.10 | 2.95 – 3.04 |
| 10 (ammonium hydroxide) | 1.95 – 2.10 | 4.96 – 5.34 | – |
| 11 (acetic anhydride) | 1.30 – 1.46 | 3.31 – 3.72 | 2.87 – 3.22 |
| 12 (NMI) | 1.30 – 1.46 | 3.31 – 3.72 | 2.89 – 3.25 |
| 14 (TCA) | 1.35 – 1.44 | 3.44 – 3.66 | 2.04 – 3.17 |
| 15 (iodine) | 1.30 – 1.44 | 3.31 – 3.66 | 2.85 – 3.15 |
| 18 (acetonitrile) | 1.30 – 1.44 | 3.31 – 3.66 | 3.31 – 3.66 |

The next table lists a sampling of the available flow tests and gives the purpose of each.

## Table 4-16
## Flow Tests

| Name of Test | Purpose |
|---|---|
| Flw to Col | Used to measure the flow rates of bottles 1-8, 11, 12, 14, 15, and 18 to column 1. |
| Set Reg1 | Used to set regulator 1, which pressurizes the phosphoramidite and tetrazole bottles (1-9). |
| Set Reg2 | Used to set regulator 2, which pressurizes bottles 11-18. |
| Flow to A* | Measures the flow rate of acetonitrile to A (bottle #1). The results are used in the "Set Dilute Flow" menu. |

Table 4-17 lists all the functions in the flow tests described in Table 4-16.

## Table 4-17
## Flow Test Procedures

| Step Numb. | Function # | Name | Step Time |
|---|---|---|---|
| **Flw to Col** | | | |
| 1 | 106 | Begin | |
| 2 | 101 | Phos Prep | 5 |
| 3 | 140 | Column 1 On | |
| 4 | 26 | G to Column | 25 |
| 5 | 104 | Interrupt | |
| 6 | 26 | G to Column | 30 |
| 7 | 104 | Interrupt | |
| 8 | 27 | C to Column | 25 |
| 9 | 104 | Interrupt | |
| 10 | 27 | C to Column | 30 |
| 11 | 104 | Interrupt | |
| 12 | 28 | T to Column | 25 |
| 13 | 104 | Interrupt | |
| 14 | 28 | T to Column | 30 |
| 15 | 104 | Interrupt | |

| Step Numb. | Function # | Name | Step Time |
|---|---|---|---|
| **Set Reg 1** | | | |
| 1 | 106 | Begin | |
| 2 | 101 | Phos Prep | 5 |
| 3 | 140 | Column 1 On | |
| 4 | 25 | A to Column | 25 |
| 5 | 104 | Interrupt | |
| 6 | 25 | A to Column | 30 |
| 7 | 141 | Column 1 Off | |
| 8 | 107 | End | |
| **Set Reg 2** | | | |
| 1 | 106 | Begin | |
| 2 | 140 | Column 1 On | |
| 3 | 40 | 14 to Column | 25 |
| 4 | 104 | Interrupt | |
| 5 | 40 | 14 to Column | 30 |

* Flow tests for flow to G, C, T, 5, etc. are also available.

# Table 4-17 (continued)

| Step Numb. | Function # | Name | Step Time |
|---|---|---|---|
| **Flw to Col (continued)** | | | |
| 16 | 29 | 5 to Column | 25 |
| 17 | 104 | Interrupt | |
| 18 | 29 | 5 to Column | 30 |
| 19 | 104 | Interrupt | |
| 20 | 30 | 6 to Column | 25 |
| 21 | 104 | Interrupt | |
| 22 | 30 | 6 to Column | 30 |
| 24 | 31 | 7 to Column | 25 |
| 25 | 104 | Interrupt | |
| 26 | 31 | 7 to Column | 30 |
| 27 | 104 | Interrupt | |
| 28 | 32 | 8 to Column | 25 |
| 29 | 104 | Interrupt | |
| 30 | 32 | 8 to Column | 30 |
| 31 | 104 | Interrupt | |
| 32 | 34 | Tet to Column | 60 |
| 33 | 104 | Interrupt | |
| 34 | 34 | Tet to Column | 30 |
| 35 | 104 | Interrupt | |
| 36 | 37 | 11 to Column | 25 |
| 37 | 104 | Interrupt | |
| 38 | 37 | 11 to Column | 30 |
| 39 | 104 | Interrupt | |
| 40 | 38 | 12 to Column | 25 |
| 41 | 104 | Interrupt | |
| 42 | 38 | 12 to Column | 30 |
| 43 | 104 | Interrupt | |
| 44 | 41 | 15 to Column | 25 |
| 45 | 104 | Interrupt | |
| 46 | 41 | 15 to Column | 30 |
| 47 | 104 | Interrupt | |
| 48 | 42 | 18 to Column | 60 |
| 49 | 104 | Interrupt | |
| 50 | 42 | 18 to Column | 30 |
| 51 | 141 | Column 1 Off | |

| Step Numb. | Function # | Name | Step Time |
|---|---|---|---|
| **Set Reg 2 (continued)** | | | |
| 6 | 141 | Column 1 Off | |
| 7 | 104 | Interrupt | |
| 8 | 142 | Column 2 On | |
| 9 | 40 | 14 to Column | 25 |
| 10 | 104 | Interrupt | |
| 11 | 40 | 14 to Column | 30 |
| 12 | 143 | Column 2 Off | |
| 13 | 104 | Interrupt | |
| 14 | 144 | Column 3 On | |
| 15 | 40 | 14 to Column | 25 |
| 16 | 104 | Interrupt | |
| 17 | 40 | 14 to Column | 30 |
| 18 | 145 | Column 3 Off | |
| 19 | 104 | Interrupt | |
| 20 | 146 | Column 4 On | |
| 21 | 40 | 14 to Column | 25 |
| 22 | 104 | Interrupt | |
| 23 | 40 | 14 to Column | 30 |
| 24 | 147 | Column 4 Off | |
| 25 | 107 | End | |
| **Set Reg3** | | | |
| 1 | 106 | Begin | |
| 2 | 140 | Column 1 On | |
| 3 | 36 | 10 to Collect | 25 |
| 4 | 104 | Interrupt | |
| 5 | 36 | 10 to Collect | 30 |
| 6 | 141 | Column 1 Off | |
| 7 | 107 | End | |
| **Flow to A** | | | |
| 1 | 106 | Begin | |
| 2 | 64 | 18 to Waste | 5 |
| 3 | 1 | Block Flush | 4 |
| 4 | 70 | 18 to A | 5 |
| 5 | 104 | Interrupt | |
| 6 | 70 | 18 to A | 30 |
| 7 | 1 | Block Flush | 4 |
| 6 | 6 | Flush to A | 10 |
| 9 | 107 | End | |

# 5

# System Description - Hardware

Section 5, System Description - Hardware, describes the components of the synthesizer and explains the chemical delivery system.


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# Introduction

The major synthesizer components are shown in Figure 5-1. (This figure illustrates a Model 392 synthesizer; see Appendix II for additional schematics of all the 392 and 394 models.) All chemical steps required for DNA synthesis take place within a reaction chamber, called the *column*. The column contains the 3'-terminal nucleoside, which is covalently attached to a *support*. The DNA chain is built by adding one base at a time to the support-bound nucleoside.

All reagents and solvents necessary for synthesis are accessed by the *chemical delivery system*. In this system, a set of solenoid valves opens to create a pathway for chemical flow. Pressure-regulated argon forces the chemicals to flow from their reservoirs through the pathway (consisting of one or more valve blocks and delivery lines) and then to the column. After completing a chemical step, the column effluent flows to the *waste bottle*, to the *trityl collection port* to collect the dimethoxytrityl cation for analysis, or to the *DNA collection vial*.

The *controller* directs and initiates all synthesizer activity and consists of a microprocessor, the software, a keyboard, a display screen, and associated electronics. Information is displayed on a four-line screen, and you give instructions by pressing the appropriate key on the keyboard.

# The Chemical Delivery System

The flow of reagents, solvents, and gas through the Model 392/394 is controlled by a positive-pressure chemical delivery system. The system components include three regulated pressure sources, twelve or fifteen reagent and solvent reservoirs (depending upon whether your instrument has five or eight base positions), delivery valves, two or four columns, a vented waste bottle, and delivery lines that interconnect the components (see Figure 5-1). All inner surfaces of the chemical delivery system are made of inert materials. The plumbing diagram shown in Figure 5-1 is simplified and does not show the valves used to pressurize and vent reagent reservoirs.

## Pressure

System pressure is provided by pre-purified (99.998) argon. Argon's high density and low oxygen contamination make it preferable to nitrogen. An argon cylinder is connected to the inlet port at the right rear of the Model 392/394 using 1/4-inch oxygen-impermeable tubing and a gas-tight connector supplied by Applied Biosystems. The pressure regulator on the tank is set to 60 psi. This high-pressure argon is used to operate the vacuum-assist system (described later in this section) and to supply argon to the regulators in the synthesizer.

An empty argon cylinder can be replaced before beginning a synthesis or when an active synthesis is interrupted. Refer to "Changing an Argon Tank" in Section 2 for replacement instructions.

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***WARNING*** ***Compressed gas cylinders must be safely attached to the wall or bench by means of approved brackets or chains. FAILURE TO DO SO COULD CAUSE THE CYLINDERS TO FALL OVER, WHICH COULD RESULT IN PHYSICAL HARM. The cylinder should always be capped when not in use. Never transport a cylinder with a regulator attached.***

---

Argon entering the synthesizer travels through a 10-micron particle filter to three pressure regulators. The regulators deliver argon to specific pressure valves used to pressurize the reagent and solvent reservoirs. (Pressure valves are further discussed under "Valves," in Section 4, and are shown in Figure 4-1.) In addition, the regulators supply argon to the columns and reagent valve blocks.

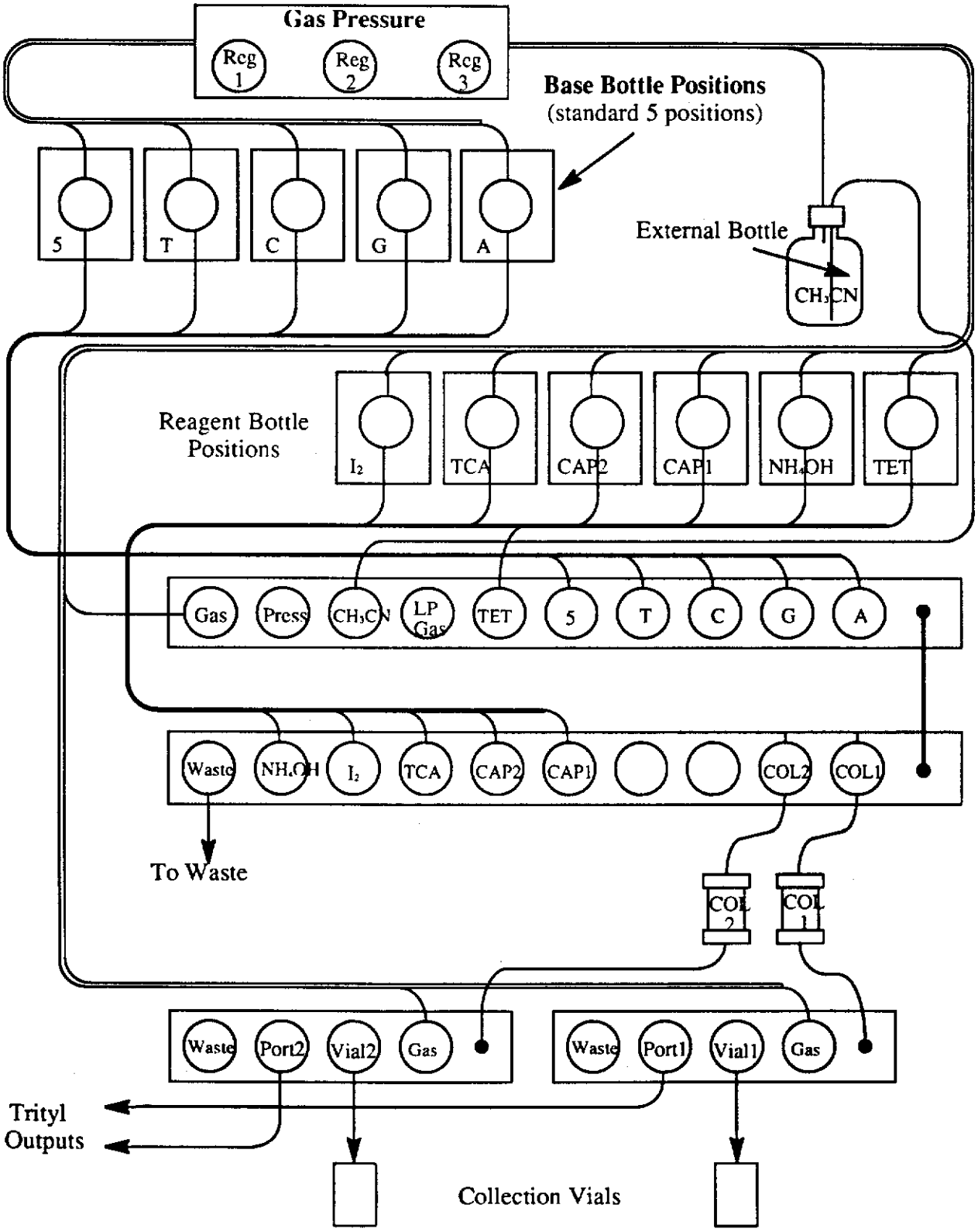


Figure 5-1. Major components of the Model 392

The phosphoramidites have one pressure valve, which supplies argon to a manifold pressurizing all five or eight phosphoramidite bottles simultaneously. For proper pressurization, bottles must be attached to *all positions*, even if some are empty. The two capping reagents—1-methylimidazole (NMI) and acetic anhydride—share a pressure valve that channels the argon to a tee to pressurize both bottles. All other reagent reservoirs—tetrazole, ammonium hydroxide, trichloroacetic acid (TCA), iodine, and acetonitrile—are pressurized by a single valve for each bottle.

## Reagent and Solvent Reservoirs

Each reservoir has a unique position on the instrument and is referred to by the number located above each receptacle. The position or reservoir numbers are 1-8 (1-5 on five-base instruments), 9, 10, 11, 12, 14, 15, and 18. Position numbers are also printed on the bottle labels. These numbers correspond to those on previous Applied Biosystems DNA synthesizers, which used all 18 positions.

Bottles 1-8 are pushed upward around a Teflon insert containing an O-ring, which forms an airtight seal inside each bottle neck. Pressing the appropriate black button above the bottle position releases the grip on the bottle and allows for its removal and subsequent replacement. When you replace a bottle, the button will return to its *out* position only when the reservoir is correctly engaged.

Bottles 9, 10, 11, 12, 14 and 15 screw snugly into a threaded cap mounted on the synthesizer. A disposable polyethylene insert forms an airtight seal between each cap and bottle.

Reservoir 18, containing acetonitrile, is a 4-liter (or sometimes a 2.5-liter) bottle that does not attach directly to the instrument. Instead, it is placed inside a protective carrier on the left side of the instrument. A cap assembly, which includes the delivery and gas lines, screws onto the bottle and connects to the synthesizer. The cap has a teflon insert and a rubber gasket. Applied Biosystems provides the cap assembly and the carrier.

### Pressure and Delivery Lines

Each bottle has an argon pressure line and a delivery line entering through the cap insert. For the phosphoramidites, Bottles 1-8, the pressure line also functions as a vent line. As shown in Figure 5-2, the argon line remains above the liquid level while the delivery line extends to the bottom of the bottle. When the correct set of valves opens, the reservoir headspace is pressurized by argon and the liquid is pushed into the delivery line and flows to its destination.

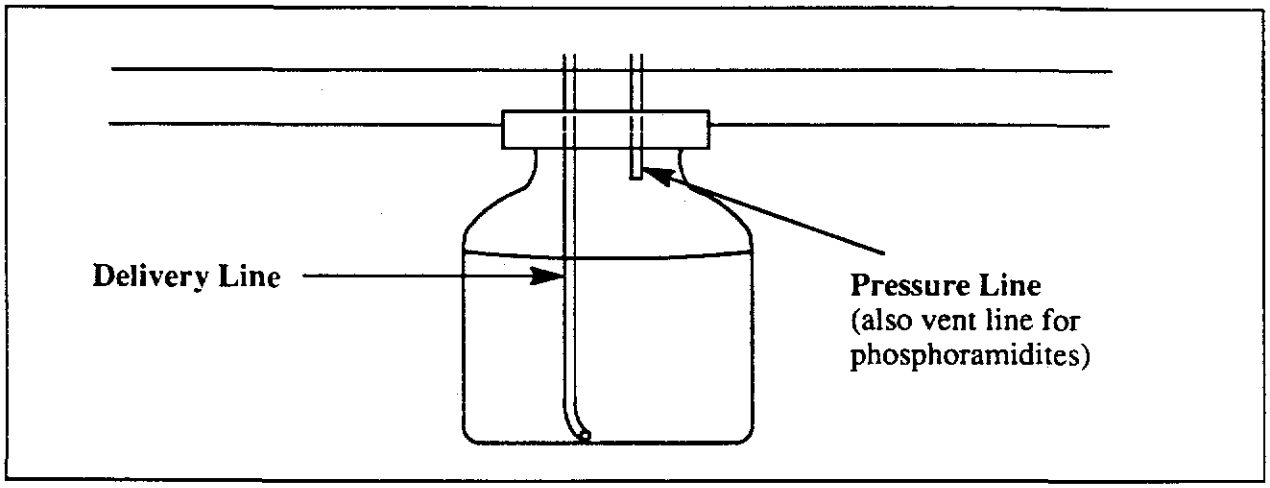


Figure 5-2. Reagent reservoir

Bottles should be be placed on all phosphoramidite and reservoir positions to maintain argon pressure and to keep the lines clean. Notice that most lines are color coded. Red lines are 0.5 mm ID. Blue lines are 0.8 mm ID. Yellow lines are 0.3 mm ID.

## Venting of Phosphoramidite Bottles

In addition to being pressurized, the phosphoramidites are vented using the pressure/vent line. When fresh phosphoramidites, which are atmosphere-sensitive, are placed on the instrument, they are purged with argon to eliminate air. A purge delivers gas through the delivery line. As the gas is passed through the bottle, the air escapes out the pressure/vent line. This is performed automatically by the bottle change procedure.

***IMPORTANT*** *The waste bottle is the low-pressure side of the delivery system and must always be kept vented to atmosphere. Be sure the vent line is properly routed to a fume hood. If the vent line is blocked, back pressure will be generated and inhibit the delivery of reagents and solvents. To verify proper ventilation requirements, see the ventilation drawing in the Model 392 and 394 DNA/RNA Synthesizer Pre-Installation Manual, included in Appendix V.*

## Delivery Valve Blocks

The Model 392/394 chemical delivery system includes two reagent valve blocks (three for eight-base instruments) and either two or four column valve blocks. The valve blocks control gas and chemical flows to the columns and exit ports.

The basic Model 392 has two 11-port reagent valve blocks and two 5-port column valve blocks. The standard Model 394 has four 5-port column valve blocks as well as an additional reagent valve block to support the standard eight base positions. Figure 5-3 is a schematic representation of all valve blocks in 2-column, 5-base instruments.

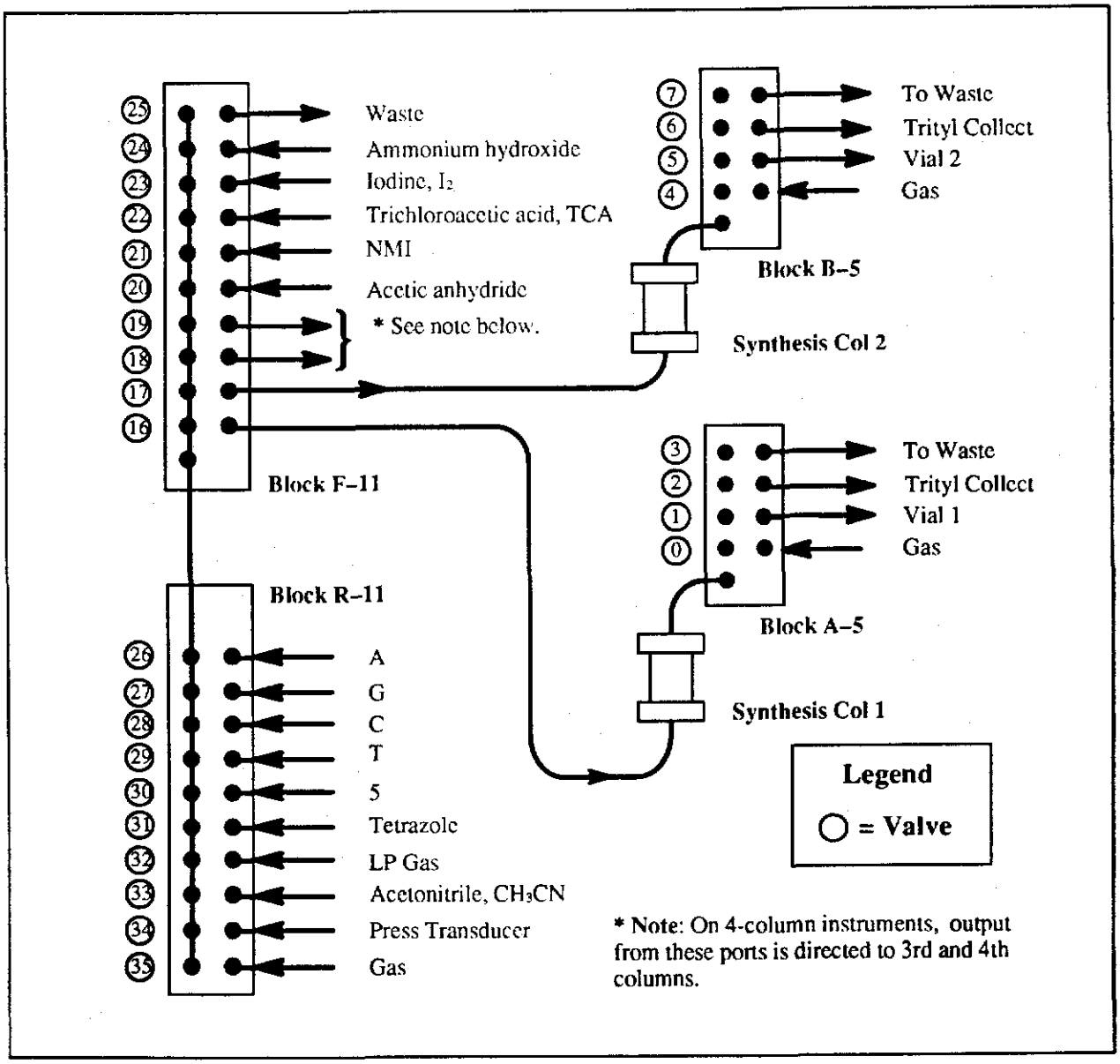


Figure 5-3. Valve blocks schematic

The two 11-port valve blocks (and the additional 5-port valve block on 8-base instruments) control the flow from the bottles to the bottom of the columns and to waste. For more detailed information than presented in Figure 5-3, see the plumbing diagrams for all versions of the synthesizer in Appendix II.

Except for the phosphoramidites and tetrazole, reagent and solvent deliveries are made to all of the active columns simultaneously. Delivery times to each column are automatically adjusted for the slight flow rate change when more columns are active.

Each 5-port column valve block directs the column effluent to the waste port, the trityl collection port, or the DNA collection vial. It also controls the argon gas used to remove or flush the reagents from the column and the column valve block.

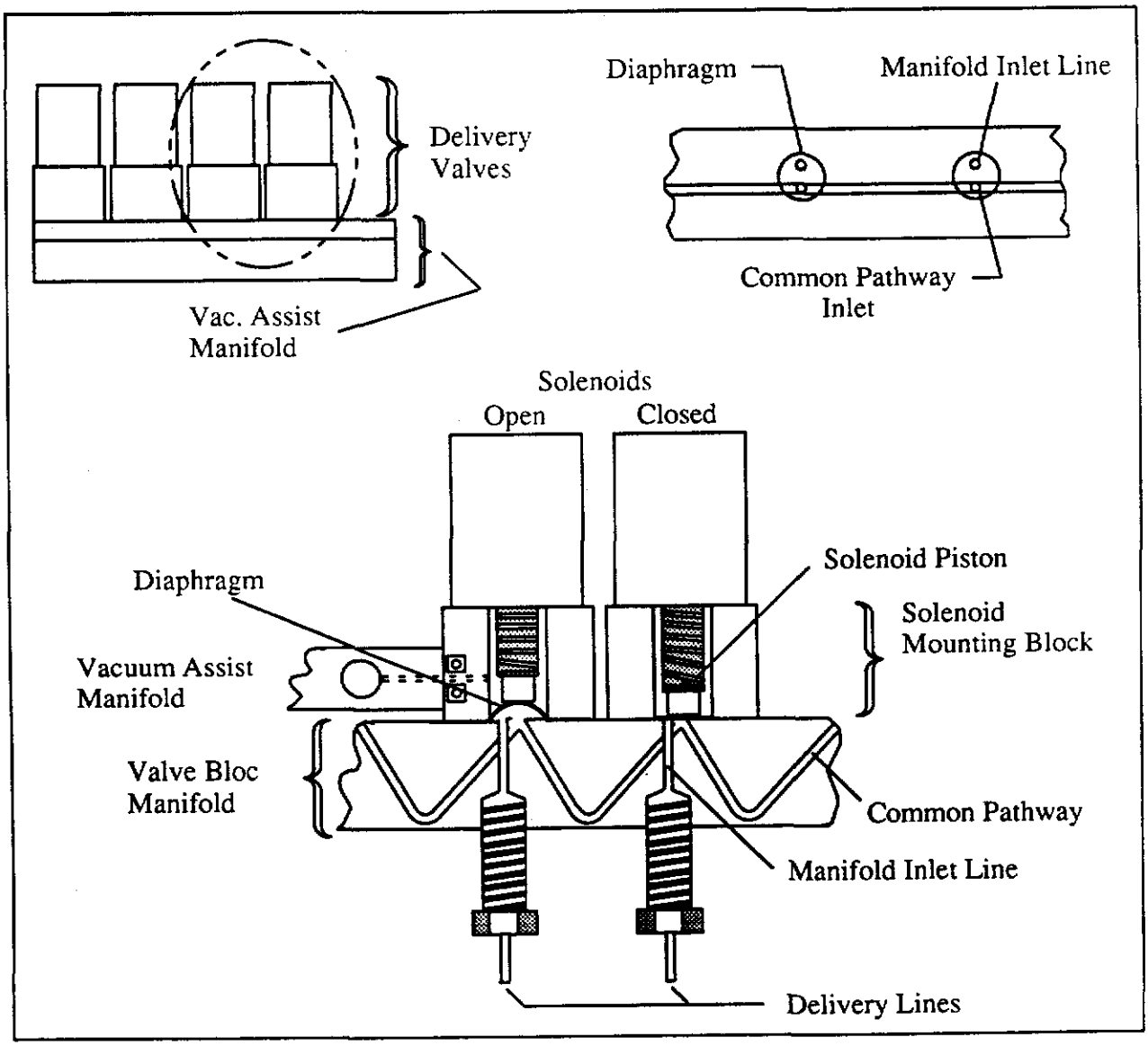


Figure 5-4. The valve block

The design of the valve blocks provides *zero dead-volume* when the solenoids are in the closed position. Delivery lines feed into each valve block and connect to the common pathway in the valve block manifold via a manifold inlet line and a solenoid-controlled diaphragm valve. See Figure 5-4.

Passage between the manifold inlet line and the common pathway of the valve block is accomplished by an open solenoid valve. When a valve opens, the solenoid piston pulls away from a diaphragm located under the piston. With vacuum assist, an open solenoid will cause the diaphragm to form a 2 µL domed chamber. The domed chamber creates a passageway between the inlet line and the common pathway. The common pathway zig-zags through the valve block manifold and passes other closed valves, which are unaffected by the flow. The direction of flow is determined by the pressures on either side of the valve block.

## Vacuum-Assist

For proper valve block operation, it is crucial that the diaphragms form a domed chamber. The vacuum assist creates a vacuum on the solenoid side of the diaphragm to create a domed chamber each time a solenoid valve opens. An aspirator pump provides vacuum assist to each valve block. The aspirator pump requires that regulated argon enter the synthesizer at a gauge pressure of approximately 60 psi.

***IMPORTANT*** *During installation, use 1/4-inch tubing. Do not use 1/8-inch tubing. It is too narrow to deliver the correct flow rate of gas.*

The vacuum gauge should always read above 13 in. Hg. When the gauge drops below 14 in. Hg (once every 2-8 hours), a valve opens to allow high-pressure argon to flow to the aspirator pump. The gas is then diverted out of the pump and creates a Venturi vacuum when a second valve opens to the vacuum-assist plumbing. A pressure drop to below 14 in. Hg more than once every 2 hours indicates a leak in the system. Contact the Applied Biosystems Technical Support Department for repair instructions.

## The Column

The initial support-bound nucleoside is contained in a disposable column that, besides the column body, has two retaining frits and two end fittings (Figure 5-5). All parts are made of inert materials. The retaining frits are porous polyethylene held in place by the end caps. The inlet and outlet are female luer fittings designed to mate with the male luer fittings on the instrument. The column is symmetrical (i.e., no top or bottom, no front or back) and can be attached to the male luer fittings in any way. Each column is color coded to show the initial nucleoside and has a unique serial number.

| Column | Color code |
|---|---|
| A | green |
| G | yellow |
| C | red |
| T | blue |

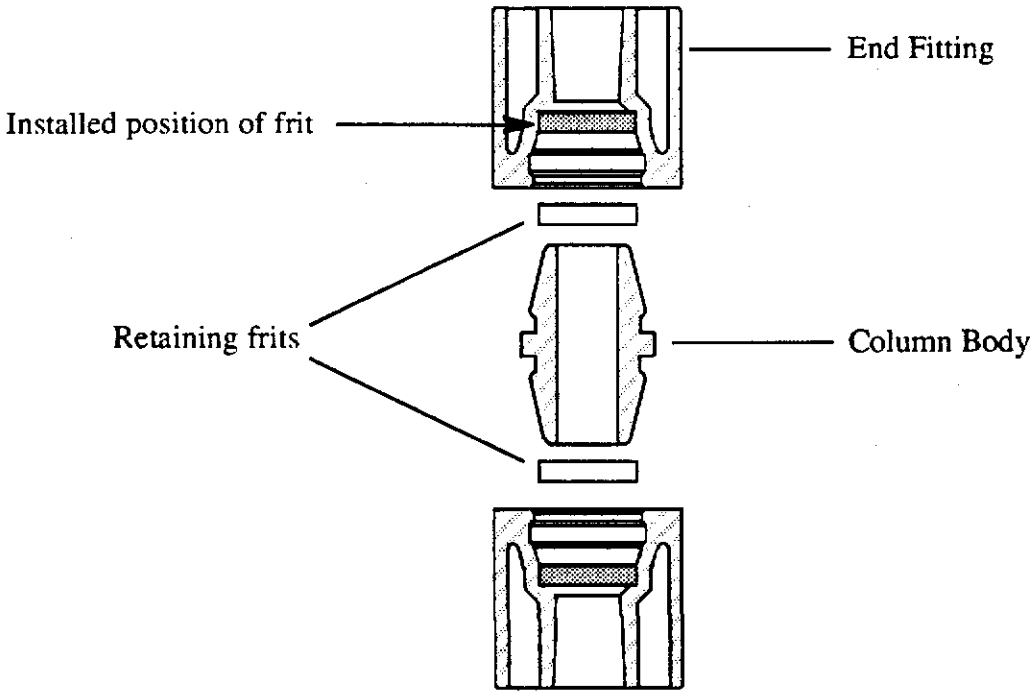


Figure 5-5. The column

The normal flow into the column is from the bottom. By sending the liquid stream upward, the CPG particles are lifted and maintained in a fluidized state. The flow rates of the solvents and reagents have been set to achieve proper mixing of the particles.

## Flow Restrictors

Reagents flow to the column through the lower luer. If you unscrew one of the lower luers, you will find a cylindrical glass flow restrictor. Reagents flow through a very small channel in the flow restrictor to the column. Small amounts of reagents can crystallize in the flow restrictor and, over time, may block the flow. This problem may be magnified if non-ABI reagents are used.

To prevent clogging, the restrictors should be cleaned once a month. To clean, boil them in water for 15 minutes and then sonicate in methanol. Sufficient matched sets of restrictors should be kept on hand to allow necessary cleaning of each set.

## Waste and Venting

The ultimate destination of most chemical deliveries is the waste bottle. The bottle is a free-standing, 10-liter polyethylene container that should be placed on the floor near the synthesizer or on a nearby bench *lower* than the instrument.

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***WARNING*** ***Synthesizer waste must be handled and disposed of properly and carefully. Depending on the synthesis cycle used, the instrument generates 1 to 2 liters of hazardous, halogenated organic liquid waste per 100 cycles of operation. When handling the waste for disposal, wear gloves, a lab coat, and eye protection, and avoid inhalation and skin contact. Place the liquid in a sealed container labeled "WASTE FLAMMABLE NOS (HALOGENATED)." All waste must be disposed of as a regulated hazardous waste according to the applicable federal, state, and local government regulations.***

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The waste line and vent line enter the cap of the waste bottle. Be sure this cap is always securely tightened. The waste line carries liquid waste from the synthesizer to the bottle. Be sure the line slopes downward toward the bottle and has no troughs that can collect waste and block the line.

The vent line carries gaseous waste to a suitable exhaust such as a fume hood. Applied Biosystems supplies a 25-foot length of tubing to connect the vent line to a fume hood. Proper venting allows operation of the Model 392/394 on an open lab bench.

***IMPORTANT*** *Prevent condensation from collecting in the vent line by continuously sloping the tubing upward toward the fume hood.*

*The waste bottle is the low-pressure side of the delivery system and must always be kept vented to atmosphere. Be sure the vent line is properly routed to a fume hood. If the vent line is blocked, back pressure will be generated. This will inhibit the delivery of reagents and solvents. See the ventilation drawing in the Model 392 and 394 DNA/RNA Synthesizer Pre-Installation Manual, included in Appendix V, to verify proper ventilation requirements.*

# The Battery

A lithium battery is provided in the Model 392/394. It should operate for several years. When the main power is turned off (either intentionally or during a power failure), all synthesis parameters are retained. These parameters include the stored DNA sequences, user-defined cycles, procedures and functions, and bottle usage information. If a power failure occurs during an active synthesis, the synthesis will be interrupted and can only be resumed when the main power returns.

The battery also maintains the synthesizer's internal clock. If a power outage does occur, the clock records the time the power failed and the time it was restored. A synthesis will then automatically resume if the elapsed time of the failure is less than the time you entered in the Power Fail menu. For futher instructions, refer to "Main Menu Option: Power Fail" in Section 3.

***WARNING*** ***The used lithium battery must be disposed of properly. The content of the battery is hazardous. Dispose of the battery in accordance with all local, state, and federal environmental and health regulations. For further information, consult the Applied Biosystems' Technical Support Department.***

# The Controller

The controller directs and initiates all synthesizer activity. Its major components are the software, the microprocessor, the display screen, the keyboard, and associated electronics.

The software defines all necessary operations for synthesis and is interpreted and executed by the microprocessor. The software is contained within a removable memory card, which plugs into the rear of the instrument. Updated software supplied by Applied Biosystems can be easily installed by removing the old card and replacing it with the new one.

***IMPORTANT*** *Before removing the memory card, always be sure the main power is off.*

Synthesis information is shown on a four-line liquid crystal display (LCD). The display can be adjusted for optimal viewing (brightness/viewing angle) by turning the knob at the left of the screen.

You interact with the instrument by selecting keys on the keyboard. The keyboard consists of several labeled keys; **0-9**; **A, G, C, T**; ◄— —► (left and right cursor keys); **Main Menu**; **Prev. Menu**; **Prev.**; **Next**; **Insert**; **Delete**; and four *soft keys* (unlabeled keys to the right of the display). The labels and actions of the soft keys change depending on the information displayed to the left of them.

The software is "menu-driven," which means that menus and pages of menus present various options and necessary information about the synthesis or status of the instrument. In response, you select an option and give instructions by pressing the appropriate key. For a complete description of all menus, refer to Section 3.

To perform automated synthesis, the software uses a cycle consisting of a series of steps which complete all chemical reactions. For further information about cycles, see "Main Menu Option: Edit Cycle," in Section 3, and "Synthesis Cycles," in Section 4.

Proper operation of the controller's electronic components are verified by a system self test. For further instructions, refer to "Main Menu Option: Self Test," in Section 3.

***IMPORTANT*** *A fan on the rear of the instrument distributes air through the electronics for cooling and should not be obstructed.*

# Fuses

The Model 392/394 has two power fuses. They are located at the rear of the instrument to the left of the fan and directly above the power line. Although the fuses have a very long life, they can stop operating. For example, if the 392/394 has no power (i.e., the fan is not working or the LCD screen is blank), a bad fuse may be causing the problem and will need replacing. Before concluding there is a bad fuse, however, make sure that the instrument is properly connected to a functional power source. For further information call the Applied Biosystems Technical Support Department.

# 6

# *Chemistry for Automated DNA/RNA Synthesis*

Section 6 explains the DNA and RNA synthesis chemistry. In addition, it contains an overview of oligonucleotide analysis and purification procedures, a description of alternative chemistries and 5' attachments, and information about quantifying and storing oligonucleotides.


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# Introduction

DNA synthesis is quite simple in concept. A reactive 3' phosphorous group of one nucleoside is coupled to the 5' hydroxyl of another nucleoside. The former is a monomer, delivered in solution. The latter is immobilized on a solid support. An internucleotide linkage is thus formed. Three other chemical reactions are necessary to prepare the growing chain of DNA for the next coupling. In this way a synthesis cycle is conducted, adding one nucleoside monomer at a time. The desired sequence and length are defined by the operator on the synthesizer.[1] When the chain is complete, the crude DNA (*oligonucleotide*) must be *cleaved* from the support and *deprotected*. If the instructions in this manual are followed, the synthesis of oligonucleotides is a reliable process and their biological activity is assured. This chapter will help you understand the synthesis chemistry and how you can attain these goals.

The *phosphoramidite* method of oligonucleotide synthesis is the chemistry of choice for most laboratories because of efficient and rapid coupling and the stability of the starting materials.[2] The synthesis is performed with the growing DNA chain attached to a solid support so that excess reagents, which are in the liquid phase, can be removed by filtration.[3] Therefore, no purification steps are required between cycles. This support material is a form of silica, controlled-pore-glass (*CPG*) beads.[4] The particle size and the pore size have been optimized for liquid transfer and mechanical strength. The synthesis cycle is depicted in Figure 6-1. The starting material is the solid support derivatized with the nucleoside, which will become the 3'-hydroxyl end of the oligonucleotide. As shown in Figure 6-2, the nucleoside is bound to the solid support through a *linker* attached at the 3'-hydroxyl. The 5'-hydroxyl is blocked with a *dimethoxytrityl* (DMT) group.

The first step of the synthesis cycle is treatment of the derivatized solid support with acid to remove the DMT group (Figure 6-4). This frees the 5'-hydroxyl for the coupling reaction. (Figure 6-6). An activated intermediate is created by simultaneously adding the phosphoramidite nucleoside monomer and tetrazole, a weak acid, to the reaction column. The tetrazole protonates the nitrogen of the phosphoramidite, making it susceptible to nucleophilic attack. This intermediate is so reactive that addition is complete within 30 seconds. As shown in Figure 6-6, the phosphoramidite is blocked at the 5'-OH with the dimethoxytrityl group.

The next step, capping, terminates any chains that did not undergo addition. Since the unreacted chains have a free 5'-OH, they can be terminated or capped by acetylation. These unreacted chains are also called "failure products." Capping is done with acetic anhydride and 1-methylimidazole.[5] Since the chains that reacted with the phosphoramidite in the previous step are still blocked with the dimethoxytrityl group, they are not affected by this step. Although capping is not ultimately required for DNA synthesis, it is highly recommended because it minimizes the length of the impurities and thus facilitates their separation from the final product (Figure 6-7).

Finally, the internucleotide linkage is converted from the phosphite to the more stable phosphotriester. Iodine is used as the oxidizing agent and water as the oxygen donor. This reaction is complete in less than 30 seconds (Figure 6-8).

After oxidation, the dimethoxytrityl group is removed with a protic acid, either trichloroacetic or dichloroacetic acid. The cycle is repeated until chain elongation is complete. At this point, the oligonucleotide is still bound to the support with protecting groups on the phosphates and the exocyclic amines of the bases A, G, and C. (T has no exocyclic amines.) The oligonucleotide is cleaved from the support by a one-hour treatment with concentrated ammonium hydroxide. Ammonia treatment also removes the cyanoethyl phosphate protecting groups.

The protecting groups on the exocyclic amines of the bases are removed by treating the crude DNA solution in ammonium hydroxide at 55° C (Figure 6-9). If standard phosphoramidites are used, deprotection requires at least 8 hours at 55° C. If FOD phosphoramidites are used, deprotection is complete in 1 hour at 55° C or 8 hours at room temperature.

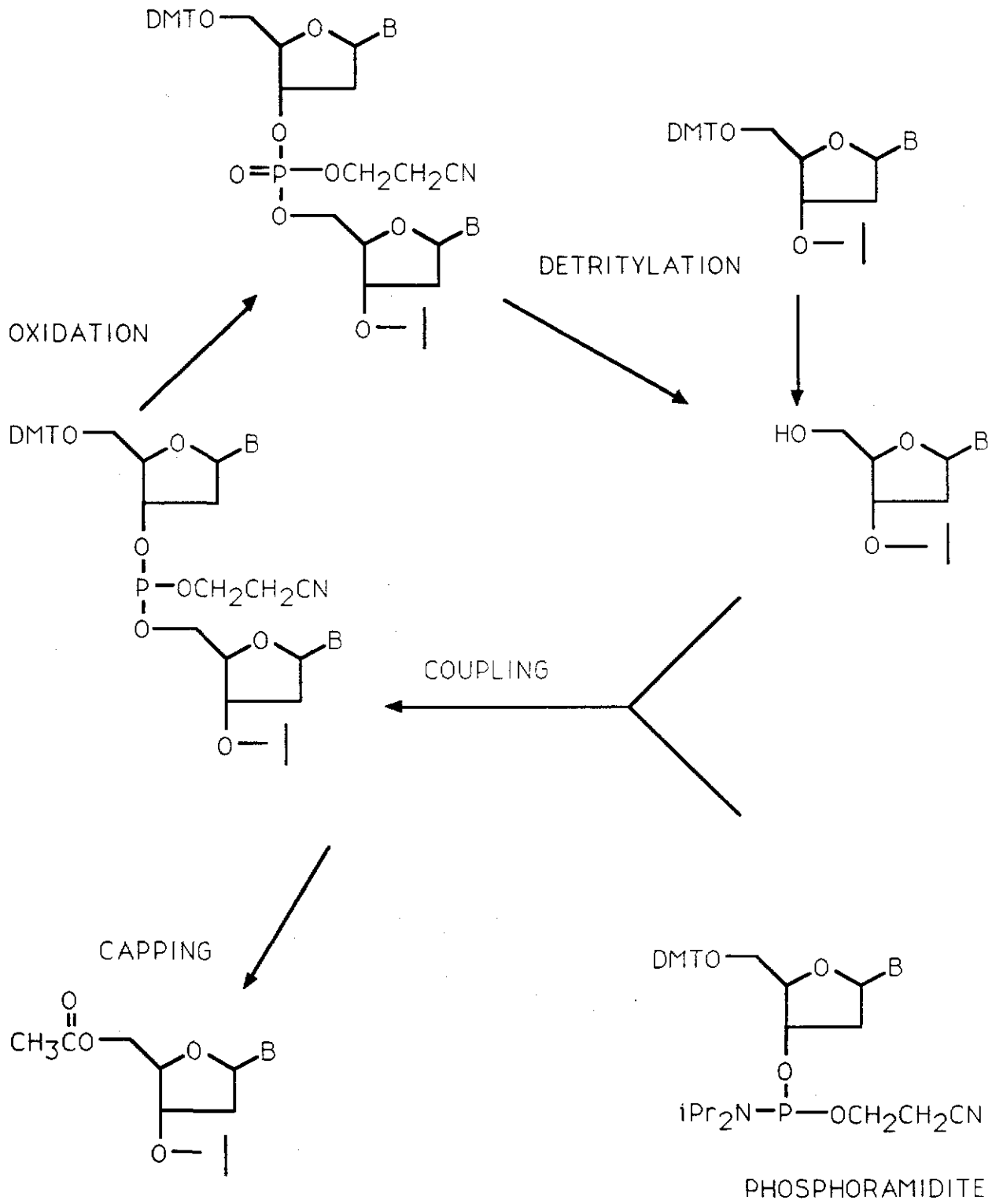



Figure 6-1. The Synthesis Cycle

# The Solid Support - CPG

The Model 392 and 394 DNA Synthesizers use a solid-phase synthesis chemistry in which the growing DNA chain remains covalently attached to an insoluble support. All reagents and solvents flow through the support, which is contained within a synthesis column.

The support used for DNA synthesis is controlled-pore glass (CPG)[4] and polystyrene. When used with the Model 392 and 394 and other Applied Biosystems reagents, the supports produce coupling efficiencies of about 98%, as measured by the trityl cation assay. This enables the synthesis of oligomers up to 175 bases in length.[6] CPG is a porous, nonswelling particle, which is about 150 microns in diameter and has 500Å pores. A wide-pore CPG support (1000Å) is also available and should be used for the synthesis of oligonucleotides greater than 60 bases.[6] Polystyrene[7] is a hydrophobic support of porous, nonswelling particles that are 50-70 microns in diameter and have 1000Å pores. The supports are covalently derivatized with one of the four nucleosides, A, G, C, or T. (See Figure 6-2 for an example of CPG derivatization.) The reactive groups on these nucleosides are blocked or protected to prevent unwanted side reactions. They are all blocked at the 5'-hydroxyl with a dimethoxytrityl group. The exocyclic amines on adenosine (A), cytidine (C), and guanosine (G) are also bound by protecting groups. The protecting groups differ for standard and fast oligonucleotide deprotection (FOD) phosphoramidites. Standard amidites use a benzoyl group to protect adenosine and cytidine ($A^{bz}$, $C^{bz}$) and an isobutyryl group to protect guanosine ($G^{ibu}$). FOD phosphoramidites use a dimethylformamidine group to protect adenosine and guanosine ($A^{dmf}$, $G^{dmf}$) and an isobutyryl group to protect cytidine ($C^{ibu}$). All of these structures are shown in Figure 6-3 (a) and (b). Thymidine does not need a protecting group because the thymine ring has no exocyclic amines.

As shown in Figure 6-2, CPG has a linker attached to its surface via a siloxane bond. All free silanol groups are capped to prevent side reactions. Polystyrene has an aminomethyl linker attached to its surface. Because the surface of polystyrene is inert, side reactions occur less frequently. The supports are derivatized by covalently attaching the 3'-hydroxyl of the nucleoside to the linker via a succinate ester bond, which is base-labile and allows for removal of the DNA from the support with ammonia. After synthesis is complete, the oligonucleotide is quantitatively cleaved, leaving a free 3'-hydroxyl.

The loading of nucleoside as measured by DMT release is typically 27-40 µmol/gram (8-12 µmol/gram for 40 nanomole columns). Prefilled columns contain 40 nanomole (polystyrene only), 0.2 µmol, 1 µmol, or 10 µmol of initial nucleoside. The Primer synthesis cycle is used in conjunction with 40 nanomole columns (for oligos of any length) or 0.2 µmol columns (for oligos less than 50 bases). Because this cycle uses 0.05 molar phosphoramidites, it offers the most economical production of oligonucleotides. The 40 nanomole or 0.2 µmol scales provide sufficient amounts of purified oligomers for most applications. The 1 µmol scale is used when greater quantities of DNA are needed. The 10 µmol scale is useful for physical studies such as

x-ray crystallography, NMR, or anti-sense applications. Wide-pore (1000Å) CPG supports are only available at the 0.2-µmol scale. The loading of nucleoside is lower, about 15 µmol/gram of 1000Å CPG support. For the synthesis of long oligonucleotides ("bigmers") on CPG, it has been shown that a lower loading of nucleoside and larger pore size are critical for success. Polystyrene is also useful for the synthesis of long oligonucleotides.

Typical quantities of oligonucleotide obtained from the different synthesis scales are given below:

| Synthesis Cycle | Crude Yield*(O.D.) (20mer) |
|---|---|
| Primer** | 20-25 at 0.2 µmol scale<br>5-10 at 40 nanomole scale |
| .2 um | 20-25 |
| 1 um | 100-120 |
| 10 um | 800-1000 |

* Yield figures based on a 20-mer sequence. Absorbance measured at 260 nm. Assuming 33 micrograms/odu.

** "Primer" is a low reagent-consumption cycle used at the 0.2 µmol and 40 nanomole scales.

With automated synthesis, the DNA is built from 3' to 5' (but entered on the synthesizer 5' to 3'). Before a synthesis can be started, one of four support-bound nucleosides (A, C, G or T) contained within a column must be placed on the instrument. This nucleoside will be the 3'-terminus of the sequence.

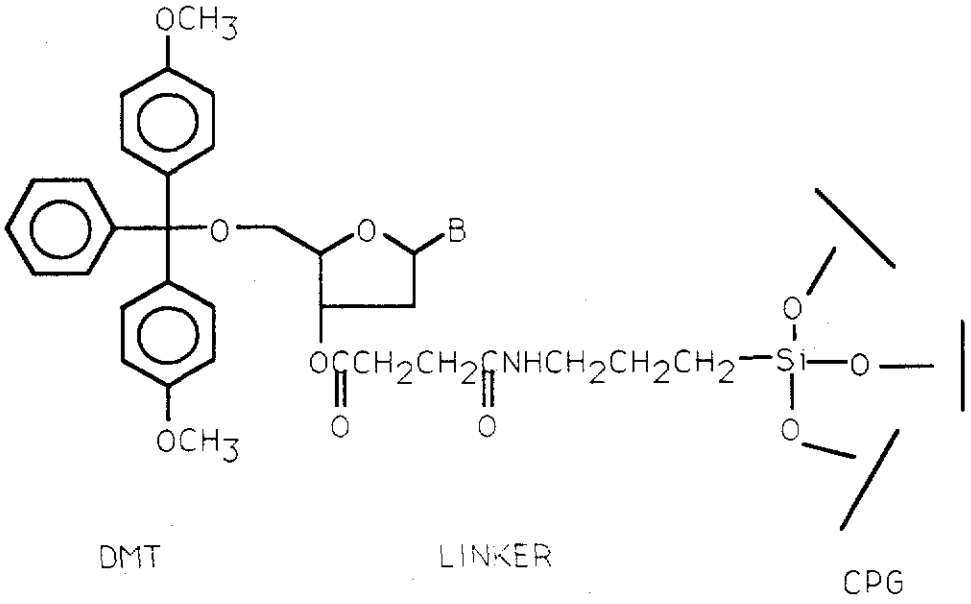
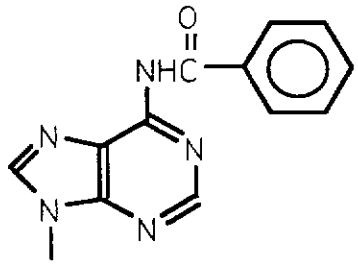
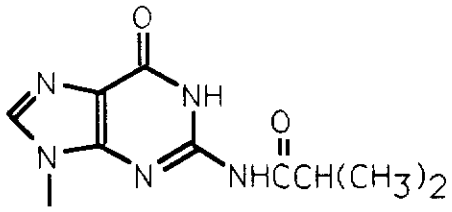



Figure 6-2. DMT-protected nuceoside

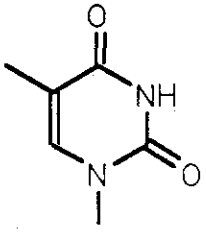
*NOTE The DMT-protected nucleoside is attached to the controlled-pore glass (CPG ) support (B = Base, A,G,C,T).*



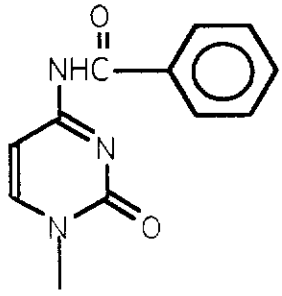

Adenosine-benzoyl protected




Guanosine-isobutyryl protected



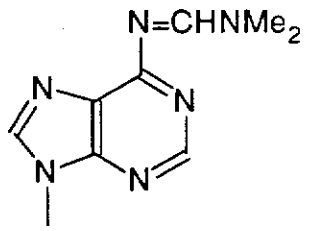

Thymidine



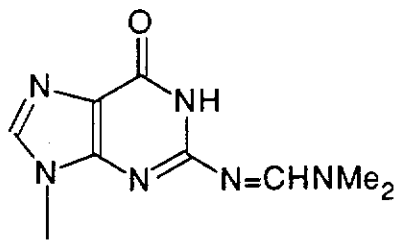

Cytidine - benzoyl protected

Figure 6-3 (a). Protected exocyclic base amines for standard phosphoramidites

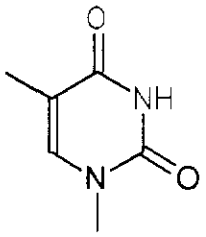
*NOTE Adenosine (A) and cytidine (C) are protected by a benzoyl group (bz), and guanosine (G) by an isobutyryl group (ib). Thymidine (T) does not need a protecting group.*



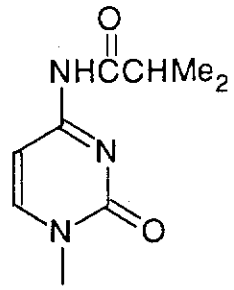

Adenosine - dimethylformamidine




Guanosine - dimethylformamidine




Thymidine




Cytidine - isobutyryl

Figure 6–3 (b). Protected exocyclic base amines for FOD phosphoramidites

*NOTE* *Adenosine (A) and guanosine (G) are protected by a dimethylformamidine group (dmf), and cytidine (C) by an isobutyryl group (ib). Thymidine (T) does not need a protecting group.*

# DNA Synthesis Chemistry Cycle

Each cycle of base addition consists of four steps:

1) Detritylation
2) Coupling
3) Capping
4) Oxidation

These reaction steps are repeated in the above order until all bases are added. Following synthesis, the DNA chain must be cleaved from the solid support and deprotected. Each step will be discussed in detail.

## Detritylation

The first step in oligonucleotide synthesis is to remove the acid-labile, dimethoxytrityl (DMT) protecting group on the 5'-hydroxyl of the support-bound nucleoside. As shown in Figure 6-4, treatment with a protic acid, TCA, will deprotect or detritylate the 5'end. This reaction will yield a reactive 5' hydroxyl, which can couple with a phosphoramidite during the following addition step.

Immediately before detritylation, the support is washed with acetonitrile to eliminate traces of the preceding reagent. Next, TCA (Bottle 14) is delivered to the column to cleave the trityl group. Detritylation under anhydrous conditions is a reversible reaction. The DMT cation is highly reactive and can re-tritylate any reactive nucleophile. Detritylation is driven to completion by the removal of the DMT cation from the synthesis column. The DMT cation is eluted by several TCA deliveries. Each is followed by a *trityl flush* in which argon passes through the column from bottom to top and pushes the liquid to the trityl collect port. Any residual TCA must be removed by an acetonitrile wash to prevent detritylation of the incoming phosphoramidite.

Following both acetonitrile washes, the remaining solvent is forced out of the column by the *trityl flush* function.

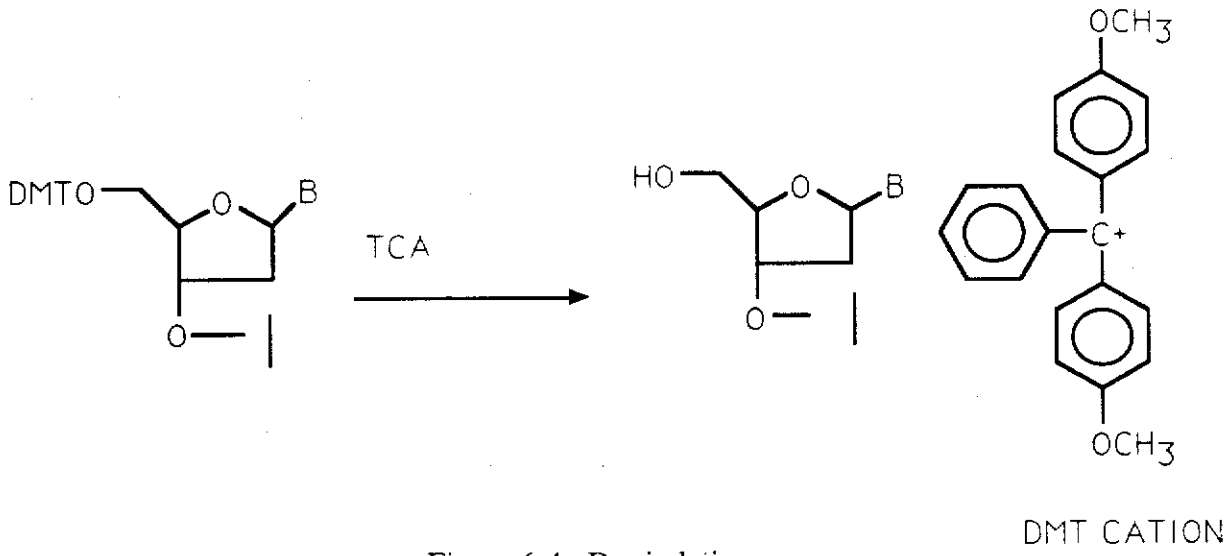



Figure 6-4. Detritylation.

*NOTE TCA is used to remove the DMT from the 5' end. This leaves a 5' hydroxyl to react with the incoming phosphoramidite in the coupling step. Quantifying the released trityl cation gives a rough indication of the step-wise yield and can be used to monitor the instrument's performance.*

## Monitoring the Trityl Cation

When the dimethoxytrityl protecting group (also referred to as DMT or trityl) is cleaved from the nucleotide, it exists as a cation (Figure 6-4). When in acid solution, this cation is relatively stable and produces a brilliant orange color. It has an absorbance maximum at about 498 nm and extinction coefficient of about 70,000 in solvents, such as acetonitrile or dichloromethane. It is easily detected and, when diluted, can be quantified spectrophotometrically.

To quantify the trityl cation released at each detritylation step, the column effluent from the TCA delivery and the subsequent wash with acetonitrile is collected. Instead of the effluent flowing into the waste bottle, it is channeled through the *trityl collection port* into tubes in a fraction collector. The Model 392/394 cycles provide for the collection of trityl effluent fractions and include a signal to advance a fraction collector.

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***WARNING Trityl material and waste must be handled with care and disposed of properly in accordance with all applicable federal, state, and local regulations for regulated hazardous waste. See the MSDSs and the waste profile in the "Safety Data" appendix for more information.***

---

Next, the absorbance of the fractions is measured to quantify the trityl released in each addition cycle. Since the trityl solution is very concentrated, it must be diluted before quantita-

tion or significant errors in the readings will occur. Typically, the trityl fraction is brought up to a volume of 10 ml with 0.1 M *p*-toluenesulfonic acid in acetonitrile. The trityl yield is then used to calculate the approximate coupling efficiency of each addition step by dividing the next step's absorbance value from the previous step's value. From this data, an overall stepwise yield can be determined and the expected product yield can be estimated.

The molar amount of DMT cation can be calculated using Beer's Law:

$A = eCl$ where: $A$ - absorbance
$e$ - extinction coefficient
$C$ - concentration
$l$ - path length

$$\text{overall yield} = \text{coupling yield}^{\text{number of couplings}}$$

For more details, see the trityl assay procedure discussed in Appendix VI.

Monitoring the trityl cation is very important, but the results must be interpreted with caution. The trityl assay is only an indirect measure of synthesis efficiency. Certainly, trityl cation yields on the order of 98-99% must be present for a good synthesis. However, high trityl yields can be present when a poor synthesis occurs. The reason for this discrepancy is that, although this chemistry is highly refined, it is not perfect. Unwanted side-reactions do occur. Some of these side-reactions contribute to the trityl cation released each cycle. In particular, a low level of extraneous chain growth other than the desired oligonucleotide occurs. In addition, sites besides the 5' hydroxyl group can participate in coupling (e.g., imperfectly capped sites on the support or branched sites on the nucleic bases).

Other scenarios for imperfect results occur if detritylation or capping is incomplete. This could happen if the synthesizer is not operating correctly, the cycle is not optimized, or the reagents are wet or impure. These events generate species other than the desired product which release the trityl cation. Low trityl yields (significantly less than 98%) always predict a less-than-optimal synthesis. In practical terms, trityl yields of less than 95% will not allow identification or purification of even short oligonucleotides. For the above reasons, the trityl assay must be viewed only as a preliminary, yet convenient, monitor of the synthesizer's performance. The assay is useful to aid in the early detection and diagnosis of instrument-related problems. Many laboratories monitor their trityl fractions only by visual inspection. With experience, a failed synthesis can be detected in this way. Evaluation of the oligonucleotide by PAGE, HPLC, or capillary electrophoresis is much more informative than the trityl assay. Synthesizer or reagent problems can be adequately diagnosed only by these methods, which are *direct* analyses of the product.

## Depurination

Trichloroacetic acid is a very effective protic acid detritylating agent. However, in the presence of protic acids, the amine-protected purines are susceptible to depurination (removal of

the purine from its sugar), with the FODs ($A^{dmf}$ and $G^{dmf}$) being less susceptible than the standard ($A^{bz}$ and $G^{ibu}$) purines. The chemical mechanism is initial protonation at N-7 of the purine ring, causing increased lability of the ribose 1' - purine N-9 bond. Cleavage of adenine and guanine bases yields a 1' hemiacetal ribose ring, the result of depurination. Oligonucleotides that contain apurinic sites are cleaved during the ammonium hydroxide treatment. Cleavage occurs at the internucleotide bonds on the 3' hydroxyl side of the apurinic deoxyribose. (This is similar in effect to the chemistry of Maxam-Gilbert sequencing.) Each purine in the oligonucleotide chain is exposed to acid at each detritylation step. Purines near the 3' end will have the longest cumulative exposure time and a greater chance for depurination. It has also been reported that a 5' terminal purine is more susceptible to depurination than an internucleotide purine.[8]

The quantity of DNA cleavage products generated by apurinic ammoniolysis is usually insignificant. If the synthesis is conducted Trityl On, for purification by OPC or trityl-on HPLC, the 5' end fragment of apurinic ammoniolysis will bear a DMT group and may complicate purification. However, when ABI reagents are used and cycles are optimized for DNA synthesis, depurination is not detectable or significant except in the case of long oligonucleotides (>80 bases) that are purine rich near the 3' end.[9] To minimize depurination, each treatment with TCA should not be extended beyond the times specified in the cycles.

*IMPORTANT* ***Do not*** *stop a synthesis while the DNA is exposed to TCA.*
***Do not*** *increase TCA delivery times.*

## Coupling

### Phosphoramidites

Phosphoramidites (shown in Figure 6-5) are chemically modified nucleosides used as the building blocks for synthesizing DNA. They are added to the support-bound nucleotide chain, one at a time, until all bases in the sequence are coupled.

The cyanoethyl phosphoramidite nucleosides have the following functional groups:

1. **A diisopropylamino on a 3' trivalent phosphorous moiety.**[10] The phosphoramidite is stable until it is made highly reactive by the activator, tetrazole.
2. **A β-cyanoethyl protecting group on the 3' phosphorous moiety.** This group prevents side reactions and aids in solubility of phosphoramidites. It is removed upon completion of the synthesis by using ammonia. During deprotection, ammonia acts as a base to remove a proton on the methylene group bearing the nitrile group. This anion is formed only in low concentration, but rapidly fragments by a β-elimination reaction to form acrylonitrile and the deprotected internucleotide phosphodiester group. Acrylonitrile then reacts irreversibly with ammonia to form 3-aminopropionitrile, an inert compound.
3. **A dimethoxytrityl (DMT) protecting group on the 5' hydroxyl.** The DMT is removed during each detritylation step leaving a reactive 5' hydroxyl available for coupling an incoming phosphoramidite.

4. **Protecting groups on the exocyclic amines of A, C, and G.** As shown in Figure 6-3, different protecting groups are used for standard ($A^{bz}$, $C^{bz}$, $G^{ibu}$) and FOD ($A^{dmf}$, $C^{ibu}$, $G^{dmf}$) phosphoramidites. These groups prevent side reactions and are removed with ammonia after completion of the synthesis. Since thymidine is unreactive and does not contain an exocyclic amine moiety, it is not protected.

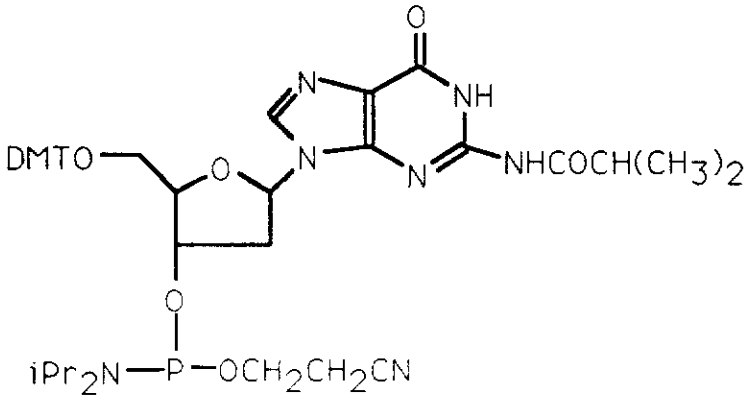


Figure 6-5. Structure of standard guanosine cyanoethyl phosphoramidite ($G^{ibu}$)

## Coupling Procedure

Before beginning the coupling step, the support is made anhydrous and free of nucleophiles (e.g., water) by an extensive wash with acetonitrile. Any extraneous nucleophiles will compete with the support-bound 5' hydroxyls for the activated phosphoramidite and will decrease coupling efficiency. The column is then dried by an argon reverse flush to remove residual acetonitrile. Tetrazole, the phosphoramidite activator, is next delivered to the column.

According to the oligonucleotide sequence, one or more of the phosphoramidites (Bottles 1 to 8) and tetrazole (Bottle 9) are then simultaneously delivered to the column. Depending on the synthesis cycle, alternate deliveries of tetrazole and then base plus tetrazole are repeated up to three times. When these reagents mix, the mild acid, tetrazole (pKa = 4.8), transfers a proton to the nitrogen of the diisopropyl group on the 3' phosphorous. (See Figure 6-6.)

This protonated amine makes a very good leaving group upon nucleophilic attack by the tetrazole to form a tetrazolyl phosphoramidite.[11] The tetrazolyl phosphoramidite is the reactive intermediate that forms the internucleotide phosphite with the support-bound 5' hydroxyl. A molar excess of tetrazole ensures that the phosphoramidite will be activated. The excess of phosphoramidite relative to free 5' hydroxyl ensures that the reaction is nearly quantitative (over 98% coupling). The coupling steps are summarized in Figure 6-6.

Mixed sequence probes are synthesized by simultaneous delivery of more than one base (AGCT5678) and tetrazole with near-equivalent coupling. Up to four bases may be speci-

fied as a mixed sequence probe. The four nucleoside phosphoramidites have slightly different reactivities, as all different molecules must.[12] The cyanoethyl phosphoramidites follow the reactivity order T > G > C > A.

When all four are delivered simultaneously, their representation (normalized to 100%) will be:

T - 30%
G - 26%
C - 24%
A - 20%

These values are slightly dependent on the cycle, location of the site in the oligonucleotide, age of the phosphoramidite solutions, etc. They have a range of about ±3% because of these variables.

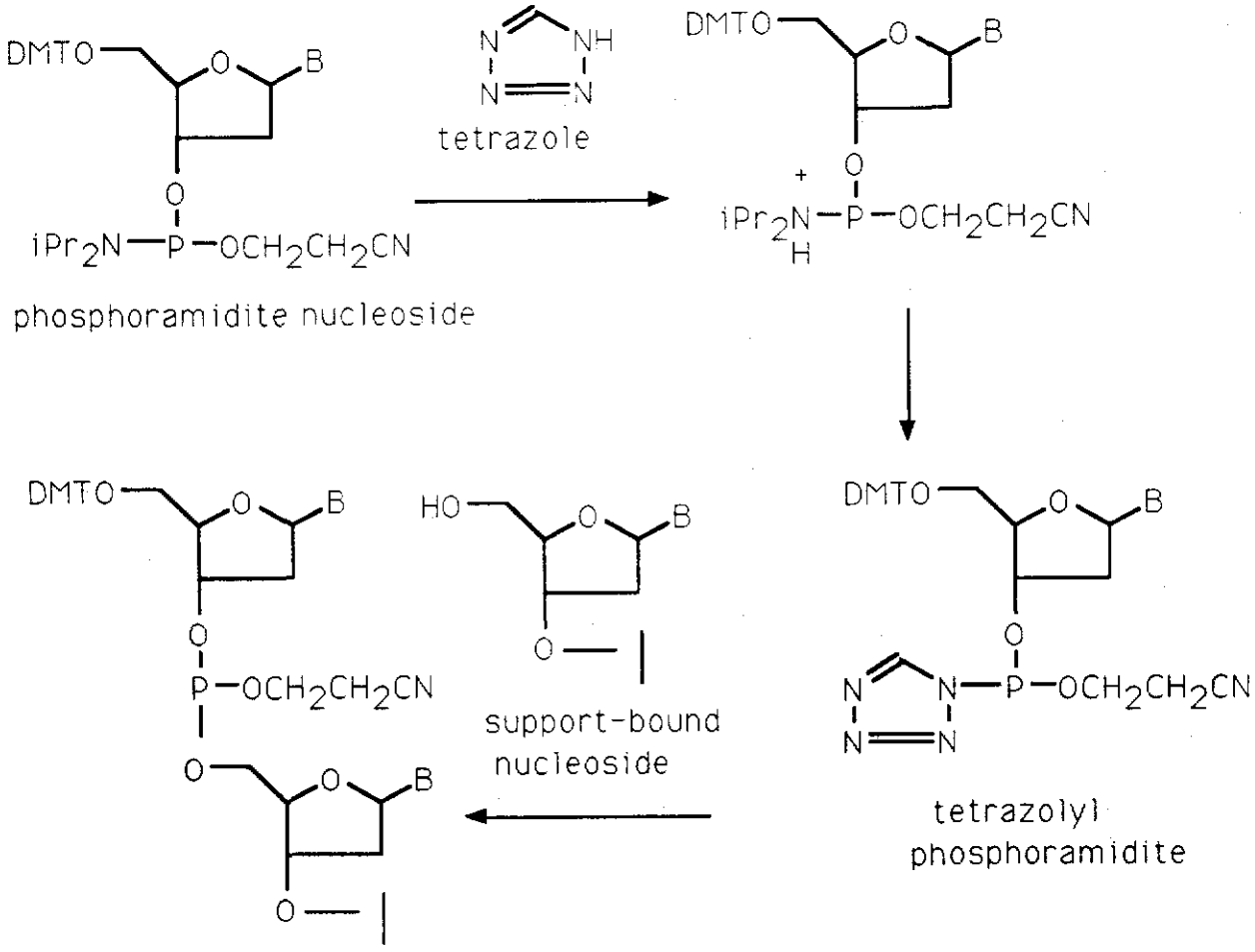


Figure 6-6. The coupling step

*NOTE Phosphoramidites and tetrazole are delivered to the column, which contains the support-bound nucleotide. The diisopropylamine is protonated and displaced by tetrazole. When the 5' OH couples to the phosphorous, a 5' to 3' internucleotide linkage is created.*

## Capping

Because coupling is not quantitative, a small percentage (about 2%) of support-bound nucleotides will fail to undergo addition. These truncated, or *failure sequences*, will remain attached to the support. If they remain in the hydroxyl form, they can propagate in subsequent coupling steps. Failure sequences with one less base than the product would then be generated making isolation of the product more difficult. Capping the remaining free hydroxyls by acetylation eliminates this problem. The capped failure sequences are then prevented from participating in the rest of the synthesis reactions.

To cap unreacted nucleotides, equal volumes of two reagents, acetic anhydride (Bottle 11) and 1-methylimidazole, NMI, (Bottle 12), are simultaneously delivered to the column. As shown in Figure 6-7, the reagents mix and create a powerful acetylating agent. The two reagents need to be segregated since the active acetylating agent is unstable. This agent reacts at the 5' hydroxyls, rendering these moieties unreactive for the remainder of the synthesis. The excess reagents are then removed by an argon reverse flush. The capping time required to acetylate the 1 or 2% unreacted 5' hydroxyls is very brief (only a few seconds). It is important to minimize this time to prevent loss of cyanoethyl groups from the internucleotide linkages and to prevent base modification byproducts. The efficiency of shorter capping time and the capping/oxidation order have been extensively demonstrated in studies at Applied Biosystems.[13]

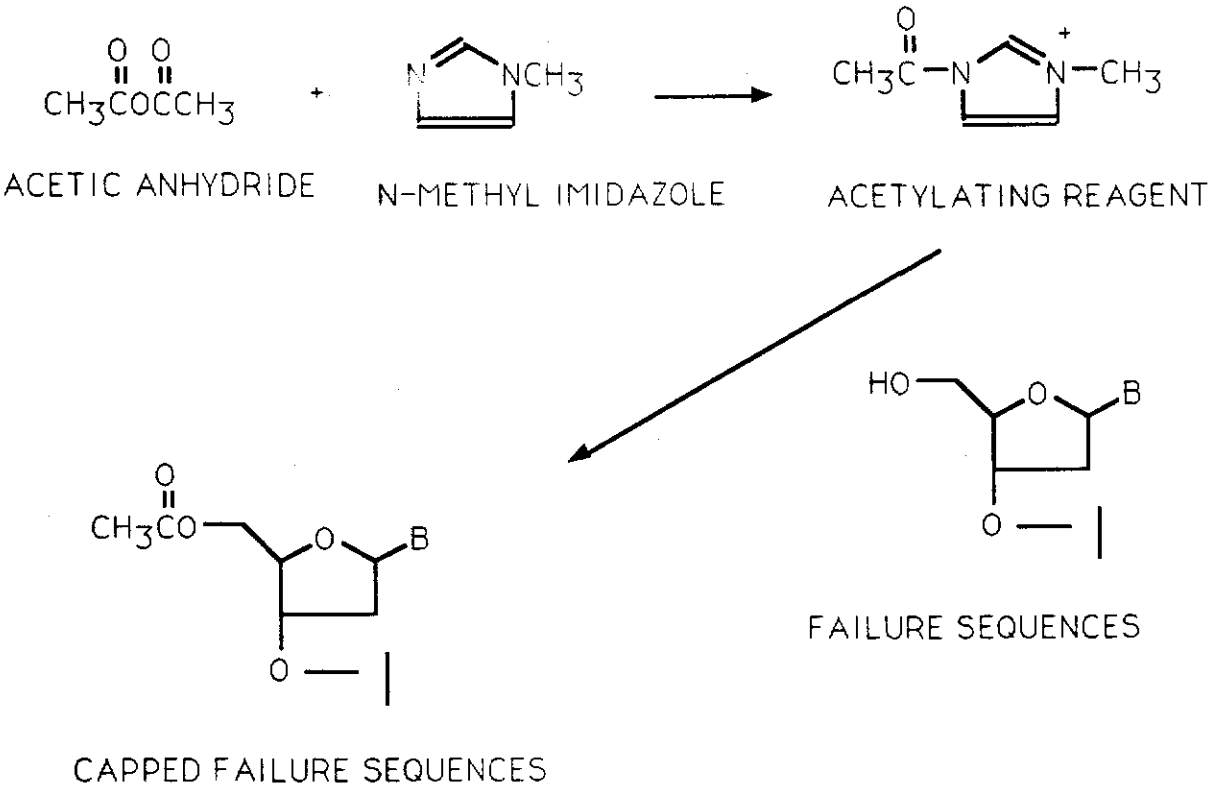


Figure 6-7. Capping of unreacted chains

*NOTE The capping reagents, acetic anhydride, and 1-methylimidazole (NMI), are used to terminate unreacted chains by acetylating the 5' hydroxyl groups.*

## Oxidation

The newly formed internucleotide linkage is a phosphite (trivalent phosphorous) triester. The phosphite linkage is unstable and is susceptible to acid and base cleavage. Therefore, immediately after capping, the trivalent phosphite triester is oxidized to a stable pentavalent phosphate triester. This is shown in Figure 6-8.

Oxidation follows capping to eliminate the possibility of traces of water from the oxidizing solution causing acetic anhydride to form acetic acid during capping. This would expose the oligonucleotides to acid as well as make capping less effective. Also, scientists at Applied Biosystems have elucidated the complex chemical pathway whereby a small side-reaction, phosphitylation of the O-6 position of guanosine, can be minimized when capping immediately follows coupling.[5, 14] O-6 phosphitylation is the initial reaction leading to potential base modification (2,6-diaminopurine) and branching structures. The enzymatic digestion/base composition assay on oligonucleotides made with a different cycle order—capping then oxidation and oxidation then capping—shows markedly different results. The Applied Biosystems standard—capping then oxidation—gives far smaller (usually undetectable) amounts of base-modified nucleosides. Cycles with oxidation followed by capping give high levels of the mutagenic modified nucleoside 2,6-diaminopurine.

Iodine is used as a mild oxidant in a basic tetrahydrofuran (THF) solution with water as the oxygen donor. When the iodine-water-pyridine-THF mixture (Bottle 15) is delivered to the column, an iodine-pyridine complex forms an adduct with the trivalent phosphorous. This adduct is decomposed by water with production of a pentavalent phosphotriester internucleotide group. The reaction is extremely fast, being quantitative in 30 seconds. The iodine solution is removed by an argon reverse flush and several acetonitrile washes, each followed by an argon reverse flush.

*NOTE Iodine may be stored at room temperature up to one year and is not sensitive to atmospheric exposure.*

***IMPORTANT*** *Do not stop the synthesis while the phosphorous is unoxidized.*

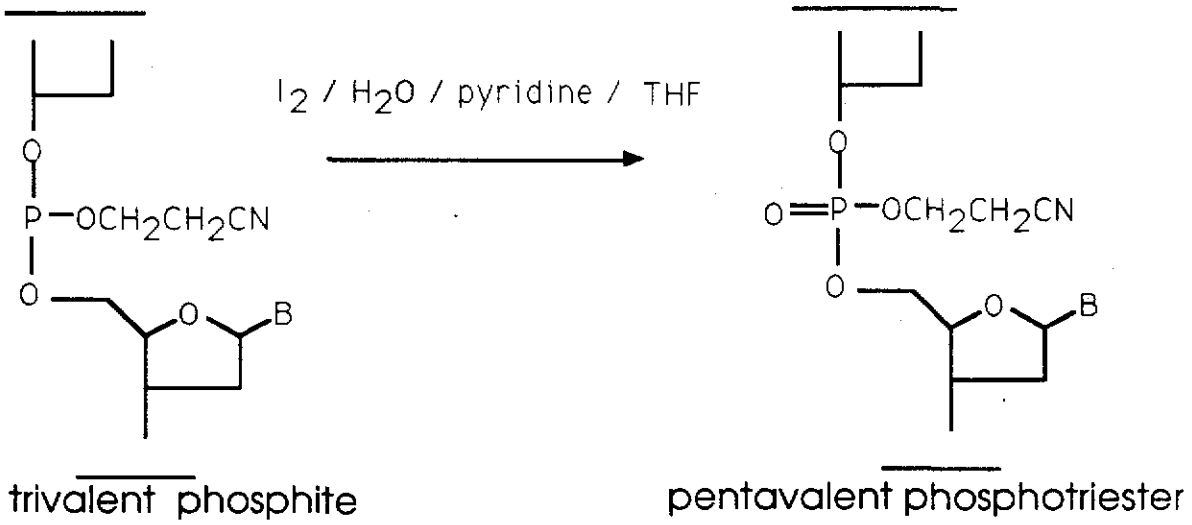


Figure 6-8. Oxidation of the trivalent phosphorous

*NOTE The unstable trivalent phosphorous of the newly formed internucleotide linkage is oxidized to a stable pentavalent phosphorous using an iodine solution.*

# Completion of the Synthesis Cycle

Following oxidation, a cycle of nucleotide addition is complete. The 5' terminus of the oligomer is protected by the dimethoxytrityl group. DNA synthesis continues by removing the 5' trityl and repeating another cycle of base addition. This is done until DNA of the specified length has been fully synthesized.

Immediately after the synthesis is complete, a trityl group may still be attached to the 5' terminus, according to the user's option. The ending method instructions may be programmed for the trityl group to remain attached (trityl on) or be cleaved (trityl off).

The oligonucleotides are usually synthesized "trityl off" when purifying by gel electrophoresis or ion exchange HPLC. Syntheses are left "trityl on" when purifying by OPC or trityl-specific, reverse-phase HPLC.

# Cleavage and Deprotection

When the synthesis is finished, the product and capped failure sequences (still attached to the support) exist as phosphate-protected, base-protected phosphotriesters. The oligonucleotides must be cleaved from the support. Also, complete deprotection is necessary to produce biologically active DNA. Phosphate deprotection and cleavage from the support are simultaneously performed automatically at the end of the synthesis cycle, while the column is still on the instrument. Base deprotection is usually completed as a separate step, off the instrument. See "Base Deprotection" on the next page for more information. For instructions on performing manual cleavage and phosphonate deprotection, refer to Appendix VI.

## Cleavage

Following synthesis, the DNA remains covalently attached to the support. The diester oligonucleotides are cleaved from the support by four consecutive treatments with fresh, concentrated ammonium hydroxide. As seen in Figure 6-9, the cleavage occurs at the base-labile ester linkage between the linker of the support and the 3' hydroxyl of the initial nucleoside. The cleaved DNA has a 3' hydroxyl.

The column effluent from each ammonium hydroxide treatment is forced into the DNA collection vial by an argon flush. The vial contains the crude mix (product and failure sequences) of base-protected oligonucleotides in ammonium hydroxide. Following cleavage, the column and the internal passages of the instrument are rinsed with acetonitrile and dried with argon. The used column and the DNA collection vial are removed. This leaves the synthesizer ready for another synthesis. The DNA is then base-deprotected, desalted, and purified for use.

*NOTE Instead of using the auto-cleavage/deprotection feature of the synthesizer, cleavage and phosphate deprotection can be accomplished manually by using the procedure described in Appendix VI.*

## Phosphate Deprotection

The cyanoethyl protecting groups are removed by treatment with ammonium hydroxide. This occurs at the same time as cleavage, making phosphate deprotection very quick and simple. (See Figure 6-9.)

## Base Deprotection

Base-protecting groups are removed by placing the vial of DNA at 55°C for at least 8 to 15 hours for standard phosphoramidites and 1 hour for FOD phosphoramidites. The FOD base-protecting groups may also be removed in 8 hours at room temperature. The deprotection process also cleaves the acetyl caps from the failure sequences. Base deprotection is an ammoniolysis reaction, in which ammonia acts as a nucleophile that attacks the carbonyl of the amide protecting groups. For effective treatment, use fresh, concentrated ammonium hydroxide during cleavage. To ensure no decrease in ammonia concentration, store the reagent in a refrigerator, tightly capped. Discard the ammonia stock 30 days after opening.

***IMPORTANT*** *Use a tightly sealed DNA collection vial that can withstand positive pressure. The vial must also have a Teflon-lined cap. Rubber-lined caps have contaminants that leach out of the cap liner during deprotection. Teflon-lined caps can be ordered from Wheaton: Part Number 240408, size 13-425.*

After completing deprotection, **cool the ammonium hydroxide-DNA solution on ice** to prevent losses from bubbling. Then remove the ammonia by vacuum. Ammonia is much easier to transfer at lower temperature than at room temperature.

---

***CAUTION*** *When drying down trityl-on syntheses, it is important to keep the oligonucleotide solution basic. Vacuum removal of the ammonia can lead to slightly acidic solution conditions, which may promote trityl removal. To maintain basic conditions during ammonia removal, add one drop of distilled triethylamine every 10 minutes. Also, avoid heating the sample.*

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If the 5' DMT group has been left on, it can be removed manually by treatment with 80% acetic acid/water for 20 minutes at room temperature. The acid is then diluted with ethanol and removed by vacuum. The ethanol addition and vacuum removal should be repeated several times. This procedure is usually done after reverse-phase HPLC or before radioactively labeling the 5' end prior to analysis by gel electrophoresis.

The deprotected, detritylated DNA has free 5' and 3' hydroxyls and is biologically active. Desalting and purification may be necessary before use in experiments. An overview of these procedures is described later in this section. Details can be found in User Bulletin 13 (Revised), found under *Users' Bulletins* at the end of this manual.

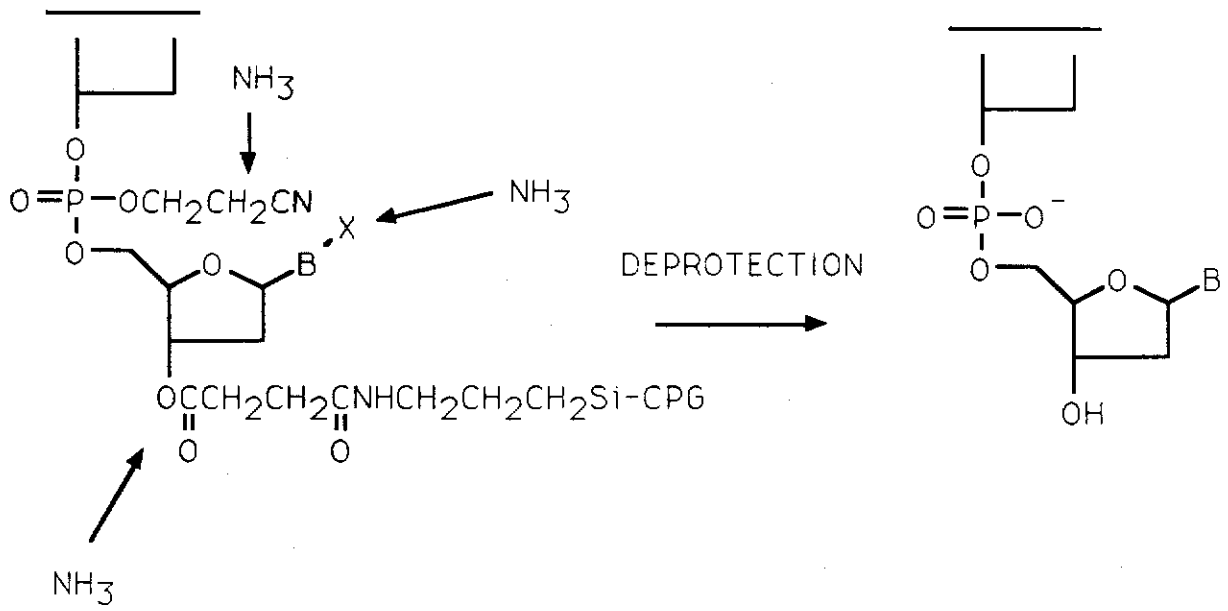


Figure 6-9. Deprotection and cleavage of β-cyanoethyl protected oligonucleotides

*NOTE Treatment with concentrated ammonium hydroxide removes the β-cyanoethyl protecting groups and cleaves the oligonucleotides from the support. The benzoyl and isobutyryl base-protecting groups of standard phosphoramidites (X) are removed by heating at 55° C in ammonia for 8 to 15 hours. The dimethylformamidine and isobutyryl protecting groups (X) of FOD phosphoramidites are removed by heating at 55° C for 1 hour or after 8 hours at room temperature.*

# Quantitation of the Oligonucleotide

Nucleic acids of any variety are most easily quantified by UV spectroscopy, measuring at or near their UV absorbance maxima, about 260 nm. A dilute aqueous solution of 1 ml or less, depending on the cuvette size, is measured by either scanning a region of about 200-350 nm or a single 260 nm wavelength measurement . A scan of an oligonucleotide will show broad absorbance with a maxima near 260 nm. Using Beer's law, the concentration of the solution and absolute quantity can be calculated. As a useful approximation, 1 optical density unit (odu; $A_{260}$) of single-stranded oligonucleotide consists of about 33 micrograms, by mass.

# Storage of the Oligonucleotide

Most applications for synthetic oligonucleotides require less DNA than even a 40 nanomole or 0.2 µmol synthesis provides. Fortunately, oligonucleotides can be stored easily, with little or no degradation for long periods of time. It is probably most convenient to store them refrigerated as a solution, in either a crude or purified state. The medium may be concentrated ammonia, used to cleave and deprotect the crude oligonucleotide, water, or dilute buffer or salt. Typical aqueous media may contain ethanol, acetonitrile, triethylammonium acetate (TEAA), EDTA, etc. It is important to keep the oligonucleotides cold to minimize degradation and bacterial growth. Alternatively, oligonucleotides may be stored dried as a pellet in a clean, dry vessel such as a micro-centrifuge tube. The solution used to elute purified oligonucleotides from OPC, 20% acetonitrile, is a convenient and stable storage medium. When stored by these means, oligonucleotides are stable for over a year. Avoid storing oligonucleotides in solutions that are mutagenic, oxidizing, or outside the pH range of 3-12.

# Analysis and Purification

This section reviews the most common methods for purification of synthetic oligonucleotides. OPC (Oligonucleotide Purification Cartridge) is a rapid, low-cost method of purifying synthetic oligonucleotides. PAGE (polyacrylamide gel electrophoresis) and HPLC (high-performance liquid chromatography) can provide a high level of purity, but require initial capital investment and are labor intensive and time consuming. A short oligonucleotide (<30 bases) made with typically high synthesis efficiency (>98% average DMT yield/cycle) may require less stringent or no purification, with efficient desalting and removal of non-nucleoside synthesis by-products sufficing. PAGE and HPLC are elaborated in detail in User Bulletin No. 13 (Revised).

## Oligonucleotide Purification Cartridge (OPC)

The *Oligonucleotide Purification Cartridge* (OPC, ABI part # 400771) was designed specifically for rapid, easy purification of synthetic oligonucleotides.[15,16] The method is based on a small, syringe-mounted cartridge containing an adsorbent material with an affinity for DMT oligonucleotides. The ammonia solution of the crude DMT oligonucleotide (10-20 crude odu) is diluted and applied directly to the cartridge. The DMT oligonucleotide product is retained. Byproducts, failure sequences not bearing a DMT group, and other impurities are not retained and are eluted. The DMT group of the OPC-bound oligonucleotide is removed with a mild acid solution; then the purified oligonucleotide is eluted (typically 1-5 odu) with about 1 ml of a 20% acetonitrile solution. The entire operation requires 15 to 20 minutes, and several OPC purifications can be conducted in parallel. The OPC operation can be easily multiplexed on a vacuum manifold (see Appendix VI). Efficient, reliable purifications are achieved with oligonucleotides up to and greater than 100 bases.

Several features of this operation are noteworthy. No sample preparation is required. The OPC material is stable to concentrated ammonia. The ammonia solution provides a *denaturing* medium, eliminating secondary structure and hydrogen-bonding, thereby eliminating co-elution of partially complementary failure sequences. The DMT group is detached and retained in the cartridge. The purified, fully deprotected oligonucleotide is eluted in a small volume of 20% acetonitrile in water, completely desalted and ready for use.

# PAGE

Polyacrylamide gel electrophoresis (PAGE) is a widely used method for the analysis and purification of oligonucleotides.[17] When charged molecules are exposed to an electric field, they will migrate with velocities determined primarily by their mass-to-charge ratios. This ratio changes linearly with the log of the molecular weight of DNA of differing chain lengths. This allows a very ordered progression of oligonucleotides with mobility decreasing as the length increases. Proper PAGE technique can provide resolution, and preparative isolation, of single-base- length differences.

*Oligonucleotide* samples for PAGE are typically prepared by drying a known amount (by measuring absorbance) and redissolving in media such as formamide/1X TBE : 9/1 or 7M urea, which are denser than the 1X TBE running buffer. These media and the gel matrix (containing 7M urea) should be denaturing to ensure disruption of secondary structure and hydrogen-bonding.

The most desirable format for PAGE of oligonucleotides is the slab gel. There are many commercial devices that essentially consist of a sandwich of two glass plates held apart by two side spacers. The thickness of the spacers determine the gel thickness. For UV shadowing, 0.75 - 1.5 mm is convenient. The acrylamide solution is poured between the glass plates and allowed to polymerize. A comb, also inserted, forms wells where the oligonuclotide is loaded. Each well forms a vertical lane during the electrophoresis. The plate length should be about 16-40 cm, depending on oligonucleotide length. The width depends only on the number of samples to be run. Dyes, such as bromophenol blue and xylene cyanol, are run either in the samples or by themselves as indicators of the migration distance of the oligonucleotides. The acrylamide concentration of the gel matrix determines the velocity of the oligonucleotides. A range of 8-20% is typical. Longer oligonucleotides require lower acrylamide concentration.

When the dyes indicate the appropriate migration distance of the oligonucleotide product, the power is turned off and the gel sandwich is disassembled. There are several methods of analysis: UV shadowing, staining, and radiolabeling/autoradiography.

## UV Shadowing

This is the simplest, easiest visualization method, and introduces the fewest artifacts into the analysis. Depending on oligonucleotide length, from 0.5 to 10 odu are loaded in a well. After electrophoresis, the gel is transferred from the plates to a clear plastic-wrapped, fluorescent TLC plate, most conveniently a plastic-backed, 20 × 20 cm plate. The gel is visualized with a UV lamp at short wavelength. The bands appear dark against the fluorescent green background. A permanent record can be made by photography through a green filter.

## Staining

Many dyes and intercalating agents, such as methylene blue, ethidium bromide, and Stains-All, will visualize oligonucleotides in a polyacrylamide matrix. After electrophoresis, the gel is transferred to a pan containing the staining agent and allowed to soak for some time period. This technique is more sensitive than UV shadowing, but is more time-consuming and uncertain. Intercalating stains (eg., ethidium bromide) are highly variable and sequence-dependent for primer length oligonucleotides and are not recommended for syntheses $\leq$ 40 bases.

### Radiolabeling

This is the most sensitive, but most laborious, method of analyzing oligonucleotides. Typically about 1 to 20 pmoles of oligonucleotide are phosphorylated enzymatically with T4 polynucleotide kinase and gamma-32P ATP to give 5' 32P phosphorylated oligonucleotides. Alternatively, 35S ATP can be used, and the oligonucleotides can be labeled at either the 5' or 3' terminus.

The radiolabeled samples are electrophoresed on the gel. The gel is wrapped in plastic and exposed to X-ray film in a dark room. An autoradiogram is generated in a time period ranging from minutes to days, depending on the specific activity of the ATP and other radiolabeling parameters. Since the film has a finite capacity for development, exposure time must be carefully monitored, usually by taking several exposures. The gel pattern on the film may be quantified by densitometry. Product identification is obvious when the appropriate tracking dye or oligonucleotide standard is also present on the gel.

## Summary

Shadowing, staining, and radiolabeling techniques can make the electrophoresis gel pattern for an oligonucleotide an excellent diagnostic tool for the course of a synthesis. Many synthesizer and reagent problems can be diagnosed by the appearance and relative amounts of the "failure bands." Also, PAGE has a distinct advantage in that many samples can be analyzed and purified concurrently. The equipment is relatively inexpensive and easy to maintain. For longer oligonucleotides ($\geq$ 80 bases), PAGE is an efficient purification method and the only analytical method. The primary disadvantages of PAGE are that it is somewhat labor intensive and dependent on good technique.

For PAGE purification, the oligonucleotide product band is located by UV-shadowing the gel material excised with a clean razor blade. The gel material that is removed should be free of failure bands, most typically the lower N-1 band. In preparative electrophoresis, the product is allowed to migrate further than in an analytical run, to maximize the separation of the product band from the N-1 band. Running a thicker gel, 2-3 mm, allows more sample to be loaded for purification. The excised gel fragment(s) are soaked in an elution buffer, such as 1X TBE (Tris Borate EDTA). The gel debris is then removed by a desalting method, such as with an OPC cartridge.

## HPLC

High-performance liquid chromatography (HPLC) is another efficient method that combines the quantitative analysis and purification of oligonucleotides. One of the advantages of HPLC is a high level of automation. Systems that allow for repetitive programmed injection, analysis, and data manipulation and storage are available. Two different types of column adsorbents are popular for oligonucleotides: reverse-phase and ion-exchange.

Reverse-phase adsorbents discriminate by the hydrophobic differences between oligonucleotides of varying lengths and sequences. When the 5' DMT is on the oligonucleotide, this group is dominant in its interaction with the support adsorbent. Reverse-phase columns also can adequately resolve "DMT-off" oligonucleotides with sufficient capacity. The mobile phase is typically a volatile buffer such as 0.1M triethyl-ammonium acetate. The oligonucleotides are eluted with a gradient of increasing organic solvent, such as acetonitrile. These conditions are non-denaturing. Occasionally, certain sequences can exhibit unpredictable HPLC elution patterns, caused by inter- or intramolecular secondary structure and hydrogen-bonding effects. Oligonucleotides, with sequences that have a high G content (especially four or more G bases in a row) often exhibit non-Watson-Crick, Hoogsteen base pairs or stacking effects. These are exemplified by multiple peaks in HPLC, caused by multiple stable conformations of the same sequence.

Ion-exchange adsorbents elute oligonucleotides based on increasing charge, i.e., chain length. An increasing salt gradient in the mobile phase is used to displace the oligonucleotide phosphate anions. The salt anions (such as ammonium sulfate or sodium phosphate) and the DNA pair with the adsorbent-bound cation, usually an alkylated ammonium species. Since ion-exchange analysis separates only on the basis of increasing charge, the desired product oligonucleotide will always elute after the lesser charged failure sequences. The high-salt mobile phase also provides a denaturing medium. Both of these factors allow easy product identification. Preparative ion-exchange HPLC requires a final desalting operation, most efficiently conducted with OPC.

For more information, consult the chapter on HPLC in User Bulletin 13 (Revised), under *Users' Bulletins* in the rear of the manual.

# Alternative Chemistries

In addition to the phosphoramidite chemistry method to prepare "normal" phosphodiester oligonucleotides, many alternative chemistries have been demonstrated on ABI synthesizers. These other chemistries include synthetic RNA, phosphate analog oligonucleotides, and chemically derivatized oligonucleotides, which can be covalently attached to other molecules.

## RNA Synthesis

RNA oligoribonucleotides can be synthesized on the Model 392/394,[18, 19] using 2' silyl 5' DMT cyanoethyl phosphoramidite RNA monomers. The RNA synthesis cycles have a longer coupling wait time (600 seconds) than the DNA synthesis cycles. RNA monomers bear a large 2' silyl protecting group and require a longer coupling time. Details can be found in Applied Biosystems' User Bulletins 47 and 53.

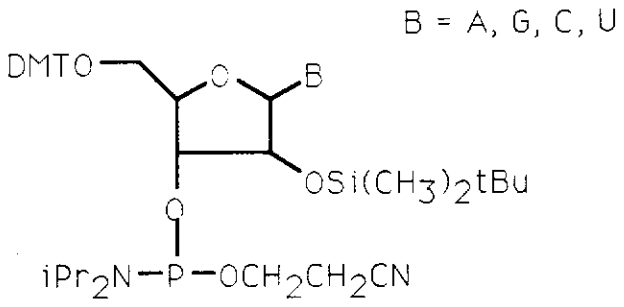


Figure 6-10. RNA phosphoramidites

## 5' Attachments

In the last several years, applications that require the covalent attachment of small molecules to oligonucleotides have been identified and developed.[20] These molecules include fluorescent dyes,[21] biotin,[22] proteins,[23] and other species that allow the identification of oligonucleotides in biological systems. In addition, oligonucleotides can be derivatized to allow attachment to a solid support.[24] In this way, an affinity matrix can be constructed to purify a sequence complementary to the support-bound sequence.

The attachment can be made at several sites on the oligonucleotide. However, the site must not impair hybridization or confer chemical instability. For these reasons and for synthesis ease and efficiency, the 5' terminus is usually the preferred location for derivatization of an oligonucleotide. See the discussion under "Aminolink 2," page 6-33.

## Phosphorothioate Synthesis

The study of phosphorothioate oligonucleotides in antisense experiments has recently intensified. Replacement of one of the nonbridging, equivalent internucleotide oxygens with sulfur results in a chiral phosphorothioate diester, resistant to nuclease metabolism *in vivo*. Specific sequences nullify transcription of mRNA in the expression of specific genes. This phenomenon is termed "the antisense effect." Potential therapeutic candidates—against HIV for example—have progressed to the preclinical, pharmacokinetic stage.

A sulfurizing reagent that significantly lowers the cost and increases the ease and efficiency of synthesizing phosphorothioate oligonucleotides is available. Tetraethylthiuram disulfide (TETD) in acetonitrile (ABI Part No. 401147) converts the internucleotide cyanoethyl phosphite to the phosphorothioate triester within 15 minutes at room temperature (see Figure 6-11). It replaces the iodine reagent, while all other reagents used for standard phosphoramidite chemistry remain the same.

When the ABI 392/394 sulfur cycles (.2 um Sulfur, 1 um Sulfur, or 10 um Sulfur) are used, the synthesis efficiency and purity of phosphorothioate oligonucleotides are comparable to that of phosphodiester oligonucleotides. Also, mixed phosphorothioate/phosphodiester DNA can be easily prepared. Sulfurization efficiency with TETD is very high. Analysis by 31P–NMR shows that sulfurization with TETD typically results in less than 1% phosphodiester contamination based on the integrated area of phosphorothioate diastereomer resonances at approximately 55 ppm and the phosphodiester region at 0 ppm. In addition, even after long exposure to the TETD reagent, analyses of oligonucleotides by enzymatic digestion show no detectable base modifications.

- TETD is stable at room temperature but must be kept tightly closed when not in use.
- TETD may be installed and removed from the synthesizer several times. It will retain peak performance for six weeks after initial use.
- Either FOD reagents or standard phosphoramidites and supports may be used.
- Phosphorothioate oligonucleotides, like phosphodiester oligos, are cleaved and deprotected at the completion of synthesis with concentrated ammonia.
- No additional post–synthesis procedures are necessary.

## Analysis/Purification

Phosphorothioate oligonucleotides may be analyzed and purified by the same techniques as normal, phosphodiester DNA: HPLC, PAGE, and OPC (see User Bulletin No. 44). Because the sulfur-containing phosphorothioates are more hydrophobic, retention times on reverse-phase HPLC are longer or require a higher organic content in the mobile phase. Product peaks may be broad relative to phosphodiester DNA. When PAGE is used, the sulfur analog has virtually the same electrophoretic mobility as normal DNA. For OPC purification, phosphorothioate oligonucleotides are eluted from the OPC with a 35% acetonitrile solution.

The following methods can also be used for phosphorothioate DNA analysis:

- MICRO–GEL100™ capillary electrophoresis, a new, high-resolution analysis method. (This is also useful for phosphodiester DNA.)
- 31P-NMR, a method that can conveniently and quantitatively measure the relative phosphodiester–to–phosphorothioate content of oligonucleotides. With high-field NMR spectrometers, a single 1–μmol synthesis provides sufficient sample to conduct analysis.

Phosphorothioates are not good substrates for polynucleotide kinase and thus do not 5' label efficiently with 32P ATP. Mixed oligonucleotides should have at least three phosphodiester linkages at the 5' end for efficient labeling.

More Details can be found in Applied Biosystems User Bulletin 58.

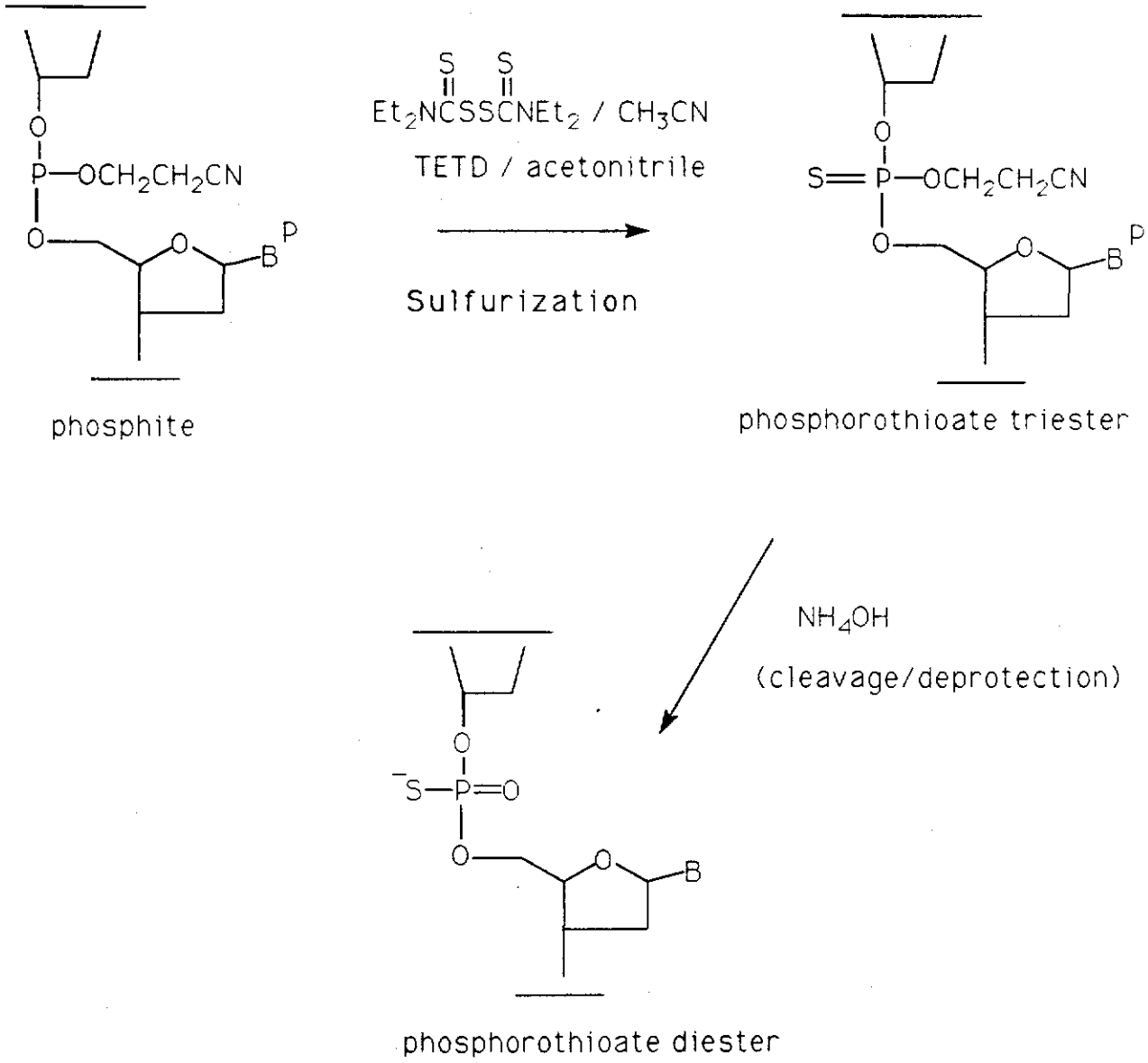


Figure 6-11. Phosphorothioate synthesis using TETD

## Aminolink 2

The 5' fluorescent dye-labeled sequencing primers used for the Applied Biosystems 373A DNA Sequencer consist of three parts: the oligonucleotide primer, a linker, and a fluorescent dye. The linker bears a highly nucleophilic primary amine group, which reacts with the electrophilic N-hydroxy succinimide group of the fluorescent dye. This linker group is created with Aminolink 2 (ABI Part No. 400808).[25]

Aminolink 2 is a phosphoramidite molecule with a six-carbon chain and a protected amine group (Figure 6-12). This reagent is handled and used like a phosphoramidite nucleoside. Activation with tetrazole forms an active intermediate that couples to the 5' hydroxyl terminus of the support-bound oligonucleotide in the final coupling cycle. Oxidation and ammonia cleavage/deprotection yields the aminolink oligonucleotide. Coupling to the fluorescent dye NHS ester or other electrophilic species is conducted in a homogeneous solution. For details on coupling to fluorescent dye NHS esters, see DNA Sequencing User Bulletin No. 11.

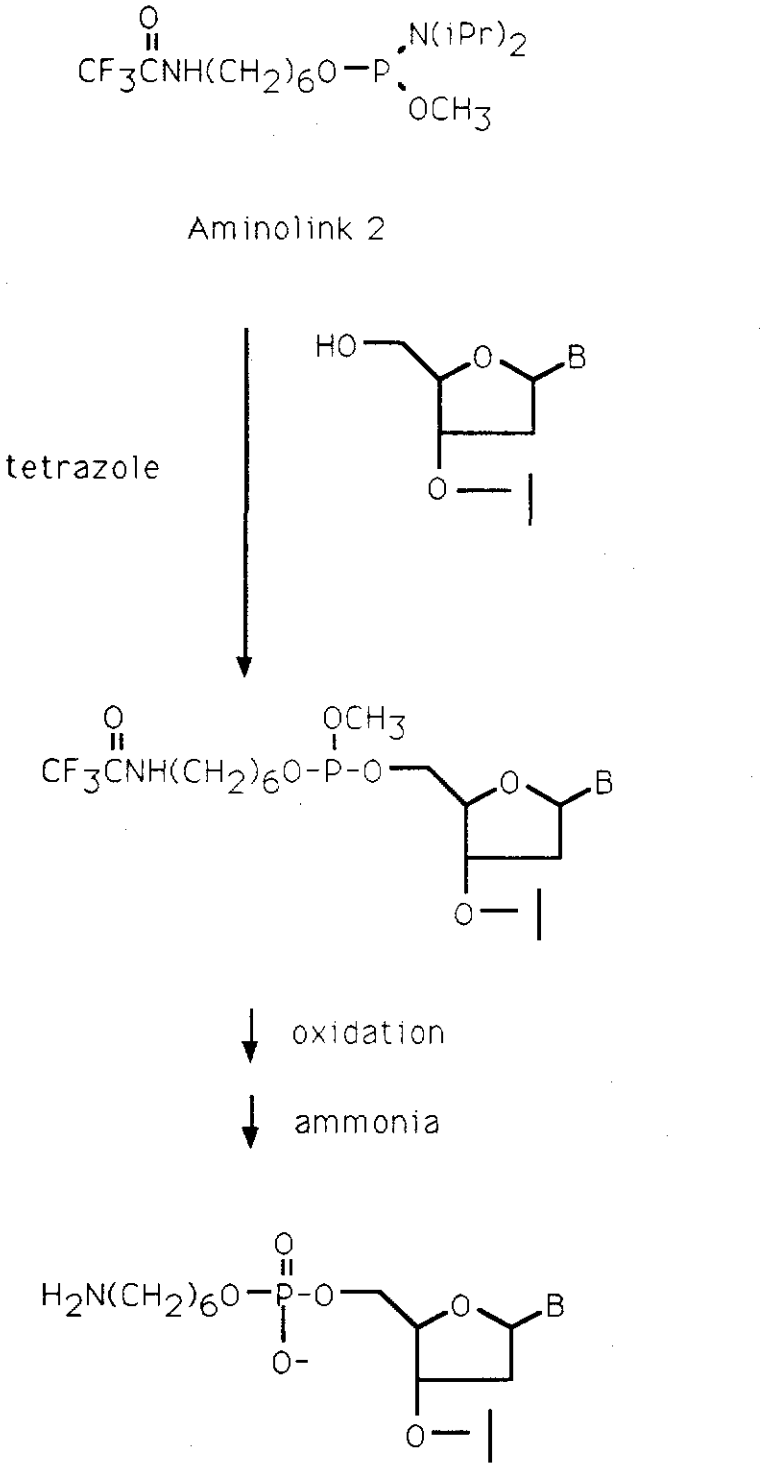


Figure 6-12. Aminolink 2 chemistry

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# *Appendix I*

# Functions, Cycles and Procedures


Table AI-1 – 392 Sequential Function List
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Table AI-2 – 392 Sequential Function List
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Table AI-3 – 394 Sequential Function List
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(four-column instrument) ..... App. I–20

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(two-column instrument) ..... App. I–23

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(four-column instrument) ..... App. I–25

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(two-column instrument) ..... App. I–28

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(four-column instrument) ..... App. I–30

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(two-column instrument) ..... App. I–32

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(four-column instrument) ..... App. I–34

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(two-column instrument) ..... App. I–37

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Table AI-28 – 394 End Procedure Listing ..... App. I–63

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Table AI-37 – 392/394 Bottle Change Procedure Listing
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Table AI-38 – 392/394 Bottle Change Procedure Listing
(Bottle 7 – eight-position only) ..... App. I–70

Table AI-39 – 392/394 Bottle Change Procedure Listing
(Bottle 8 – eight-position only) ..... App. I–70

Table AI-40 – 392/394 Bottle Change Procedure Listing
(Bottle 9 – all instruments) ..... App. I–71

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Table AI-42 – 392/394 Bottle Change Procedure Listing
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(Bottle 12 – all instruments) ..... App. I–72

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(eight-position instrument) ..... App. I–78

# Table AI-1
# 392 Sequential Function List
# (two-column/five-base instrument)

| Numb. | Name | Valve List | Numb. | Name | Valve List |
|---|---|---|---|---|---|
| 1 | Block Flush | 35,25,0,3,4,7 | 58 | TET to Waste | 49,31,25 |
| 2 | Reverse Flush | 25,0,16,4,17 | 59 | 10 to Waste | 47,24,25 |
| 3 | Trityl Flush | 35,16,2,17,6 | 60 | 11 to Waste | 53,20,25 |
| 4 | Flush to Waste | 35,16,3,17,7 | 61 | 12 to Waste | 53,21,25 |
| 5 | Flush to Colect | 35,16,1,17,5 | 62 | 14 to Waste | 52,22,25 |
| 6 | Flush to A | 32,46,26 | 63 | 15 to Waste | 51,23,25 |
| 7 | Flush to G | 32,46,27 | 64 | 18 to Waste | 54,33,25 |
| 8 | Flush to C | 32,46,28 | 65-69 | INVALID FNCTION | |
| 9 | Flush to T | 32,46,29 | 70 | 18 to A | 54,46,33,26 |
| 10 | Flush to 5 | 32,46,30 | 71 | 18 to G | 54,46,33,27 |
| 11- 13 | INVALID FNCTION | | 72 | 18 to C | 54,33,46,28 |
| 14 | Flush to Bases | 32,46 | 73 | 18 to T | 54,33,46,29 |
| 15 | Flush to Tet | 35,31 | 74 | 18 to 5 | 54,33,46,30 |
| 16 | Flush to 10 | 35,24 | 75-77 | INVALID FNCTION | |
| 17 | Flush to 11 + 12 | 35,20,21 | 78 | 18 to Tet | 54,33,31 |
| 18 | Flush to 14 + 15 | 35,22,23 | 79 | 18 to 10 | 54,33,24 |
| 19 | Flush to 18 | 33,32 | 80 | 18 to 11 | 54,33,20 |
| 20- 24 | INVALID FNCTION | | 81 | 18 to 12 | 54,33,21 |
| 25 | A to Column | 45,26,16, 3,17, 7 | 82 | 18 to 11 + 12 | 54,33,20,21 |
| 26 | G to Column | 45,27,16, 3,17, 7 | 83 | 18 to 14 | 54,33,22 |
| 27 | C to Column | 45,28,16, 3,17, 7 | 84 | 18 to 15 | 54,33,23 |
| 28 | T to Column | 45,29,16, 3,17, 7 | 85 | 18 to 14 + 15 | 54,33,22,23 |
| 29 | 5 to Column | 45,30,16, 3,17, 7 | 86 | 18 To Bases | 54,33,46 |
| 30-32 | INVALID FNCTION | | 87-89 | INVALID FNCTION | |
| 33 | B+Tet to Column | 45,49,31,16, 3,17, 7 | 90 | 15 to A | 51,46,23,26 |
| 34 | Tet to Column | 49,31,16, 3,17, 7 | 91 | 15 to G | 51,46,23,27 |
| 35 | 5+Tet to Column | 45,30,49,31,16, 3,17, 7 | 92 | 15 to C | 51,46,23,28 |
| 36 | 10 to Collect | 47,24,16, 1,17, 5 | 93 | 15 to T | 51,46,23,29 |
| 37 | 11 to Column | 53,20,16, 3,17, 7 | 94 | 15 to Bases | 51,46,23 |
| 38 | 12 to Column | 53,21,16, 3,17, 7 | 95- 99 | INVALID FNCTION | |
| 39 | Cap to Column | 53,20,21,16,3,17,7 | 100 | 10 Vent | 48 |
| 40 | 14 to Column | 52,22,16,3,17,7 | 101 | Phos Prep | 45,49 |
| 41 | 15 to Column | 51,23,16,3,17,7 | 102 | Cap Prep | 53 |
| 42 | 18 to Column | 54,33,16,3,17,7 | 103 | Wait | |
| 43 | Push to Column | 54,33,16, 3,17, 7 | 104 | Interrupt | |
| 44-49 | INVALID FNCTION | | 105 | Start Detrityl | |
| 50 | A to Waste | 45,26,25 | 106 | Begin | |
| 51 | G to Waste | 45,27,25 | 107 | End | |
| 52 | C to Waste | 45,28,25 | 108 | Block to Col | 16, 3,17,7 |
| 53 | T to Waste | 45,29,25 | 109 | Waste - Port | |
| 54 | 5 to Waste | 45,30,25 | 110 | Waste - Bottle | |
| 55-57 | INVALID FNCTION | | 111 | Block Vent | 25, 3, 7 |
| | | | 112 | Trityl Advance | |

# Table AI-1 (cont.)

| Numb. | Name | Valve List |
|---|---|---|
| 113 | End Advance | |
| 114 | Vent Bases | 46 |
| 115 | Prep 10 | 47 |
| 116-119 | INVALID FNCTION | |
| 120 | Advance FC | 59 |
| 121 | Relay 1 On | 59 |
| 122 | Relay 1 Off | 59 |
| 123 | Relay 1 Pulse | 59 |
| 124 | Relay 2 On | 60 |
| 125 | Relay 2 Off | 60 |
| 126 | Relay 2 Pulse | 60 |
| 127 | Relay 3 On | 61 |
| 128 | Relay 3 Off | 61 |
| 129 | Relay 3 Pulse | 61 |
| 130 | Relay 4 On | 62 |
| 131 | Relay 4 Off | 62 |
| 132 | Relay 4 Pulse | 62 |
| 133 | Relay 5 On | 63 |
| 134 | Relay 5 Off | 63 |
| 135-139 | INVALID FNCTION | |
| 140 | Column 1 On | |
| 141 | Column 1 Off | |
| 142 | Column 2 On | |
| 143 | Column 2 Off | |
| 144-149 | INVALID FNCTION | |
| 150 | Bottle 01 On | |
| 151 | Bottle 01 Off | |
| 152 | Bottle 02 On | |
| 153 | Bottle 02 Off | |
| 154 | Bottle 03 On | |
| 155 | Bottle 03 Off | |
| 156 | Bottle 04 On | |
| 157 | Bottle 04 Off | |
| 158 | Bottle 05 On | |
| 159 | Bottle 05 Off | |
| 160-169 | INVALID FNCTION | |
| 170 | Dilute 'A' | 54,46,33,26 |
| 171 | Dilute 'G' | 54,46,33,27 |
| 172 | Dilute 'C' | 54,33,46,28 |
| 173 | Dilute 'T' | 54,33,46,29 |
| 174 | Dilute '5' | 54,33,46,30 |
| 175-199 | INVALID FNCTION | |
| 200 | User01 | |
| 201 | User02 | |
| 202 | User03 | |
| 203 | User04 | |
| 204 | User05 | |
| 205 | User06 | |
| 206 | User07 | |
| 207 | User08 | |
| 208 | User09 | |
| 209 | User10 | |
| 210 | User11 | |
| 211 | User12 | |
| 212 | User13 | |
| 213 | User14 | |
| 214 | User15 | |
| 215 | User16 | |
| 216 | User17 | |
| 217 | User18 | |
| 218 | User19 | |
| 219 | User20 | |
| 220 | User21 | |
| 221 | User22 | |
| 222 | User23 | |
| 223 | User24 | |
| 224 | User25 | |

# Table AI-2
# 392 Sequential Function List
# (two-column/eight-base instrument)

| Numb. | Name | Valve List | Numb. | Name | Valve List |
|---|---|---|---|---|---|
| 1 | Block Flush | 40,35,25,0,3,4,7 | 52 | C to Waste | 45,28,25 |
| 2 | Reverse Flush | 25,0,16,4,17 | 53 | T to Waste | 45,29,25 |
| 3 | Trityl Flush | 40,35,16,2,17,6 | 54 | 5 to Waste | 45,30,25 |
| 4 | Flush to Waste | 40,35,16,3,17,7 | 55 | 6 to Waste | 45,31,25 |
| 5 | Flush to Collect | 40,35,16,1,17,5 | 56 | 7 to Waste | 45,32,25 |
| 6 | Flush to A | 43,35,46,26 | 57 | 8 to Waste | 45,33,25 |
| 7 | Flush to G | 43,35,46,27 | 58 | TET to Waste | 49,34,25 |
| 8 | Flush to C | 43,35,46,28 | 59 | 10 to Waste | 47,24,25 |
| 9 | Flush to T | 43,35,46,29 | 60 | 11 to Waste | 53,20,25 |
| 10 | Flush to 5 | 43,35,46,30 | 61 | 12 to Waste | 53,21,25 |
| 11 | Flush to 6 | 43,35,46,31 | 62 | 14 to Waste | 52,22,25 |
| 12 | Flush to 7 | 43,35,46,32 | 63 | 15 to Waste | 51,23,25 |
| 13 | Flush to 8 | 43,35,46,33 | 64 | 18 to Waste | 54,42,35,25 |
| 14 | Flush to Bases | 43,35,46 | 65-69 | INVALID FNCTION | |
| 15 | Flush to Tet | 40,35,34 | 70 | 18 to A | 54,42,46,35,26 |
| 16 | Flush to 10 | 40,35,24 | 71 | 18 to G | 54,42,46,35,27 |
| 17 | Flush to 11 + 12 | 40,35,20,21 | 72 | 18 to C | 54,42,46,35,28 |
| 18 | Flush to 14 + 15 | 40,35,22,23 | 73 | 18 to T | 54,42,46,35,29 |
| 19 | Flush to 18 | 43,42 | 74 | 18 to 5 | 54,42,46,35,30 |
| 20- 24 | INVALID FNCTION | | 75 | 18 to 6 | 54,42,46,35,31 |
| 25 | A to Column | 45,26,16,3,17,7 | 76 | 18 to 7 | 54,42,46,35,32 |
| 26 | G to Column | 45,27,16,3,17,7 | 77 | 18 to 8 | 54,42,46,35,33 |
| 27 | C to Column | 45,28,16, 3,17,7 | 78 | 18 to Tet | 54,42,35,34 |
| 28 | T to Column | 45,29,16, 3,17,7 | 79 | 18 to 10 | 54,42,35,24 |
| 29 | 5 to Column | 45,30,16, 3,17,7 | 80 | 18 to 11 | 54,42,35,20 |
| 30 | 6 to Column | 45,31,16,3,17,7 | 81 | 18 to 12 | 54,42,35,21 |
| 31 | 7 to Column | 45,32,16,3,17,7 | 82 | 18 to 11 + 12 | 54,42,35,20,21 |
| 32 | 8 to Column | 45,33,16,3,17,7 | 83 | 18 to 14 | 54,42,35,22 |
| 33 | B+Tet to Column | 45,49,34,16,3,17,7 | 84 | 18 to 15 | 54,42,35,23 |
| 34 | Tet to Column | 49,34,16,3,17, 7 | 85 | 18 to 14 + 15 | 54,42,35,22,23 |
| 35 | 5+Tet to Column | 45,30,49,34,16,3,17,7 | 86 | 18 To Bases | 54,42,35,46 |
| 36 | 10 to Collect | 47,24,16,1,17, 5 | 87- 89 | INVALID FNCTION | |
| 37 | 11 to Column | 53,20,16,3,17,7 | 90 | 15 to A | 51,46,23,26 |
| 38 | 12 to Column | 53,21,16, 3,17,7 | 91 | 15 to G | 51,46,23,27 |
| 39 | Cap to Column | 53,20,21,16,3,17,7 | 92 | 15 to C | 51,46,23,28 |
| 40 | 14 to Column | 52,22,16,3,17,7 | 93 | 15 to T | 51,46,23,29 |
| 41 | 15 to Column | 51,23,16,3,17,7 | 94 | 15 to Bases | 51,46,23 |
| 42 | 18 to Column | 54,42,35,16,3,17,7 | 95- 99 | INVALID FNCTION | |
| 43 | Push to Column | 54,42,35,16,3,17,7 | 100 | 10 Vent | 48 |
| 44-49 | INVALID FNCTION | | 101 | Phos Prep | 45,49 |
| 50 | A to Waste | 45,26,25 | 102 | Cap Prep | 53 |
| 51 | G to Waste | 45,27,25 | 103 | Wait | |

# Table AI-2 (cont.)

| Numb. | Name | Valve List |
|---|---|---|
| 104 | Interrupt | |
| 105 | Start Detrityl | |
| 106 | Begin | |
| 107 | End | |
| 108 | Block to Col | 16,3,17,7 |
| 109 | Waste - Port | |
| 110 | Waste - Bottle | |
| 111 | Block Vent | 25, 3, 7 |
| 112 | Trityl Advance | |
| 113 | End Advance | |
| 114 | Vent Bases | 46 |
| 115 | Prep 10 | 47 |
| 116-119 | INVALID FNCTION | |
| 120 | Advance FC | 59 |
| 121 | Relay 1 On | 59 |
| 122 | Relay 1 Off | 59 |
| 123 | Relay 1 Pulse | 59 |
| 124 | Relay 2 On | 60 |
| 125 | Relay 2 Off | 60 |
| 126 | Relay 2 Pulse | 60 |
| 127 | Relay 3 On | 61 |
| 128 | Relay 3 Off | 61 |
| 129 | Relay 3 Pulse | 61 |
| 130 | Relay 4 On | 62 |
| 131 | Relay 4 Off | 62 |
| 132 | Relay 4 Pulse | 62 |
| 133 | Relay 5 On | 63 |
| 134 | Relay 5 Off | 63 |
| 135-139 | INVALID FNCTION | |
| 140 | Column 1 On | |
| 141 | Column 1 Off | |
| 142 | Column 2 On | |
| 143 | Column 1 Off | |
| 144-149 | INVALID FNCTION | |
| 150 | Bottle01 On | |
| 151 | Bottle01 Off | |
| 152 | Bottle02 On | |
| 153 | Bottle02 Off | |
| 154 | Bottle03 On | |
| 155 | Bottle03 Off | |
| 156 | Bottle04 On | |
| 157 | Bottle04 Off | |
| 158 | Bottle05 On | |
| 159 | Bottle05 Off | |
| 160 | Bottle06 On | |
| 161 | Bottle06 Off | |
| 162 | Bottle07 On | |
| 163 | Bottle07 Off | |
| 164 | Bottle08 On | |
| 165 | Bottle08 Off | |
| 166-169 | INVALID FNCTION | |
| 170 | Dilute 'A' | 54,42,46,35,26 |
| 171 | Dilute 'G' | 54,42,46,35,27 |
| 172 | Dilute 'C' | 54,42,46,35,28 |
| 173 | Dilute 'T' | 54,42,46,35,29 |
| 174 | Dilute '5' | 54,42,46,35,30 |
| 175 | Dilute '6' | 54,42,46,35,31 |
| 176 | Dilute '7' | 54,42,46,35,32 |
| 177 | Dilute '8' | 54,42,46,35,33 |
| 178-199 | INVALID FNCTION | |
| 200 | User01 | |
| 201 | User02 | |
| 202 | User03 | |
| 203 | User04 | |
| 204 | User05 | |
| 205 | User06 | |
| 206 | User07 | |
| 207 | User08 | |
| 208 | User09 | |
| 209 | User10 | |
| 210 | User11 | |
| 211 | User12 | |
| 212 | User13 | |
| 213 | User14 | |
| 214 | User15 | |
| 215 | User16 | |
| 216 | User17 | |
| 217 | User18 | |
| 218 | User19 | |
| 219 | User20 | |
| 220 | User21 | |
| 221 | User22 | |
| 222 | User 23 | |
| 223 | User 24 | |
| 224 | User 25 | |

# Table AI-3
# 394 Sequential Function List
# (four-column/five-base instrument)

| Numb. | Name | Valve List | Numb. | Name | Valve List |
|---|---|---|---|---|---|
| 1 | Block Flush | 35,25,0,3,4,7,8,11,12,15 | 53 | T to Waste | 45,29,25 |
| 2 | Reverse Flush | 25,0,16,4,17,8,18,12,19 | 54 | 5 to Waste | 45,30,25 |
| 3 | Trityl Flush | 35,16,2,17,6,18,10,19,14 | 55-57 | INVALID FNCTION | |
| 4 | Flush to Waste | 35,16,3,17,7,18,11,19,15 | 58 | TET to Waste | 49,31,25 |
| 5 | Flush to Collect | 35,16,1,17,5,18,9,19,13 | 59 | 10 to Waste | 47,24,25 |
| 6 | Flush to A | 32,46,26 | 60 | 11 to Waste | 53,20,25 |
| 7 | Flush to G | 32,46,27 | 61 | 12 to Waste | 53,21,25 |
| 8 | Flush to C | 32,46,28 | 62 | 14 to Waste | 52,22,25 |
| 9 | Flush to T | 32,46,29 | 63 | 15 to Waste | 51,23,25 |
| 10 | Flush to 5 | 32,46,30 | 64 | 18 to Waste | 54,33,25 |
| 11- 13 | INVALID FNCTION | | 65-69 | INVALID FNCTION | |
| 14 | Flush to Bases | 32,46 | 70 | 18 to A | 54,46,33,26 |
| 15 | Flush to Tet | 35,31 | 71 | 18 to G | 54,46,33,27 |
| 16 | Flush to 10 | 35,24 | 72 | 18 to C | 54,33,46,28 |
| 17 | Flush to 11 + 12 | 35,20,21 | 73 | 18 to T | 54,33,46,29 |
| 18 | Flush to 14 + 15 | 35,22,23 | 74 | 18 to 5 | 54,33,46,30 |
| 19 | Flush to 18 | 33,32 | 75-77 | INVALID FNCTION | |
| 20- 24 | INVALID FNCTION | | 78 | 18 to Tet | 54,33,31 |
| 25 | A to Column | 45,26,16,3,17, 7,18,11,19,15 | 79 | 18 to 10 | 54,33,24 |
| 26 | G to Column | 45,27,16,3,17, 7,18,11,19,15 | 80 | 18 to 11 | 54,33,20 |
| 27 | C to Column | 45,28,16,3,17, 7,18,11,19,15 | 81 | 18 to 12 | 54,33,21 |
| 28 | T to Column | 45,29,16,3,17, 7,18,11,19,15 | 82 | 18 to 11 + 12 | 54,33,20,21 |
| 29 | 5 to Column | 45,30,16,3,17, 7,18,11,19,15 | 83 | 18 to 14 | 54,33,22 |
| 30-32 | INVALID FNCTION | | 84 | 18 to 15 | 54,33,23 |
| 33 | B+Tet to Column | 45,49,31,16, 3,17,7,18,11, | 85 | 18 to 14 + 15 | 54,33,22,23 |
| | | 19,15 | 86 | 18 To Bases | 51,33,46 |
| 34 | Tet to Column | 49,31,16,3,17,7,18,11,19,15 | 87-89 | INVALID FNCTION | |
| 35 | 5+Tet to Column | 45,30,49,31,16, 3,17, 7,18 | 90 | 15 to A | 51,46,23,26 |
| | | 11,19,15 | 91 | 15 to G | 51,46,23,27 |
| 36 | 10 to Collect | 47,24,16,1,17,5,18,9,19,13 | 92 | 15 to C | 51,46,23,28 |
| 37 | 11 to Column | 53,20,16,3,17,7,18,11,19,15 | 93 | 15 to T | 51,46,23,29 |
| 38 | 12 to Column | 53,21,16,3,17,7,18,11,19,15 | 94 | 15 to Bases | 51,46,23 |
| 39 | Cap to Column | 53,20,21,16,3,17,7,18,11, | 95- 99 | INVALID FNCTION | |
| | | 19,15 | 100 | 10 Vent | 48 |
| 40 | 14 to Column | 52,22,16,3,17,7,18,11,19,15 | 101 | Phos Prep | 45,49 |
| 41 | 15 to Column | 51,23,16,3,17,7,18,11,19,15 | 102 | Cap Prep | 53 |
| 42 | 18 to Column | 54,33,16,3,17,7,18,11,19,15 | 103 | Wait | |
| 43 | Push to Column | 54,33,16,3,17,7,18,11,19,15 | 104 | Interrupt | |
| 44-49 | INVALID FNCTION | | 105 | Start Detrityl | |
| 50 | A to Waste | 45,26,25 | 106 | Begin | |
| 51 | G to Waste | 45,27,25 | 107 | End | |
| 52 | C to Waste | 45,28,25 | 108 | Block to Col | 16,3,17,7,18,11,19,15 |
| | | | 109 | Waste - Port | |

# Table AI-3 (cont.)

| Numb. | Name | Valve List |
|---|---|---|
| 110 | Waste - Bottle | |
| 111 | Block Vent | 25, 3, 7,11,15 |
| 112 | Trityl Advance | |
| 113 | End Advance | |
| 114 | Vent Bases | 46 |
| 115 | Prep 10 | 47 |
| 116-119 | INVALID FNCTION | |
| 120 | Advance FC | 59 |
| 121 | Relay 1 On | 59 |
| 122 | Relay 1 Off | 59 |
| 123 | Relay 1 Pulse | 59 |
| 124 | Relay 2 On | 60 |
| 125 | Relay 2 Off | 60 |
| 126 | Relay 2 Pulse | 60 |
| 127 | Relay 3 On | 61 |
| 128 | Relay 3 Off | 61 |
| 129 | Relay 3 Pulse | 61 |
| 130 | Relay 4 On | 62 |
| 131 | Relay 4 Off | 62 |
| 132 | Relay 4 Pulse | 62 |
| 133 | Relay 5 On | 63 |
| 134 | Relay 5 Off | 63 |
| 135-139 | INVALID FNCTION | |
| 140 | Column 1 On | |
| 141 | Column 1 Off | |
| 142 | Column 2 On | |
| 143 | Column 2 Off | |
| 144 | Column 3 On | |
| 145 | Column 3 Off | |
| 146 | Column 4 On | |
| 147 | Column 4 Off | |
| 148-149 | INVALID FNCTION | |
| 150 | Bottle 01 On | |
| 151 | Bottle 01 Off | |
| 152 | Bottle 02 On | |
| 153 | Bottle 02 Off | |
| 154 | Bottle 03 On | |
| 155 | Bottle 03 Off | |
| 156 | Bottle 04 On | |
| 157 | Bottle 04 Off | |
| 158 | Bottle 05 On | |
| 159 | Bottle 05 Off | |
| 160-169 | INVALID FNCTION | |
| 170 | Dilute 'A' | 54,46,33,26 |
| 171 | Dilute 'G' | 54,46,33,27 |
| 172 | Dilute 'C' | 54,33,46,28 |
| 173 | Dilute 'T' | 54,33,46,29 |
| 174 | Dilute '5' | 54,33,46,30 |
| 175-199 | INVALID FNCTION | |
| 200 | User01 | |
| 201 | User02 | |
| 202 | User03 | |
| 203 | User04 | |
| 204 | User05 | |
| 205 | User06 | |
| 206 | User07 | |
| 207 | User08 | |
| 208 | User09 | |
| 209 | User10 | |
| 210 | User11 | |
| 211 | User12 | |
| 212 | User13 | |
| 213 | User14 | |
| 214 | User15 | |
| 215 | User16 | |
| 216 | User17 | |
| 217 | User18 | |
| 218 | User19 | |
| 219 | User20 | |
| 220 | User21 | |
| 221 | User22 | |
| 222 | User23 | |
| 223 | User24 | |
| 224 | User25 | |

# Table AI-4
# 394 Sequential Function List
# (four-column/eight-base instrument)

| Numb. | Name | Valve List | Numb. | Name | Valve List |
|---|---|---|---|---|---|
| 1 | Block Flush | 40,35,25,0,3,4,7,8,11,12,15 | 43 | Push to Column | 54,42,35,16,3,17,7,18, 11, 19,15 |
| 2 | Reverse Flush | 25,0,16,4,17,8,18,12,19 | | | |
| 3 | Trityl Flush | 40,35,16,2,17,6,18,10,19,14 | 44-49 | INVALID FNCTION | |
| 4 | Flush to Waste | 40,35,16,3,17,7,18,11,19,15 | 50 | A to Waste | 45,26,25 |
| 5 | Flush to Colect | 40,35,16,1,17,5,18,9,19,13 | 51 | G to Waste | 45,27,25 |
| 6 | Flush to A | 43,35,46,26 | 52 | C to Waste | 45,28,25 |
| 7 | Flush to G | 43,35,46,27 | 53 | T to Waste | 45,29,25 |
| 8 | Flush to C | 43,35,46,28 | 54 | 5 to Waste | 45,30,25 |
| 9 | Flush to T | 43,35,46,29 | 55 | 6 to Waste | 45,31,25 |
| 10 | Flush to 5 | 43,35,46,30 | 56 | 7 to Waste | 45,32,25 |
| 11 | Flush to 6 | 43,35,46,31 | 57 | 8 to Waste | 45,33,25 |
| 12 | Flush to 7 | 43,35,46,32 | 58 | TET to Waste | 49,34,25 |
| 13 | Flush to 8 | 43,35,46,33 | 59 | 10 to Waste | 47,24,25 |
| 14 | Flush to Bases | 43,35,46 | 60 | 11 to Waste | 53,20,25 |
| 15 | Flush to Tet | 40,35,34 | 61 | 12 to Waste | 53,21,25 |
| 16 | Flush to 10 | 40,35,24 | 62 | 14 to Waste | 52,22,25 |
| 17 | Flush to 11 + 12 | 40,35,20,21 | 63 | 15 to Waste | 51,23,25 |
| 18 | Flush to 14 + 15 | 40,35,22,23 | 64 | 18 to Waste | 54,42,35,25 |
| 19 | Flush to 18 | 43,42 | 65-69 | INVALID FNCTION | |
| 20- 24 | INVALID FNCTION | | 70 | 18 to A | 54,42,46,35,26 |
| 25 | A to Column | 45,26,16,3,17,7,18,11,19,15 | 71 | 18 to G | 54,42,46,35,27 |
| 26 | G to Column | 45,27,16,3,17,7,18,11,19,15 | 72 | 18 to C | 54,42,46,35,28 |
| 27 | C to Column | 45,28,16,3,17,7,18,11,19,15 | 73 | 18 to T | 54,42,46,35,29 |
| 28 | T to Column | 45,29,16,3,17,7,18,11,19,15 | 74 | 18 to 5 | 54,42,46,35,30 |
| 29 | 5 to Column | 45,30,16,3,17,7,18,11,19,15 | 75 | 18 to 6 | 54,42,46,35,31 |
| 30 | 6 to Column | 45,31,16,3,17,7,18,11,19,15 | 76 | 18 to 7 | 54,42,46,35,32 |
| 31 | 7 to Column | 45,32,16,3,17,7,18,11,19,15 | 77 | 18 to 8 | 54,42,46,35,33 |
| 32 | 8 to Column | 45,33,16,3,17,7,18,11,19,15 | 78 | 18 to Tet | 54,42,35,34 |
| 33 | B+Tet to Column | 45,49,34,16,3,17,7,18,11, 19,15 | 79 | 18 to 10 | 54,42,35,24 |
| | | | 80 | 18 to 11 | 54,42,35,20 |
| 34 | Tet to Column | 49,34,16,3,17,7,18,11,19,15 | 81 | 18 to 12 | 54,42,35,21 |
| | | | 82 | 18 to 11 + 12 | 54,42,35,20,21 |
| 35 | 5+Tet to Column | 45,30,49,34,16,3,17,7,18, 11,19,15 | 83 | 18 to 14 | 54,42,35,22 |
| | | | 84 | 18 to 15 | 54,42,35,23 |
| 36 | 10 to Collect | 47,24,16,1,17,5,18,9,19,13 | 85 | 18 to 14 +15 | 54,42,35,22,23 |
| 37 | 11 to Column | 53,20,16,3,17,7,18,11,19,15 | 85 | 18 to Bases | 54,42,35,46 |
| 38 | 12 to Column | 53,21,16,3,17,7,18,11,19,15 | 87- 89 | INVALID FNCTION | |
| 39 | Cap to Column | 53,20,21,16,3,17,7,18,11, 19,15 | 90 | 15 to A | 51,46,23,26 |
| | | | 91 | 15 to G | 51,46,23,27 |
| 40 | 14 to Column | 52,22,16,3,17,7,18,11,19,15 | 92 | 15 to C | 51,46,23,28 |
| 41 | 15 to Column | 51,23,16,3,17,7,18,11,19,15 | 93 | 15 to T | 51,46,23,29 |
| 42 | 18 to Column | 54,42,35,16,3,17,7,18,11, 19,15 | 94 | 15 to Bases | 51,46,23 |

# Table AI-4 (cont.)

| Numb. | Name | Valve List |
|---|---|---|
| 95- 99 | INVALID FNCTION | |
| 100 | 10 Vent | 48 |
| 101 | Phos Prep | 45,49 |
| 102 | Cap Prep | 53 |
| 103 | Wait | |
| 104 | Interrupt | |
| 105 | Start Detrityl | |
| 106 | Begin | |
| 107 | End | |
| 108 | Block to Col | 16,3,17,7,18,11,19,15 |
| 109 | Waste - Port | |
| 110 | Waste - Bottle | |
| 111 | Block Vent | 25, 3, 7,11,15 |
| 112 | Trityl Advance | |
| 113 | End Advance | |
| 114 | Vent Bases | |
| 115 | Prep 10 | |
| 116-119 | INVALID FNCTION | |
| 120 | Advance FC | 59 |
| 121 | Relay 1 On | 59 |
| 122 | Relay 1 Off | 59 |
| 123 | Relay 1 Pulse | 59 |
| 124 | Relay 2 On | 60 |
| 125 | Relay 2 Off | 60 |
| 126 | Relay 2 Pulse | 60 |
| 127 | Relay 3 On | 61 |
| 128 | Relay 3 Off | 61 |
| 129 | Relay 3 Pulse | 61 |
| 130 | Relay 4 On | 62 |
| 131 | Relay 4 Off | 62 |
| 132 | Relay 4 Pulse | 62 |
| 133 | Relay 5 On | 63 |
| 134 | Relay 5 Off | 63 |
| 135-139 | INVALID FNCTION | |
| 140 | Column 1 On | |
| 141 | Column 1 Off | |
| 142 | Column 2 On | |
| 143 | Column 2 Off | |
| 144 | Column 3 On | |
| 145 | Column 3 Off | |
| 146 | Column 4 On | |
| 147 | Column 4 Off | |
| 148-149 | INVALID FNCTION | |

| Numb. | Name | Valve List |
|---|---|---|
| 150 | Bottle01 On | |
| 151 | Bottle01 Off | |
| 152 | Bottle02 On | |
| 153 | Bottle02 Off | |
| 154 | Bottle03 On | |
| 155 | Bottle03 Off | |
| 156 | Bottle04 Off | |
| 157 | Bottle04 Off | |
| 158 | Bottle05 On | |
| 159 | Bottle05 Off | |
| 160 | Bottle06 On | |
| 161 | Bottle06 Off | |
| 162 | Bottle07 On | |
| 163 | Bottle07 Off | |
| 164 | Bottle08 On | |
| 165 | Bottle08 Off | |
| 166-169 | INVALID FNCTION | |
| 170 | Dilute 'A' | 54,42,46,35,26 |
| 171 | Dilute 'G' | 54,42,46,35,27 |
| 172 | Dilute 'C' | 54,42,46,35,28 |
| 173 | Dilute 'T' | 54,42,46,35,29 |
| 174 | Dilute '5' | 54,42,46,35,30 |
| 175 | Dilute '6' | 54,42,46,35,31 |
| 176 | Dilute '7' | 54,42,46,35,32 |
| 177 | Dilute '8' | 54,42,46,35,33 |
| 178-199 | INVALID FNCTION | |
| 200 | User01 | |
| 201 | User02 | |
| 202 | User03 | |
| 203 | User04 | |
| 204 | User05 | |
| 205 | User06 | |
| 206 | User07 | |
| 207 | User08 | |
| 208 | User09 | |
| 209 | User10 | |
| 210 | User11 | |
| 211 | User12 | |
| 212 | User13 | |
| 213 | User14 | |
| 214 | User15 | |
| 215 | User16 | |
| 216 | User17 | |

# Table AI-4 (cont.)

| Numb. | Name | Valve List | Numb. | Name | Valve List |
|---|---|---|---|---|---|
| 217 | User18 | | | | |
| 218 | User19 | | | | |
| 219 | User20 | | | | |
| 220 | User21 | | | | |
| 221 | User22 | | | | |
| 222 | User23 | | | | |
| 223 | User24 | | | | |
| 224 | User25 | | | | |

# Table AI-5
# Primer Cycle
# (two-column instrument)

**Name : Primer** **9:53:57P, 1/ 4/91**
**Steps: 83**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 106 | Begin | | | | | | | Yes |
| 2 | 64 | 18 to Waste | 3.0 | | | | | | Yes |
| 3 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 2 | Reverse Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | | | | | Yes |
| 7 | 140 | Column 1 On | | | | | | | Yes |
| 8 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 34 | Tet to Column | 1.0 | Yes, | Yes | Yes | Yes | Yes | Yes |
| 10 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 11 | 34 | Tet to Column | 1.3 | Yes | Yes | Yes | Yes | Yes | Yes |
| 12 | 4 | Flush to Waste | 0.6 | No | No | No | No | Yes | Yes |
| 13 | 141 | Column 1 Off | | | | | | | Yes |
| 14 | 142 | Column 2 On | | | | | | | Yes |
| 15 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 16 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 17 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 18 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 19 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 20 | 34 | Tet to Column | 1.6 | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 4 | Flush to Waste | 0.6 | No | No | No | No | Yes | Yes |
| 22 | 143 | Column 2 Off | | | | | | | Yes |
| 23 | 4 | Flush to Waste | 0.9 | Yes | Yes | Yes | Yes | Yes | Yes |
| 24 | 103 | Wait | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 25 | 2 | Reverse Flush | 0.4 | Yes | Yes | Yes | Yes | Yes | Yes |
| 26 | 103 | Wait | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 27 | 4 | Flush to Waste | 0.7 | Yes | Yes | Yes | Yes | Yes | Yes |
| 28 | 103 | Wait | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 29 | 2 | Reverse Flush | 0.4 | Yes | Yes | Yes | Yes | Yes | Yes |
| 30 | 103 | Wait | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 31 | 4 | Flush to Waste | 0.7 | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 103 | Wait | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 33 | 2 | Reverse Flush | 0.4 | Yes | Yes | Yes | Yes | Yes | Yes |
| 34 | 103 | Wait | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 35 | 4 | Flush to Waste | 0.7 | Yes | Yes | Yes | Yes | Yes | Yes |
| 36 | 102 | Cap Prep | 3.0 | | | | | | Yes |
| 37 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 38 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |

# Table AI-5 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|
| 40 | 39 | Cap to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 41 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 43 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 44 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 45 | 41 | 15 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 64 | 18 to Waste | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 47 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 48 | 103 | Wait | 15.0 | | | | | | Yes |
| 48 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 50 | 4 | Flush to Waste | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 51 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 53 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 54 | 105 | Start Detrityl | | | | | | | Yes |
| 55 | 64 | 18 to Waste | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 56 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 57 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 58 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 59 | 112 | Trityl Advance | | | | | | | Yes |
| 60 | 109 | Waste - Port | | | | | | | Yes |
| 61 | 120 | Advance FC | | | | | | | Yes |
| 62 | 120 | Advance FC | | | | | | | Yes |
| 63 | 120 | Advance FC | | | | | | | Yes |
| 64 | 120 | Advance FC | | | | | | | Yes |
| 65 | 113 | End Advance | | | | | | | Yes |
| 66 | 40 | 14 to Column | 6.0 | No | No | Yes | Yes | Yes | No |
| 67 | 3 | Trityl Flush | 5.0 | No | No | Yes | Yes | Yes | No |
| 68 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 69 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 70 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 71 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 72 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 73 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 74 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 75 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 76 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 77 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | No |
| 78 | 3 | Trityl Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | No |
| 79 | 110 | Waste - Bottle | | | | | | | Yes |
| 80 | 42 | 18 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 81 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 82 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 83 | 107 | End | | | | | | | Yes |

# Table AI-6
# Primer Cycle
# (four-column instrument)

**Name : Primer** **1:00:46A, 1/ 1/91**
**Steps: 101**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 106 | Begin | | | | | | | | | | Yes |
| 2 | 64 | 18 to Waste | 3.0 | | | | | | | | | Yes |
| 3 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 2 | Reverse Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | | | | | | | | Yes |
| 7 | 140 | Column 1 On | | | | | | | | | | Yes |
| 8 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 10 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 11 | 34 | Tet to Column | 1.3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 12 | 4 | Flush to Waste | 0.6 | No | No | No | No | Yes | Yes | Yes | Yes | Yes |
| 13 | 141 | Column 1 Off | | | | | | | | | | Yes |
| 14 | 142 | Column 2 On | | | | | | | | | | Yes |
| 15 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 16 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 17 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 18 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 19 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 20 | 34 | Tet to Column | 1.6 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 4 | Flush to Waste | 0.6 | No | No | No | No | Yes | Yes | Yes | Yes | Yes |
| 22 | 143 | Column 2 Off | | | | | | | | | | Yes |
| 23 | 144 | Column 3 On | | | | | | | | | | Yes |
| 24 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 25 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 26 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 27 | 34 | Tet to Column | 1.0 | | | | | | | | | Yes |
| 28 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 29 | 34 | Tet to Column | 1.7 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 30 | 4 | Flush to Waste | 0.6 | No | No | No | No | Yes | Yes | Yes | Yes | Yes |
| 31 | 145 | Column 3 Off | | | | | | | | | | Yes |
| 32 | 146 | Column 4 On | | | | | | | | | | Yes |
| 33 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 34 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 35 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 36 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 37 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 38 | 34 | Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 4 | Flush to Waste | 0.6 | No | No | No | No | Yes | Yes | Yes | Yes | Yes |

# Table AI-6 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 40 | 147 | Column 4 Off | | | | | | | | | | Yes |
| 41 | 4 | Flush to Waste | 0.9 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 103 | Wait | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 2 | Reverse Flush | 0.4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 44 | 103 | Wait | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 45 | 4 | Flush to Waste | 0.7 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 103 | Wait | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 47 | 2 | Reverse Flush | 0.4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 48 | 103 | Wait | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 4 | Flush to Waste | 0.7 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 50 | 103 | Wait | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 51 | 2 | Reverse Flush | 0.4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 103 | Wait | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 53 | 4 | Flush to Waste | 0.7 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 54 | 102 | Cap Prep | 3.0 | | | | | | | | | Yes |
| 55 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 56 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 57 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 58 | 39 | Cap to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 59 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 60 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 61 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 62 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 63 | 41 | 15 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 64 | 64 | 18 to Waste | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 65 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 66 | 103 | Wait | 15.0 | | | | | | | | | Yes |
| 67 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 68 | 4 | Flush to Waste | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 69 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 70 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 71 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 72 | 105 | Start Detrityl | | | | | | | | | | Yes |
| 73 | 64 | 18 to Waste | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 74 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 75 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 76 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 77 | 112 | Trityl Advance | | | | | | | | | | Yes |
| 78 | 109 | Waste - Port | | | | | | | | | | Yes |
| 79 | 120 | Advance FC | | | | | | | | | | Yes |
| 80 | 120 | Advance FC | | | | | | | | | | Yes |
| 81 | 120 | Advance FC | | | | | | | | | | Yes |
| 82 | 120 | Advance FC | | | | | | | | | | Yes |
| 83 | 113 | End Advance | | | | | | | | | | Yes |

# Table AI-6 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 84 | 40 | 14 to Column | 6.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 85 | 3 | Trityl Flush | 5.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 86 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 87 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 88 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 89 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 90 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 91 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 92 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 93 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 94 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 95 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 96 | 3 | Trityl Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 97 | 110 | Waste - Bottle | | | | | | | | | | Yes |
| 98 | 42 | 18 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 99 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 100 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 101 | 107 | End | | | | | | | | | | Yes |

# Table AI-7
# .2 um CE Cycle
# (two-column instrument)

**Name : .2 um CE** **9:55:13P, 1/ 4/91**
**Steps: 73**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A G C T 5 | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 64 | 18 to Waste | 3.0 | | Yes |
| 3 | 42 | 18 to Column | 10.0 | Yes Yes Yes Yes Yes | Yes |
| 4 | 2 | Reverse Flush | 8.0 | Yes Yes Yes Yes Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | Yes |
| 7 | 140 | Column 1 On | | | Yes |
| 8 | 111 | Block Vent | 2.0 | Yes Yes Yes Yes Yes | Yes |
| 9 | 58 | Tet to Waste | 1.7 | | Yes |
| 10 | 33 | B+Tet to Column | 2.0 | Yes Yes Yes Yes Yes | Yes |
| 11 | 34 | Tet to Column | 1.0 | Yes Yes Yes Yes Yes | Yes |
| 12 | 33 | B+Tet to Column | 1.5 | Yes Yes Yes Yes Yes | Yes |
| 13 | 43 | Push to Column | | | Yes |
| 14 | 141 | Column 1 Off | | | Yes |
| 15 | 142 | Column 2 On | | | Yes |
| 16 | 64 | 18 to Waste | 4.0 | | Yes |
| 17 | 1 | Block Flush | 3.0 | Yes Yes Yes Yes Yes | Yes |
| 18 | 111 | Block Vent | 2.0 | Yes Yes Yes Yes Yes | Yes |
| 19 | 58 | Tet to Waste | 1.7 | | Yes |
| 20 | 33 | B+Tet to Column | 2.0 | Yes Yes Yes Yes Yes | Yes |
| 21 | 34 | Tet to Column | 1.0 | Yes Yes Yes Yes Yes | Yes |
| 22 | 33 | B+Tet to Column | 1.5 | Yes Yes Yes Yes Yes | Yes |
| 23 | 43 | Push to Column | | | Yes |
| 24 | 143 | Column 2 Off | | | Yes |
| 25 | 103 | Wait | 25.0 | Yes Yes Yes Yes Yes | Yes |
| 26 | 102 | Cap Prep | 3.0 | | Yes |
| 27 | 64 | 18 to Waste | 4.0 | | Yes |
| 28 | 2 | Reverse Flush | 5.0 | Yes Yes Yes Yes Yes | Yes |
| 29 | 1 | Block Flush | 3.0 | Yes Yes Yes Yes Yes | Yes |
| 30 | 39 | Cap to Column | 10.0 | Yes Yes Yes Yes Yes | Yes |
| 31 | 103 | Wait | 5.0 | Yes Yes Yes Yes Yes | Yes |
| 32 | 64 | 18 to Waste | 4.0 | | Yes |
| 33 | 2 | Reverse Flush | 5.0 | Yes Yes Yes Yes Yes | Yes |
| 34 | 1 | Block Flush | 3.0 | Yes Yes Yes Yes Yes | Yes |
| 35 | 41 | 15 to Column | 8.0 | Yes Yes Yes Yes Yes | Yes |
| 36 | 64 | 18 to Waste | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 37 | 1 | Block Flush | 3.0 | Yes Yes Yes Yes Yes | Yes |
| 38 | 103 | Wait | 15.0 | Yes Yes Yes Yes Yes | Yes |
| 39 | 42 | 18 to Column | 10.0 | Yes Yes Yes Yes Yes | Yes |

## Table AI-7 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|
| 40 | 4 | Flush to Waste | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 41 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 44 | 105 | Start Detrityl | | | | | | | Yes |
| 45 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 46 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 47 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 48 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 112 | Trityl Advance | | | | | | | Yes |
| 50 | 109 | Waste - Port | | | | | | | Yes |
| 51 | 120 | Advance FC | | | | | | | Yes |
| 52 | 120 | Advance FC | | | | | | | Yes |
| 53 | 120 | Advance FC | | | | | | | Yes |
| 54 | 120 | Advance FC | | | | | | | Yes |
| 55 | 113 | End Advance | | | | | | | Yes |
| 56 | 40 | 14 to Column | 6.0 | No | No | Yes | Yes | Yes | No |
| 57 | 3 | Trityl Flush | 5.0 | No | No | Yes | Yes | Yes | No |
| 58 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 59 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 60 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 61 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 62 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 63 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 64 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 65 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 66 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 67 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | No |
| 68 | 3 | Trityl Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | No |
| 69 | 110 | Waste - Bottle | | | | | | | Yes |
| 70 | 42 | 18 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 71 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 72 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 73 | 107 | End | | | | | | | Yes |

# Table AI-8
# .2 um CE Cycle
# (four-column instrument)

**Name : .2 um CE** **1:02:14A, 1/ 1/91**
**Steps: 93**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 106 | Begin | | | | | | | | | | Yes |
| 2 | 64 | 18 to Waste | 3.0 | | | | | | | | | Yes |
| 3 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 2 | Reverse Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | | | | | | | | Yes |
| 7 | 140 | Column 1 On | | | | | | | | | | Yes |
| 8 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 10 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 11 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 12 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 13 | 43 | Push to Column | | | | | | | | | | Yes |
| 14 | 141 | Column 1 Off | | | | | | | | | | Yes |
| 15 | 142 | Column 2 On | | | | | | | | | | Yes |
| 16 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 17 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 18 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 19 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 20 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 22 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 23 | 43 | Push to Column | | | | | | | | | | Yes |
| 24 | 143 | Column 2 Off | | | | | | | | | | Yes |
| 25 | 144 | Column 3 On | | | | | | | | | | Yes |
| 26 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 27 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 28 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 29 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 30 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 31 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

## Table AI-8 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 33 | 43 | Push to Column | | | | | | | | | | Yes |
| 34 | 145 | Column 3 Off | | | | | | | | | | Yes |
| 35 | 146 | Column 4 On | | | | | | | | | | Yes |
| 36 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 37 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 38 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 40 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 41 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 43 | Push to Column | | | | | | | | | | Yes |
| 44 | 147 | Column 4 Off | | | | | | | | | | Yes |
| 45 | 103 | Wait | 25.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 102 | Cap Prep | 3.0 | | | | | | | | | Yes |
| 47 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 48 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 50 | 39 | Cap to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 51 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 53 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 54 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 55 | 41 | 15 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 56 | 64 | 18 to Waste | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 57 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 58 | 103 | Wait | 15.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 59 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 60 | 4 | Flush to Waste | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 61 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 62 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 63 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 64 | 105 | Start Detrityl | | | | | | | | | | Yes |
| 65 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 66 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 67 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 68 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 69 | 112 | Trityl Advance | | | | | | | | | | Yes |
| 70 | 109 | Waste - Port | | | | | | | | | | Yes |
| 71 | 120 | Advance FC | | | | | | | | | | Yes |
| 72 | 120 | Advance FC | | | | | | | | | | Yes |
| 73 | 120 | Advance FC | | | | | | | | | | Yes |
| 74 | 120 | Advance FC | | | | | | | | | | Yes |

# Table AI-8 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 75 | 113 | End Advance | | | | | | | | | | Yes |
| 76 | 40 | 14 to Column | 6.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 77 | 3 | Trityl Flush | 5.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 78 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 79 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 80 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 81 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 82 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 83 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 84 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 85 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 86 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 87 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 88 | 3 | Trityl Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 89 | 110 | Waste - Bottle | | | | | | | | | | Yes |
| 90 | 42 | 18 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 91 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 92 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 93 | 107 | End | | | | | | | | | | Yes |

# Table AI-9
# 1.0 um CE Cycle
# (two-column instrument)

**Name : 1.0 um CE** **9:56:21P, 1/ 4/91**
**Steps: 73**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 106 | Begin | | | | | | | Yes |
| 2 | 64 | 18 to Waste | 3.0 | | | | | | Yes |
| 3 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 2 | Reverse Flush | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | | | | | Yes |
| 7 | 140 | Column 1 On | | | | | | | Yes |
| 8 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 58 | Tet to Waste | 1.7 | | | | | | Yes |
| 10 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 11 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 12 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 13 | 43 | Push to Column | | | | | | | Yes |
| 14 | 141 | Column 1 Off | | | | | | | Yes |
| 15 | 142 | Column 2 On | | | | | | | Yes |
| 16 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 17 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 18 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 19 | 58 | Tet to Waste | 1.7 | | | | | | Yes |
| 20 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 22 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 23 | 43 | Push to Column | | | | | | | Yes |
| 24 | 143 | Column 2 Off | | | | | | | Yes |
| 25 | 103 | Wait | 25.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 26 | 102 | Cap Prep | 3.0 | | | | | | Yes |
| 27 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 28 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 29 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 30 | 39 | Cap to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 31 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 33 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 34 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 35 | 41 | 15 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 36 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 37 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 38 | 103 | Wait | 15.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 40 | 4 | Flush to Waste | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes |

## Table AI-9 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|
| 41 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 44 | 105 | Start Detrityl | | | | | | | Yes |
| 45 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 46 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 47 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 48 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 112 | Trityl Advance | | | | | | | Yes |
| 50 | 109 | Waste - Port | | | | | | | Yes |
| 51 | 120 | Advance FC | | | | | | | Yes |
| 52 | 120 | Advance FC | | | | | | | Yes |
| 53 | 120 | Advance FC | | | | | | | Yes |
| 54 | 120 | Advance FC | | | | | | | Yes |
| 55 | 113 | End Advance | | | | | | | Yes |
| 56 | 40 | 14 to Column | 6.0 | No | No | Yes | Yes | Yes | No |
| 57 | 3 | Trityl Flush | 5.0 | No | No | Yes | Yes | Yes | No |
| 58 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 59 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 60 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 61 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 62 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 63 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 64 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 65 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 66 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 67 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | No |
| 68 | 3 | Trityl Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | No |
| 69 | 110 | Waste - Bottle | | | | | | | Yes |
| 70 | 42 | 18 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 71 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 72 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 73 | 107 | End | | | | | | | Yes |

# Table AI-10
# 1.0 um CE Cycle
# (four-column instrument)

**Name : 1.0 um CE** **1:03:38A, 1/ 1/91**
**Steps: 93**

| Step Numb. | Function # Name | Step Time | Step Active for Bases A G C T 5 6 7 8 | Safe Step |
|---|---|---|---|---|
| 1 | 106 Begin | | | Yes |
| 2 | 64 18 to Waste | 3.0 | | Yes |
| 3 | 42 18 to Column | 10.0 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 4 | 2 Reverse Flush | 10.0 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 5 | 1 Block Flush | 4.0 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 6 | 101 Phos Prep | 3.0 | | Yes |
| 7 | 140 Column 1 On | | | Yes |
| 8 | 111 Block Vent | 2.0 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 9 | 58 Tet to Waste | 1.7 | | Yes |
| 10 | 33 B+Tet to Column | 2.5 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 11 | 34 Tet to Column | 1.0 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 12 | 33 B+Tet to Column | 2.5 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 13 | 43 Push to Column | | | Yes |
| 14 | 141 Column 1 Off | | | Yes |
| 15 | 142 Column 2 On | | | Yes |
| 16 | 64 18 to Waste | 4.0 | | Yes |
| 17 | 1 Block Flush | 3.0 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 18 | 111 Block Vent | 2.0 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 19 | 58 Tet to Waste | 1.7 | | Yes |
| 20 | 33 B+Tet to Column | 2.5 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 21 | 34 Tet to Column | 1.0 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 22 | 33 B+Tet to Column | 2.5 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 23 | 43 Push to Column | | | Yes |
| 24 | 143 Column 2 Off | | | Yes |
| 25 | 144 Column 3 On | | | Yes |
| 26 | 64 18 to Waste | 4.0 | | Yes |
| 27 | 1 Block Flush | 3.0 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 28 | 111 Block Vent | 2.0 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 29 | 58 Tet to Waste | 1.7 | | Yes |
| 30 | 33 B+Tet to Column | 2.5 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 31 | 34 Tet to Column | 1.0 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 32 | 33 B+Tet to Column | 2.5 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |
| 33 | 43 Push to Column | | | Yes |
| 34 | 145 Column 3 Off | | | Yes |
| 35 | 146 Column 4 On | | | Yes |
| 36 | 64 18 to Waste | 4.0 | | Yes |
| 37 | 1 Block Flush | 3.0 | Yes Yes Yes Yes Yes Yes Yes Yes | Yes |

## Table AI-10 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 38 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 40 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 41 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 43 | Push to Column | | | | | | | | | | Yes |
| 44 | 147 | Column 4 Off | | | | | | | | | | Yes |
| 45 | 103 | Wait | 25.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 102 | Cap Prep | 3.0 | | | | | | | | | Yes |
| 47 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 48 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 50 | 39 | Cap to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 51 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 53 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 54 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 55 | 41 | 15 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 56 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 57 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 58 | 103 | Wait | 15.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 59 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 60 | 4 | Flush to Waste | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 61 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 62 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 63 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 64 | 105 | Start Detrityl | | | | | | | | | | Yes |
| 65 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 66 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 67 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 68 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 69 | 112 | Trityl Advance | | | | | | | | | | Yes |
| 70 | 109 | Waste - Port | | | | | | | | | | Yes |
| 71 | 120 | Advance FC | | | | | | | | | | Yes |
| 72 | 120 | Advance FC | | | | | | | | | | Yes |
| 73 | 120 | Advance FC | | | | | | | | | | Yes |
| 74 | 120 | Advance FC | | | | | | | | | | Yes |
| 75 | 113 | End Advance | | | | | | | | | | Yes |
| 76 | 40 | 14 to Column | 6.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 77 | 3 | Trityl Flush | 5.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |

# Table AI-10 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 78 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 79 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 80 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 81 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 82 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 83 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 84 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 85 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 86 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 87 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 88 | 3 | Trityl Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 89 | 110 | Waste - Bottle | | | | | | | | | | Yes |
| 90 | 42 | 18 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 91 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 92 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 93 | 107 | End | | | | | | | | | | Yes |

# Table AI-11
# 10 um CE Cycle
# (two-column instrument)

**Name : 10 um CE** **9:57:28P, 1/ 4/91**
**Steps: 62**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A G C T 5 | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 64 | 18 to Waste | 4.0 | | Yes |
| 3 | 42 | 18 to Column | 80.0 | Yes Yes Yes Yes Yes | Yes |
| 4 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | Yes |
| 7 | 140 | Column 1 On | | | Yes |
| 8 | 34 | Tet to Column | 1.0 | Yes Yes Yes Yes Yes | Yes |
| 9 | 33 | B+Tet to Column | 22.5 | Yes Yes Yes Yes Yes | Yes |
| 10 | 141 | Column 1 Off | | | Yes |
| 11 | 142 | Column 2 On | | | Yes |
| 12 | 64 | 18 to Waste | 5.0 | | Yes |
| 13 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 14 | 34 | Tet to Column | 1.0 | Yes Yes Yes Yes Yes | Yes |
| 15 | 33 | B+Tet to Column | 22.5 | Yes Yes Yes Yes Yes | Yes |
| 16 | 143 | Column 2 Off | | | Yes |
| 17 | 103 | Wait | 40.0 | Yes Yes Yes Yes Yes | Yes |
| 18 | 64 | 18 to Waste | 5.0 | | Yes |
| 19 | 42 | 18 to Column | 30.0 | Yes Yes Yes Yes Yes | Yes |
| 20 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 21 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 22 | 102 | Cap Prep | 5.0 | | Yes |
| 23 | 39 | Cap to Column | 45.0 | Yes Yes Yes Yes Yes | Yes |
| 24 | 103 | Wait | 10.0 | Yes Yes Yes Yes Yes | Yes |
| 25 | 64 | 18 to Waste | 5.0 | | Yes |
| 26 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 27 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 28 | 41 | 15 to Column | 40.0 | Yes Yes Yes Yes Yes | Yes |
| 29 | 103 | Wait | 20.0 | Yes Yes Yes Yes Yes | Yes |
| 30 | 64 | 18 to Waste | 5.0 | | Yes |
| 31 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 32 | 42 | 18 to Column | 50.0 | Yes Yes Yes Yes Yes | Yes |
| 33 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 34 | 42 | 18 to Column | 50.0 | Yes Yes Yes Yes Yes | Yes |
| 35 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 36 | 42 | 18 to Column | 50.0 | Yes Yes Yes Yes Yes | Yes |

# Table AI-11 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|
| 37 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 38 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 40 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 41 | 105 | Start Detrityl | | | | | | | Yes |
| 42 | 64 | 18 to Waste | 5.0 | | | | | | Yes |
| 43 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 44 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 45 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 112 | Trityl Advance | | | | | | | Yes |
| 47 | 109 | Waste - Port | | | | | | | Yes |
| 48 | 120 | Advance FC | | | | | | | Yes |
| 49 | 120 | Advance FC | | | | | | | Yes |
| 50 | 120 | Advance FC | | | | | | | Yes |
| 51 | 120 | Advance FC | | | | | | | Yes |
| 52 | 113 | End Advance | | | | | | | Yes |
| 53 | 40 | 14 to Column | 110.0 | Yes | Yes | Yes | Yes | Yes | No |
| 54 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | No |
| 55 | 64 | 18 to Waste | 5.0 | | | | | | No |
| 56 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | No |
| 57 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | No |
| 58 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | No |
| 59 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 60 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 61 | 110 | Waste - Bottle | | | | | | | Yes |
| 62 | 107 | End | | | | | | | Yes |

# Table AI-12
# 10 um CE Cycle
# (four-column instrument)

**Name : 10 um CE** **1:05:00A, 1/ 1/91**
**Steps: 74**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 106 | Begin | | | | | | | | | | Yes |
| 2 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 3 | 42 | 18 to Column | 80.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | | | | | | | | Yes |
| 7 | 140 | Column 1 On | | | | | | | | | | Yes |
| 8 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 33 | B+Tet to Column | 22.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 10 | 141 | Column 1 Off | | | | | | | | | | Yes |
| 11 | 142 | Column 2 On | | | | | | | | | | Yes |
| 12 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 13 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 14 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 15 | 33 | B+Tet to Column | 22.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 16 | 143 | Column 2 Off | | | | | | | | | | Yes |
| 17 | 144 | Column 3 On | | | | | | | | | | Yes |
| 18 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 19 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 20 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 33 | B+Tet to Column | 22.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 22 | 145 | Column 3 Off | | | | | | | | | | Yes |
| 23 | 146 | Column 4 On | | | | | | | | | | Yes |
| 24 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 25 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 26 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 27 | 33 | B+Tet to Column | 22.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 28 | 147 | Column 4 Off | | | | | | | | | | Yes |
| 29 | 103 | Wait | 40.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 30 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 31 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 33 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 34 | 102 | Cap Prep | 5.0 | | | | | | | | | Yes |
| 35 | 39 | Cap to Column | 45.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 36 | 103 | Wait | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 37 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 38 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 40 | 41 | 15 to Column | 40.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 41 | 103 | Wait | 20.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

## Table AI-12 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 42 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 43 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 44 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 45 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 47 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 48 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 50 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 51 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 53 | 105 | Start Detrityl | | | | | | | | | | Yes |
| 54 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 55 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 56 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 57 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 58 | 112 | Trityl Advance | | | | | | | | | | Yes |
| 59 | 109 | Waste - Port | | | | | | | | | | Yes |
| 69 | 120 | Advance FC | | | | | | | | | | Yes |
| 61 | 120 | Advance FC | | | | | | | | | | Yes |
| 62 | 120 | Advance FC | | | | | | | | | | Yes |
| 63 | 120 | Advance FC | | | | | | | | | | Yes |
| 64 | 113 | End Advance | | | | | | | | | | Yes |
| 65 | 40 | 14 to Column | 110.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 66 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 67 | 64 | 18 to Waste | 5.0 | | | | | | | | | No |
| 68 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 69 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 70 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 71 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 72 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 73 | 110 | Waste - Bottle | | | | | | | | | | Yes |
| 74 | 107 | End | | | | | | | | | | |

# Table AI-13
# .2 um RNA Cycle
# (two-column instrument)

**Name : .2 um RNA** **9:58:28P, 1/ 4/91**
**Steps: 73**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 106 | Begin | | | | | | | Yes |
| 2 | 64 | 18 to Waste | 3.0 | | | | | | Yes |
| 3 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 2 | Reverse Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | | | | | Yes |
| 7 | 140 | Column 1 On | | | | | | | Yes |
| 8 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 58 | Tet to Waste | 1.7 | | | | | | Yes |
| 10 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 11 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 12 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 13 | 43 | Push to Column | | | | | | | Yes |
| 14 | 141 | Column 1 Off | | | | | | | Yes |
| 15 | 142 | Column 2 On | | | | | | | Yes |
| 16 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 17 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 18 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 19 | 58 | Tet to Waste | 1.7 | | | | | | Yes |
| 20 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 22 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 23 | 43 | Push to Column | | | | | | | Yes |
| 24 | 143 | Column 2 Off | | | | | | | Yes |
| 25 | 103 | Wait | 600.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 26 | 102 | Cap Prep | 3.0 | | | | | | Yes |
| 27 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 28 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 29 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 30 | 39 | Cap to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 31 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 33 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 34 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 35 | 41 | 15 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 36 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 37 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |

# Table AI-13 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|
| 38 | 103 | Wait | 15.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 40 | 4 | Flush to Waste | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 41 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 44 | 105 | Start Detrityl | | | | | | | Yes |
| 45 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 46 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 47 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 48 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 112 | Trityl Advance | | | | | | | Yes |
| 50 | 109 | Waste - Port | | | | | | | Yes |
| 51 | 120 | Advance FC | | | | | | | Yes |
| 52 | 120 | Advance FC | | | | | | | Yes |
| 53 | 120 | Advance FC | | | | | | | Yes |
| 54 | 120 | Advance FC | | | | | | | Yes |
| 55 | 113 | End Advance | | | | | | | Yes |
| 56 | 40 | 14 to Column | 6.0 | No | No | Yes | Yes | Yes | No |
| 57 | 3 | Trityl Flush | 5.0 | No | No | Yes | Yes | Yes | No |
| 58 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 59 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 60 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 61 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 62 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 63 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 64 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 65 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 66 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 67 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | No |
| 68 | 3 | Trityl Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | No |
| 69 | 110 | Waste - Bottle | | | | | | | Yes |
| 70 | 42 | 18 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 71 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 72 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 73 | 107 | End | | | | | | | Yes |

# Table AI-14
## .2 um RNA Cycle
## (four-column instrument)

**Name : .2 um RNA** **1:06:09A, 1/ 1/91**
**Steps: 93**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 106 | Begin | | | | | | | | | | Yes |
| 2 | 64 | 18 to Waste | 3.0 | | | | | | | | | Yes |
| 3 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 2 | Reverse Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | | | | | | | | Yes |
| 7 | 140 | Column 1 On | | | | | | | | | | Yes |
| 8 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 10 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 11 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 12 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 13 | 43 | Push to Column | | | | | | | | | | Yes |
| 14 | 141 | Column 1 Off | | | | | | | | | | Yes |
| 15 | 142 | Column 2 On | | | | | | | | | | Yes |
| 16 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 17 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 18 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 19 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 20 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 22 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 23 | 43 | Push to Column | | | | | | | | | | Yes |
| 24 | 143 | Column 2 Off | | | | | | | | | | Yes |
| 25 | 144 | Column 3 On | | | | | | | | | | Yes |
| 26 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 27 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 28 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 29 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 30 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 31 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 33 | 43 | Push to Column | | | | | | | | | | Yes |
| 34 | 145 | Column 3 Off | | | | | | | | | | Yes |
| 35 | 146 | Column 4 On | | | | | | | | | | Yes |
| 36 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 37 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 38 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 40 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

# Table AI-14 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 41 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 43 | Push to Column | | | | | | | | | | Yes |
| 44 | 147 | Column 4 Off | | | | | | | | | | Yes |
| 45 | 103 | Wait | 600.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 102 | Cap Prep | 3.0 | | | | | | | | | Yes |
| 47 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 48 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 50 | 39 | Cap to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 51 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 53 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 54 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 55 | 41 | 15 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 56 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 57 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 58 | 103 | Wait | 15.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 59 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 60 | 4 | Flush to Waste | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 61 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 62 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 63 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 64 | 105 | Start Detrityl | | | | | | | | | | Yes |
| 65 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 66 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 67 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 68 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 69 | 112 | Trityl Advance | | | | | | | | | | Yes |
| 70 | 109 | Waste - Port | | | | | | | | | | Yes |
| 71 | 120 | Advance FC | | | | | | | | | | Yes |
| 72 | 120 | Advance FC | | | | | | | | | | Yes |
| 73 | 120 | Advance FC | | | | | | | | | | Yes |
| 74 | 120 | Advance FC | | | | | | | | | | Yes |
| 75 | 113 | End Advance | | | | | | | | | | Yes |
| 76 | 40 | 14 to Column | 6.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 77 | 3 | Trityl Flush | 5.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 78 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 79 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 80 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 81 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 82 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 83 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 84 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 85 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |

## Table AI-14 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 86 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 87 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 88 | 3 | Trityl Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 89 | 110 | Waste - Bottle | | | | | | | | | | Yes |
| 90 | 42 | 18 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 91 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 92 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 93 | 107 | End | | | | | | | | | | Yes |

# Table AI-15
# 1.0 um RNA Cycle
# (two-column instrument)

**Name : 1.0 um RNA** **10:12:51P, 1/ 4/91**
**Steps: 73**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 106 | Begin | | | | | | | Yes |
| 2 | 64 | 18 to Waste | 3.0 | | | | | | Yes |
| 3 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 2 | Reverse Flush | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | | | | | Yes |
| 7 | 140 | Column 1 On | | | | | | | Yes |
| 8 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 58 | Tet to Waste | 1.7 | | | | | | Yes |
| 10 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 11 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 12 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 13 | 43 | Push to Column | | | | | | | Yes |
| 14 | 141 | Column 1 Off | | | | | | | Yes |
| 15 | 142 | Column 2 On | | | | | | | Yes |
| 16 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 17 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 18 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 19 | 58 | Tet to Waste | 1.7 | | | | | | Yes |
| 20 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 22 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 23 | 43 | Push to Column | | | | | | | Yes |
| 24 | | Column 2 Off | | | | | | | Yes |
| 25 | 103 | Wait | 600.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 26 | 102 | Cap Prep | 3.0 | | | | | | Yes |
| 27 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 28 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 29 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 30 | 39 | Cap to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 31 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 33 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 34 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 35 | 41 | 15 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 36 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 37 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 38 | 103 | Wait | 15.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 40 | 4 | Flush to Waste | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes |

# Table AI-15 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|
| 41 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 44 | 105 | Start Detrityl | | | | | | | Yes |
| 45 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 46 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 47 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 48 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 112 | Trityl Advance | | | | | | | Yes |
| 50 | 109 | Waste - Port | | | | | | | Yes |
| 51 | 120 | Advance FC | | | | | | | Yes |
| 52 | 120 | Advance FC | | | | | | | Yes |
| 53 | 120 | Advance FC | | | | | | | Yes |
| 54 | 120 | Advance FC | | | | | | | Yes |
| 55 | 113 | End Advance | | | | | | | Yes |
| 56 | 40 | 14 to Column | 6.0 | No | No | Yes | Yes | Yes | No |
| 57 | 3 | Trityl Flush | 5.0 | No | No | Yes | Yes | Yes | No |
| 58 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 59 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 60 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 61 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 62 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 63 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 64 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 65 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 66 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 67 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | No |
| 68 | 3 | Trityl Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | No |
| 69 | 110 | Waste - Bottle | | | | | | | Yes |
| 70 | 42 | 18 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 71 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 72 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 73 | 107 | End | | | | | | | Yes |

# Table AI-16
# 1.0 um RNA Cycle
# (four-column instrument)

**Name : 1.0 um RNA** **1:07:31A, 1/ 1/91**
**Steps: 93**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 106 | Begin | | | | | | | | | | Yes |
| 2 | 64 | 18 to Waste | 3.0 | | | | | | | | | Yes |
| 3 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 2 | Reverse Flush | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | | | | | | | | Yes |
| 7 | 140 | Column 1 On | | | | | | | | | | Yes |
| 8 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 10 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 11 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 12 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 13 | 43 | Push to Column | | | | | | | | | | Yes |
| 14 | 141 | Column 1 Off | | | | | | | | | | Yes |
| 15 | 142 | Column 2 On | | | | | | | | | | Yes |
| 16 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 17 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 18 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 19 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 20 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 22 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 23 | 43 | Push to Column | | | | | | | | | | Yes |
| 24 | 143 | Column 2 Off | | | | | | | | | | Yes |
| 25 | 144 | Column 3 On | | | | | | | | | | Yes |
| 26 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 27 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 28 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 29 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 30 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 31 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 33 | 43 | Push to Column | | | | | | | | | | Yes |
| 34 | 145 | Column 3 Off | | | | | | | | | | Yes |
| 35 | 146 | Column 4 On | | | | | | | | | | Yes |
| 36 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 37 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 38 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 40 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 41 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

# Table AI-16 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 42 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 43 | Push to Column | | | | | | | | | | Yes |
| 44 | 147 | Column 4 Off | | | | | | | | | | Yes |
| 45 | 103 | Wait | 600.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 102 | Cap Prep | 3.0 | | | | | | | | | Yes |
| 47 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 48 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 50 | 39 | Cap to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 51 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 53 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 54 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 55 | 41 | 15 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 56 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 57 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 58 | 103 | Wait | 15.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 59 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 60 | 4 | Flush to Waste | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 61 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 62 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 63 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 64 | 105 | Start Detrityl | | | | | | | | | | Yes |
| 65 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 66 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 67 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 68 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 69 | 112 | Trityl Advance | | | | | | | | | | Yes |
| 70 | 109 | Waste - Port | | | | | | | | | | Yes |
| 71 | 120 | Advance FC | | | | | | | | | | Yes |
| 72 | 120 | Advance FC | | | | | | | | | | Yes |
| 73 | 120 | Advance FC | | | | | | | | | | Yes |
| 74 | 120 | Advance FC | | | | | | | | | | Yes |
| 75 | 113 | End Advance | | | | | | | | | | Yes |
| 76 | 40 | 14 to Column | 6.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 77 | 3 | Trityl Flush | 5.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 78 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 79 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 80 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 81 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 82 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 83 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 84 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 85 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |

## Table AI-16 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 86 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 87 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 88 | 3 | Trityl Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 89 | 110 | Waste - Bottle | | | | | | | | | | Yes |
| 90 | 42 | 18 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 91 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 92 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 93 | 107 | End | | | | | | | | | | Yes |

# Table AI-17
# 10 um RNA Cycle
# (two-column instrument)

**Name : 10 um RNA** **10:15:09, 1/ 4/91**
**Steps: 62**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A G C T 5 | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 64 | 18 to Waste | 4.0 | | Yes |
| 3 | 42 | 18 to Column | 80.0 | Yes Yes Yes Yes Yes | Yes |
| 4 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | Yes |
| 7 | 140 | Column 1 On | | | Yes |
| 8 | 34 | Tet to Column | 1.0 | Yes Yes Yes Yes Yes | Yes |
| 9 | 33 | B+Tet to Column | 22.5 | Yes Yes Yes Yes Yes | Yes |
| 10 | 141 | Column 1 Off | | | Yes |
| 11 | 142 | Column 2 On | | | Yes |
| 12 | 64 | 18 to Waste | 5.0 | | Yes |
| 13 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 14 | 34 | Tet to Column | 1.0 | Yes Yes Yes Yes Yes | Yes |
| 15 | 33 | B+Tet to Column | 22.5 | Yes Yes Yes Yes Yes | Yes |
| 16 | 143 | Column 2 Off | | | Yes |
| 17 | 103 | Wait | 600.0 | Yes Yes Yes Yes Yes | Yes |
| 18 | 64 | 18 to Waste | 5.0 | Yes Yes Yes Yes Yes | Yes |
| 19 | 42 | 18 to Column | 30.0 | Yes Yes Yes Yes Yes | Yes |
| 20 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 21 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 22 | 102 | Cap Prep | 5.0 | | Yes |
| 23 | 39 | Cap to Column | 45.0 | Yes Yes Yes Yes Yes | Yes |
| 24 | 103 | Wait | 10.0 | Yes Yes Yes Yes Yes | Yes |
| 25 | 64 | 18 to Waste | 5.0 | | Yes |
| 26 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 27 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 28 | 41 | 15 to Column | 40.0 | Yes Yes Yes Yes Yes | Yes |
| 29 | 103 | Wait | 20.0 | Yes Yes Yes Yes Yes | Yes |
| 30 | 64 | 18 to Waste | 5.0 | | Yes |
| 31 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 32 | 42 | 18 to Column | 50.0 | Yes Yes Yes Yes Yes | Yes |
| 33 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 34 | 42 | 18 to Column | 50.0 | Yes Yes Yes Yes Yes | Yes |
| 35 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 36 | 42 | 18 to Column | 50.0 | Yes Yes Yes Yes Yes | Yes |
| 37 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 38 | 42 | 18 to Column | 50.0 | Yes Yes Yes Yes Yes | Yes |
| 39 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 40 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |

# Table AI-17 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|
| 41 | 105 | Start Detrityl | | | | | | | Yes |
| 42 | 64 | 18 to Waste | 5.0 | | | | | | Yes |
| 43 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 44 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 45 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 112 | Trityl Advance | | | | | | | Yes |
| 47 | 109 | Waste - Port | | | | | | | Yes |
| 48 | 120 | Advance FC | | | | | | | Yes |
| 49 | 120 | Advance FC | | | | | | | Yes |
| 50 | 120 | Advance FC | | | | | | | Yes |
| 51 | 120 | Advance FC | | | | | | | Yes |
| 52 | 113 | End Advance | | | | | | | Yes |
| 53 | 40 | 14 to Column | 110.0 | Yes | Yes | Yes | Yes | Yes | No |
| 54 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | No |
| 55 | 64 | 18 to Waste | 5.0 | | | | | | No |
| 56 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | No |
| 57 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | No |
| 58 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | No |
| 59 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | No |
| 60 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 61 | 110 | Waste - Bottle | | | | | | | Yes |
| 62 | 107 | End | | | | | | | Yes |

# Table AI-18
# 10 um RNA Cycle
# (four-column instrument)

**Name : 10 um RNA** **1:08:52A, 1/ 1/91**
**Steps: 74**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 106 | Begin | | | | | | | | | | Yes |
| 2 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 3 | 42 | 18 to Column | 80.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | | | | | | | | Yes |
| 7 | 140 | Column 1 On | | | | | | | | | | Yes |
| 8 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 33 | B+Tet to Column | 22.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 10 | 141 | Column 1 Off | | | | | | | | | | Yes |
| 11 | 142 | Column 2 On | | | | | | | | | | Yes |
| 12 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 13 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 14 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 15 | 33 | B+Tet to Column | 22.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 16 | 143 | Column 2 Off | | | | | | | | | | Yes |
| 17 | 144 | Column 3 On | | | | | | | | | | Yes |
| 18 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 19 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 20 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 33 | B+Tet to Column | 22.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 22 | 145 | Column 3 Off | | | | | | | | | | Yes |
| 23 | 146 | Column 4 On | | | | | | | | | | Yes |
| 24 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 25 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 26 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 27 | 33 | B+Tet to Column | 22.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 28 | 147 | Column 4 Off | | | | | | | | | | Yes |
| 29 | 103 | Wait | 600.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 30 | 64 | 18 to Waste | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 31 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 33 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 34 | 102 | Cap Prep | 5.0 | | | | | | | | | Yes |
| 35 | 39 | Cap to Column | 45.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 36 | 103 | Wait | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 37 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 38 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 40 | 41 | 15 to Column | 40.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 41 | 103 | Wait | 20.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

# Table AI-18 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 42 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 43 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 44 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 45 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 47 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 48 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 50 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 51 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 53 | 105 | Start Detrityl | | | | | | | | | | Yes |
| 54 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 55 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 56 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 57 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 58 | 112 | Trityl Advance | | | | | | | | | | Yes |
| 59 | 109 | Waste - Port | | | | | | | | | | Yes |
| 60 | 120 | Advance FC | | | | | | | | | | Yes |
| 61 | 120 | Advance FC | | | | | | | | | | Yes |
| 62 | 120 | Advance FC | | | | | | | | | | Yes |
| 63 | 120 | Advance FC | | | | | | | | | | Yes |
| 64 | 113 | End Advance | | | | | | | | | | Yes |
| 65 | 40 | 14 to Column | 110.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 66 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 67 | 64 | 18 to Waste | 5.0 | | | | | | | | | No |
| 68 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 69 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 70 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 71 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 72 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 73 | 110 | Waste - Bottle | | | | | | | | | | Yes |
| 74 | 107 | End | | | | | | | | | | Yes |

# Table AI-19
# .2 um Sulfur Cycle
# (two-column instrument)

**Name : .2 um Sulfur** **10:16:08P, 1/ 4/91**
**Steps: 78**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A G C T 5 | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 64 | 18 to Waste | 3.0 | | Yes |
| 3 | 42 | 18 to Column | 10.0 | Yes Yes Yes Yes Yes | Yes |
| 4 | 2 | Reverse Flush | 8.0 | Yes Yes Yes Yes Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | Yes |
| 7 | 140 | Column 1 On | | | Yes |
| 8 | 111 | Block Vent | 2.0 | Yes Yes Yes Yes Yes | Yes |
| 9 | 58 | Tet to Waste | 1.7 | | Yes |
| 10 | 33 | B+Tet to Column | 2.0 | Yes Yes Yes Yes Yes | Yes |
| 11 | 34 | Tet to Column | 1.0 | Yes Yes Yes Yes Yes | Yes |
| 12 | 33 | B+Tet to Column | 1.5 | Yes Yes Yes Yes Yes | Yes |
| 13 | 43 | Push to Column | | | Yes |
| 14 | 141 | Column 1 Off | | | Yes |
| 15 | 142 | Column 2 On | | | Yes |
| 16 | 64 | 18 to Waste | 4.0 | | Yes |
| 17 | 1 | Block Flush | 3.0 | Yes Yes Yes Yes Yes | Yes |
| 18 | 111 | Block Vent | 2.0 | Yes Yes Yes Yes Yes | Yes |
| 19 | 58 | Tet to Waste | 1.7 | | Yes |
| 20 | 33 | B+Tet to Column | 2.0 | Yes Yes Yes Yes Yes | Yes |
| 21 | 34 | Tet to Column | 1.0 | Yes Yes Yes Yes Yes | Yes |
| 22 | 33 | B+Tet to Column | 1.5 | Yes Yes Yes Yes Yes | Yes |
| 23 | 43 | Push to Column | | | Yes |
| 24 | 143 | Column 2 Off | | | Yes |
| 25 | 103 | Wait | 25.0 | Yes Yes Yes Yes Yes | Yes |
| 26 | 2 | Reverse Flush | 5.0 | Yes Yes Yes Yes Yes | Yes |
| 27 | 1 | Block Flush | 3.0 | Yes Yes Yes Yes Yes | Yes |
| 28 | 41 | 15 to Column | 8.0 | Yes Yes Yes Yes Yes | Yes |
| 29 | 64 | 18 to Waste | 4.0 | | Yes |
| 30 | 103 | Wait | 900.0 | Yes Yes Yes Yes Yes | Yes |
| 31 | 42 | 18 to Column | 10.0 | Yes Yes Yes Yes Yes | Yes |
| 32 | 4 | Flush to Waste | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 33 | 42 | 18 to Column | 10.0 | Yes Yes Yes Yes Yes | Yes |
| 34 | 2 | Reverse Flush | 5.0 | Yes Yes Yes Yes Yes | Yes |
| 35 | 1 | Block Flush | 3.0 | Yes Yes Yes Yes Yes | Yes |
| 36 | 102 | Cap Prep | 3.0 | | Yes |
| 37 | 64 | 18 to Waste | 4.0 | | Yes |
| 38 | 2 | Reverse Flush | 5.0 | Yes Yes Yes Yes Yes | Yes |
| 39 | 1 | Block Flush | 3.0 | Yes Yes Yes Yes Yes | Yes |

## Table AI-19 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|
| 40 | 39 | Cap to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 41 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 43 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 44 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 45 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 47 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 48 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 105 | Start Detrityl | | | | | | | Yes |
| 50 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 51 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 53 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 54 | 112 | Trityl Advance | | | | | | | Yes |
| 55 | 109 | Waste - Port | | | | | | | Yes |
| 56 | 120 | Advance FC | | | | | | | Yes |
| 57 | 120 | Advance FC | | | | | | | Yes |
| 58 | 120 | Advance FC | | | | | | | Yes |
| 59 | 120 | Advance FC | | | | | | | Yes |
| 60 | 113 | End Advance | | | | | | | Yes |
| 61 | 40 | 14 to Column | 6.0 | No | No | Yes | Yes | Yes | No |
| 62 | 3 | Trityl Flush | 5.0 | No | No | Yes | Yes | Yes | No |
| 63 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 64 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 65 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 66 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 67 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 68 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 69 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | No |
| 70 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 71 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | No |
| 72 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | No |
| 73 | 3 | Trityl Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | No |
| 74 | 110 | Waste - Bottle | | | | | | | No |
| 75 | 42 | 18 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 76 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 77 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 78 | 107 | End | | | | | | | Yes |

# Table AI-20
# .2 um Sulfur Cycle
# (four-column instrument)

**Name : .2 um Sulfur** **1:10:01A, 1/ 1/91**
**Steps: 98**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 106 | Begin | | | | | | | | | | Yes |
| 2 | 64 | 18 to Waste | 3.0 | | | | | | | | | Yes |
| 3 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 2 | Reverse Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | 101 | *Phos Prep* | 3.0 | | | | | | | | | Yes |
| 7 | 140 | Column 1 On | | | | | | | | | | Yes |
| 8 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 10 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 11 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 12 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 13 | 43 | Push to Column | | | | | | | | | | Yes |
| 14 | 141 | Column 1 Off | | | | | | | | | | Yes |
| 15 | 142 | Column 2 On | | | | | | | | | | Yes |
| 16 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 17 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 18 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 19 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 20 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 22 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 23 | 43 | Push to Column | | | | | | | | | | Yes |
| 24 | 143 | Column 2 Off | | | | | | | | | | Yes |
| 25 | 144 | *Column 3 On* | | | | | | | | | | Yes |
| 26 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 27 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 28 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 29 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 30 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 31 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

# Table AI-20 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 33 | 43 | Push to Column | | | | | | | | | | Yes |
| 34 | 145 | Column 3 Off | | | | | | | | | | Yes |
| 35 | 146 | Column 4 On | | | | | | | | | | Yes |
| 36 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 37 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 38 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 40 | 33 | B+Tet to Column | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 41 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 33 | B+Tet to Column | 1.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 43 | Push to Column | | | | | | | | | | Yes |
| 44 | 147 | Column 4 Off | | | | | | | | | | Yes |
| 45 | 103 | Wait | 25.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 47 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 48 | 41 | 15 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 50 | 103 | Wait | 900.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 51 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 4 | Flush to Waste | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 53 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 54 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 55 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 56 | 102 | Cap Prep | 3.0 | | | | | | | | | Yes |
| 57 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 58 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 59 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 60 | 39 | Cap to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 61 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 62 | 64 | 18 to Waste | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 63 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 64 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 65 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 66 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 67 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 68 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 69 | 105 | Start Detrityl | | | | | | | | | | Yes |
| 70 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 71 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 72 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 73 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 74 | 112 | Trityl Advance | | | | | | | | | | Yes |
| 75 | 109 | Waste - Port | | | | | | | | | | Yes |
| 76 | 120 | Advance FC | | | | | | | | | | Yes |

## Table AI-20 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 77 | 120 | Advance FC | | | | | | | | | | Yes |
| 78 | 120 | Advance FC | | | | | | | | | | Yes |
| 79 | 120 | Advance FC | | | | | | | | | | Yes |
| 80 | 113 | End Advance | | | | | | | | | | Yes |
| 81 | 40 | 14 to Column | 6.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 82 | 3 | Trityl Flush | 5.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 83 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 84 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 85 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 86 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 87 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 88 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 89 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 90 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 91 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 92 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 93 | 3 | Trityl Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 94 | 110 | Waste - Bottle | | | | | | | | | | No |
| 95 | 42 | 18 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 96 | 2 | Reverse Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 97 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 98 | 107 | End | | | | | | | | | | Yes |

# Table AI-21
# 1.0 um Sulfur Cycle
# (two-column instrument)

**Name : 1.0 um Sulfur** **10:18:38P, 1/ 4/91**

**Steps: 85**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 106 | Begin | | | | | | | Yes |
| 2 | 64 | 18 to Waste | 3.0 | | | | | | Yes |
| 3 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 2 | Reverse Flush | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | | | | | Yes |
| 7 | 140 | Column 1 On | | | | | | | Yes |
| 8 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 58 | Tet to Waste | 1.7 | | | | | | Yes |
| 10 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 11 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 12 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 13 | 43 | Push to Column | | | | | | | Yes |
| 14 | 141 | Column 1 Off | | | | | | | Yes |
| 15 | 142 | Column 2 On | | | | | | | Yes |
| 16 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 17 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 18 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 19 | 58 | Tet to Waste | 1.7 | | | | | | Yes |
| 20 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 22 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes |
| 23 | 43 | Push to Column | | | | | | | Yes |
| 24 | 143 | Column 2 Off | | | | | | | Yes |
| 25 | 103 | Wait | 25.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 26 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 27 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 28 | 41 | 15 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 29 | 64 | 18 to Waste | 4.0 | | | | | | Yes |
| 30 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 31 | 103 | Wait | 900.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 33 | 4 | Flush to Waste | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 34 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 35 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 36 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 37 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 38 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 40 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes |

# Table AI-21 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A G C T 5 | Safe Step |
|---|---|---|---|---|---|
| 41 | 102 | Cap Prep | 3.0 | | Yes |
| 42 | 64 | 18 to Waste | 4.0 | | Yes |
| 43 | 2 | Reverse Flush | 7.0 | Yes Yes Yes Yes Yes | Yes |
| 44 | 1 | Block Flush | 3.0 | Yes Yes Yes Yes Yes | Yes |
| 45 | 39 | Cap to Column | 10.0 | Yes Yes Yes Yes Yes | Yes |
| 46 | 103 | Wait | 5.0 | Yes Yes Yes Yes Yes | Yes |
| 47 | 64 | 18 to Waste | 4.0 | | Yes |
| 48 | 2 | Reverse Flush | 7.0 | Yes Yes Yes Yes Yes | Yes |
| 49 | 42 | 18 to Column | 10.0 | Yes Yes Yes Yes Yes | Yes |
| 50 | 2 | Reverse Flush | 7.0 | Yes Yes Yes Yes Yes | Yes |
| 51 | 42 | 18 to Column | 10.0 | Yes Yes Yes Yes Yes | Yes |
| 52 | 2 | Reverse Flush | 7.0 | Yes Yes Yes Yes Yes | Yes |
| 53 | 42 | 18 to Column | 10.0 | Yes Yes Yes Yes Yes | Yes |
| 54 | 2 | Reverse Flush | 7.0 | Yes Yes Yes Yes Yes | Yes |
| 55 | 1 | Block Flush | 3.0 | Yes Yes Yes Yes Yes | Yes |
| 56 | 105 | Start Detrityl | | | Yes |
| 57 | 64 | 18 to Waste | 4.0 | | Yes |
| 58 | 42 | 18 to Column | 10.0 | Yes Yes Yes Yes Yes | Yes |
| 59 | 2 | Reverse Flush | 7.0 | Yes Yes Yes Yes Yes | Yes |
| 60 | 1 | Block Flush | 3.0 | Yes Yes Yes Yes Yes | Yes |
| 61 | 112 | Trityl Advance | | | Yes |
| 62 | 109 | Waste - Port | | | Yes |
| 63 | 120 | Advance FC | | | Yes |
| 64 | 120 | Advance FC | | | Yes |
| 65 | 120 | Advance FC | | | Yes |
| 66 | 120 | Advance FC | | | Yes |
| 67 | 113 | End Advance | | | Yes |
| 68 | 40 | 14 to Column | 6.0 | No No Yes Yes Yes | No |
| 69 | 3 | Trityl Flush | 5.0 | No No Yes Yes Yes | No |
| 70 | 40 | 14 to Column | 6.0 | Yes Yes Yes Yes Yes | No |
| 71 | 103 | Wait | 5.0 | Yes Yes Yes Yes Yes | No |
| 72 | 3 | Trityl Flush | 5.0 | Yes Yes Yes Yes Yes | No |
| 73 | 40 | 14 to Column | 6.0 | Yes Yes Yes Yes Yes | No |
| 74 | 103 | Wait | 5.0 | Yes Yes Yes Yes Yes | No |
| 75 | 3 | Trityl Flush | 5.0 | Yes Yes Yes Yes Yes | No |
| 76 | 40 | 14 to Column | 6.0 | Yes Yes Yes Yes Yes | No |
| 77 | 103 | Wait | 5.0 | Yes Yes Yes Yes Yes | No |
| 78 | 3 | Trityl Flush | 5.0 | Yes Yes Yes Yes Yes | No |
| 79 | 42 | 18 to Column | 10.0 | Yes Yes Yes Yes Yes | No |
| 80 | 3 | Trityl Flush | 8.0 | Yes Yes Yes Yes Yes | No |
| 81 | 110 | Waste - Bottle | | | No |
| 82 | 42 | 18 to Column | 8.0 | Yes Yes Yes Yes Yes | Yes |
| 83 | 2 | Reverse Flush | 7.0 | Yes Yes Yes Yes Yes | Yes |
| 84 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 85 | 107 | End | | | Yes |

# Table AI-22
# 1.0 um Sulfur Cycle
# (four-column instrument)

**Name : 1.0 um Sulfur** **1:11:28A, 1/ 1/91**
**Steps: 104**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 106 | Begin | | | | | | | | | | Yes |
| 2 | 64 | 18 to Waste | 3.0 | | | | | | | | | Yes |
| 3 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 2 | Reverse Flush | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | | | | | | | | Yes |
| 7 | 140 | Column 1 On | | | | | | | | | | Yes |
| 8 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 10 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 11 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 12 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 13 | 43 | Push to Column | | | | | | | | | | Yes |
| 14 | 141 | Column 1 Off | | | | | | | | | | Yes |
| 15 | 142 | Column 2 On | | | | | | | | | | Yes |
| 16 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 17 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 18 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 19 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 20 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 22 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 23 | 43 | Push to Column | | | | | | | | | | Yes |
| 24 | 143 | Column 2 Off | | | | | | | | | | Yes |
| 25 | 144 | Column 3 On | | | | | | | | | | Yes |
| 26 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 27 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 28 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 29 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 30 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 31 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 33 | 43 | Push to Column | | | | | | | | | | Yes |
| 34 | 145 | Column 3 Off | | | | | | | | | | Yes |
| 35 | 146 | Column 4 On | | | | | | | | | | Yes |
| 36 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 37 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 38 | 111 | Block Vent | 2.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 58 | Tet to Waste | 1.7 | | | | | | | | | Yes |
| 40 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

# Table AI-22 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 41 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 33 | B+Tet to Column | 2.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 43 | Push to Column | | | | | | | | | | Yes |
| 44 | 147 | Column 4 Off | | | | | | | | | | Yes |
| 45 | 103 | Wait | 25.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 47 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 48 | 41 | 15 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 50 | 103 | Wait | 900.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 51 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 4 | Flush to Waste | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 53 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 54 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 55 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 56 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 57 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 58 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 59 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 60 | 102 | Cap Prep | 3.0 | | | | | | | | | Yes |
| 61 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 62 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 63 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 64 | 39 | Cap to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 65 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 66 | 64 | 18 to Waste | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 67 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 68 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 69 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 70 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 71 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 72 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 73 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 74 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 75 | 105 | Start Detrityl | | | | | | | | | | Yes |
| 76 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 77 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 78 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 79 | 1 | Block Flush | 3.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 80 | 112 | Trityl Advance | | | | | | | | | | Yes |
| 81 | 109 | Waste - Port | | | | | | | | | | Yes |
| 82 | 120 | Advance FC | | | | | | | | | | Yes |

# Table AI-22 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 83 | 120 | Advance FC | | | | | | | | | | Yes |
| 84 | 120 | Advance FC | | | | | | | | | | Yes |
| 85 | 120 | Advance FC | | | | | | | | | | Yes |
| 86 | 113 | End Advance | | | | | | | | | | Yes |
| 87 | 40 | 14 to Column | 6.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 88 | 3 | Trityl Flush | 5.0 | No | No | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 89 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 90 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 91 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 92 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 93 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 94 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 95 | 40 | 14 to Column | 6.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 96 | 103 | Wait | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 97 | 3 | Trityl Flush | 5.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 98 | 42 | 18 to Column | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 99 | 3 | Trityl Flush | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 100 | 110 | Waste - Bottle | | | | | | | | | | No |
| 101 | 42 | 18 to Column | 8.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 102 | 2 | Reverse Flush | 7.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 103 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 104 | 107 | End | | | | | | | | | | Yes |

# Table AI-23
# 10 um Sulfur Cycle
# (two-column instrument)

**Name : 10 um CE** **10:20:06P, 1/ 4/91**
**Steps: 70**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A G C T 5 | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 64 | 18 to Waste | 4.0 | | Yes |
| 3 | 42 | 18 to Column | 80.0 | Yes Yes Yes Yes Yes | Yes |
| 4 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | Yes |
| 7 | 140 | Column 1 On | | | Yes |
| 8 | 34 | Tet to Column | 1.0 | Yes Yes Yes Yes Yes | Yes |
| 9 | 33 | B+Tet to Column | 22.5 | Yes Yes Yes Yes Yes | Yes |
| 10 | 141 | Column 1 Off | | | Yes |
| 11 | 142 | Column 2 On | | | Yes |
| 12 | 64 | 18 to Waste | 5.0 | | Yes |
| 13 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 14 | 34 | Tet to Column | 1.0 | Yes Yes Yes Yes Yes | Yes |
| 15 | 33 | B+Tet to Column | 22.5 | Yes Yes Yes Yes Yes | Yes |
| 16 | 143 | Column 2 Off | | | Yes |
| 17 | 103 | Wait | 40.0 | Yes Yes Yes Yes Yes | Yes |
| 18 | 64 | 18 to Waste | 5.0 | | Yes |
| 19 | 42 | 18 to Column | 30.0 | Yes Yes Yes Yes Yes | Yes |
| 20 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 21 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 22 | 41 | 15 to Column | 40.0 | Yes Yes Yes Yes Yes | Yes |
| 23 | 64 | 18 to Waste | 5.0 | | Yes |
| 24 | 103 | Wait | 900.0 | Yes Yes Yes Yes Yes | Yes |
| 25 | 42 | 18 to Column | 50.0 | Yes Yes Yes Yes Yes | Yes |
| 26 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 27 | 42 | 18 to Column | 50.0 | Yes Yes Yes Yes Yes | Yes |
| 28 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 29 | 42 | 18 to Column | 50.0 | Yes Yes Yes Yes Yes | Yes |
| 30 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 31 | 42 | 18 to Column | 50.0 | Yes Yes Yes Yes Yes | Yes |
| 32 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 33 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 34 | 102 | Cap Prep | 5.0 | | Yes |
| 35 | 39 | Cap to Column | 45.0 | Yes Yes Yes Yes Yes | Yes |
| 36 | 103 | Wait | 10.0 | Yes Yes Yes Yes Yes | Yes |
| 37 | 64 | 18 to Waste | 5.0 | | Yes |
| 38 | 2 | Reverse Flush | 35.0 | Yes Yes Yes Yes Yes | Yes |
| 39 | 1 | Block Flush | 4.0 | Yes Yes Yes Yes Yes | Yes |
| 40 | 42 | 18 to Column | 50.0 | Yes Yes Yes Yes Yes | Yes |

## Table AI-23 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|
| 41 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 44 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 45 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 47 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 48 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 105 | Start Detrityl | | | | | | | Yes |
| 50 | 64 | 18 to Waste | 5.0 | | | | | | Yes |
| 51 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 53 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 54 | 112 | Trityl Advance | | | | | | | Yes |
| 55 | 109 | Waste - Port | | | | | | | Yes |
| 56 | 120 | Advance FC | | | | | | | Yes |
| 57 | 120 | Advance FC | | | | | | | Yes |
| 58 | 120 | Advance FC | | | | | | | Yes |
| 59 | 120 | Advance FC | | | | | | | Yes |
| 60 | 113 | End Advance | | | | | | | Yes |
| 61 | 40 | 14 to Column | 110.0 | Yes | Yes | Yes | Yes | Yes | No |
| 62 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | No |
| 63 | 64 | 18 to Waste | 5.0 | | | | | | No |
| 64 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | No |
| 65 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | No |
| 66 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 67 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 68 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes |
| 69 | 110 | Waste - Bottle | | | | | | | Yes |
| 70 | 107 | End | | | | | | | Yes |

# Table AI-24
# 10 um Sulfur Cycle
# (four-column instrument)

**Name : 10 um Sulfur** **1:13:02A, 1/ 1/91**
**Steps: 82**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 106 | Begin | | | | | | | | | | Yes |
| 2 | 64 | 18 to Waste | 4.0 | | | | | | | | | Yes |
| 3 | 42 | 18 to Column | 80.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | 101 | Phos Prep | 3.0 | | | | | | | | | Yes |
| 7 | 140 | Column 1 On | | | | | | | | | | Yes |
| 8 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 33 | B+Tet to Column | 22.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 10 | 141 | Column 1 Off | | | | | | | | | | Yes |
| 11 | 142 | Column 2 On | | | | | | | | | | Yes |
| 12 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 13 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 14 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 15 | 33 | B+Tet to Column | 22.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 16 | 143 | Column 2 Off | | | | | | | | | | Yes |
| 17 | 144 | Column 3 On | | | | | | | | | | Yes |
| 18 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 19 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 20 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 33 | B+Tet to Column | 22.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 22 | 145 | Column 3 Off | | | | | | | | | | Yes |
| 23 | 146 | Column 4 On | | | | | | | | | | Yes |
| 24 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 25 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 26 | 34 | Tet to Column | 1.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 27 | 33 | B+Tet to Column | 22.5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 28 | 147 | Column 4 Off | | | | | | | | | | Yes |
| 29 | 103 | Wait | 40.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 30 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 31 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 33 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 34 | 41 | 15 to Column | 40.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 35 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 36 | 103 | Wait | 900.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 37 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 38 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 40 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

# Table AI-24 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases A | G | C | T | 5 | 6 | 7 | 8 | Safe Step |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 41 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 44 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 45 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 102 | Cap Prep | 5.0 | | | | | | | | | Yes |
| 47 | 39 | Cap to Column | 45.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 48 | 103 | Wait | 10.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 50 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 51 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 53 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 54 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 55 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 56 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 57 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 58 | 42 | 18 to Column | 50.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 59 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 60 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 61 | 105 | Start Detrityl | | | | | | | | | | Yes |
| 62 | 64 | 18 to Waste | 5.0 | | | | | | | | | Yes |
| 63 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 64 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 65 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 66 | 112 | Trityl Advance | | | | | | | | | | Yes |
| 67 | 109 | Waste - Port | | | | | | | | | | Yes |
| 68 | 120 | Advance FC | | | | | | | | | | Yes |
| 69 | 120 | Advance FC | | | | | | | | | | Yes |
| 70 | 120 | Advance FC | | | | | | | | | | Yes |
| 71 | 120 | Advance FC | | | | | | | | | | Yes |
| 72 | 113 | End Advance | | | | | | | | | | Yes |
| 73 | 40 | 14 to Column | 110.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 74 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 75 | 64 | 18 to Waste | 5.0 | | | | | | | | | No |
| 76 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 77 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 78 | 42 | 18 to Column | 30.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 79 | 2 | Reverse Flush | 35.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 80 | 1 | Block Flush | 4.0 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 81 | 110 | Waste - Bottle | | | | | | | | | | Yes |
| 82 | 107 | End | | | | | | | | | | Yes |

# Table AI-25
# ABI Begin Procedure Listing

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 103 | Begin | | | Yes |
| 2 | 101 | Phos Prep | 10.0 | Pressurizes the phosphoramidite and tetrazole reservoirs prior to delivery. | Yes |
| 3 | 50 | A to Waste | 2.0 | Steps 3-11 fill the delivery lines with fresh | Yes |
| 4 | 51 | G to Waste | 2.0 | reagent by delivering the phosphoramidites . | Yes |
| 5 | 52 | C to Waste | 2.0 | and tetrazole to waste. | Yes |
| 6 | 53 | T to Waste | 2.0 | | Yes |
| 7 | 54 | 5 to Waste | 2.0 | | Yes |
| 8 | 55 | 6 to Waste | 2.0 | | Yes |
| 9 | 56 | 7 to Waste | 2.0 | | Yes |
| 10 | 57 | 8 to Waste | 2.0 | | Yes |
| 11 | 58 | Tet to Waste | 2.0 | | Yes |
| 12 | 64 | 18 to Waste | 10.0 | Removes the phosphoramidite and tetrazole left in the reagent valve block. Rinses the valve block. | Yes |
| 13 | 1 | Block Flush | 10.0 | Removes acetonitrile from the reagent valve | Yes |
| 14 | 107 | End | | blocks. | Yes |

# Table AI-26
# 394 End Procedure Listing

**Name : End CESS** **6:49:37A, 1/ 4/91**
**Steps: 30**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 2 | Reverse Flush | 60.0 | Flushes columns and column blocks in reverse. | Yes |
| 3 | 115 | Prep 10 | 15.0 | Prepares ammonium hydroxide for delivery | Yes |
| 4 | 36 | 10 to Collect | 7.0 | Fills column with ammonium hydroxide. | Yes |
| 5 | 64 | 18 to Waste | 9.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 6 | 1 | Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 7 | 103 | Wait | 900.0 | Ammonium hydroxide cleaves oligo from support. | Yes |
| 8 | 36 | 10 to Collect | 7.0 | Ammon. hydrox. delivery to col. & collection vial. | Yes |
| 9 | 64 | 18 to Waste | 7.0 | 2nd rinse with acetonitrile. | Yes |
| 10 | 1 | Block Flush | 5.0 | 2nd block flush. | Yes |
| 11 | 103 | Wait | 900.0 | | Yes |
| 12 | 36 | 10 to Collect | 7.0 | | Yes |
| 13 | 64 | 18 to Waste | 7.0 | | Yes |
| 14 | 1 | Block Flush | 5.0 | | Yes |
| 15 | 103 | Wait | 900.0 | | Yes |
| 16 | 36 | 10 to Collect | 7.0 | | Yes |
| 17 | 64 | 18 to Waste | 7.0 | | Yes |
| 18 | 1 | Block Flush | 5.0 | | Yes |
| 19 | 103 | Wait | 900.0 | | Yes |
| 20 | 5 | Flush to Collect | 9.0 | Flushes ammonium hydroxide into collect. vial. | Yes |
| 21 | 36 | 10 to Collect | 7.0 | | Yes |
| 22 | 5 | Flush to Collect | 9.0 | | Yes |
| 23 | 2 | Reverse Flush | 60.0 | | Yes |
| 24 | 1 | Block Flush | 4.0 | | Yes |
| 25 | 64 | 18 to Waste | 7.0 | | Yes |
| 26 | 42 | 18 to Column | 30.0 | | Yes |
| 27 | 2 | Reverse Flush | 60.0 | | Yes |
| 28 | 1 | Block Flush | 10.0 | | Yes |
| 29 | 100 | 10 Vent | 30.0 | | Yes |
| 30 | 107 | End | | | Yes |

# Table AI-27
# 394 End Procedure Listing

**Name : End CE** **6:48:27P, 1/ 4/91**
**Steps: 30**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 2 | Reverse Flush | 60.0 | Flushes columns and column blocks in reverse. | Yes |
| 3 | 115 | Prep 10 | 15.0 | Prepares ammonium hydroxide for delivery | Yes |
| 4 | 36 | 10 to Collect | 11.0 | Fills column with ammonium hydroxide. | Yes |
| 5 | 64 | 18 to Waste | 5.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 6 | 1 | Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 7 | 103 | Wait | 900.0 | Ammonium hydroxide cleaves oligo from support. | Yes |
| 8 | 36 | 10 to Collect | 11.0 | Ammon. hydrox. delivery to col. & collection vial. | Yes |
| 9 | 64 | 18 to Waste | 5.0 | 2nd rinse with acetonitrile. | Yes |
| 10 | 1 | Block Flush | 5.0 | 2nd block flush. | Yes |
| 11 | 103 | Wait | 900.0 | | Yes |
| 12 | 36 | 10 to Collect | 11.0 | | Yes |
| 13 | 64 | 18 to Waste | 5.0 | | Yes |
| 14 | 1 | Block Flush | 5.0 | | Yes |
| 15 | 103 | Wait | 900.0 | | Yes |
| 16 | 36 | 10 to Collect | 11.0 | | Yes |
| 17 | 64 | 18 to Waste | 5.0 | | Yes |
| 18 | 1 | Block Flush | 5.0 | | Yes |
| 19 | 103 | Wait | 900.0 | | Yes |
| 20 | 5 | Flush to Collect | 9.0 | Flushes ammonium hydroxide into collect. vial. | Yes |
| 21 | 36 | 10 to Collect | 9.0 | | Yes |
| 22 | 5 | Flush to Collect | 9.0 | | Yes |
| 23 | 2 | Reverse Flush | 60.0 | | Yes |
| 24 | 1 | Block Flush | 4.0 | | Yes |
| 25 | 64 | 18 to Waste | 5.0 | | Yes |
| 26 | 42 | 18 to Column | 30.0 | | Yes |
| 27 | 2 | Reverse Flush | 60.0 | | Yes |
| 28 | 1 | Block Flush | 10.0 | | Yes |
| 29 | 100 | 10 Vent | 30.0 | | Yes |
| 30 | 107 | End | | | Yes |

# Table AI-28
# 394 End Procedure Listing

**Name : End CE 10** **6:50:12P, 1/ 4/91**
**Steps: 29**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 2 | Reverse Flush | 60.0 | Flushes columns and column blocks in reverse. | Yes |
| 3 | 115 | Prep 10 | 15.0 | Prepares ammonium hydroxide for delivery | Yes |
| 4 | 36 | 10 to Collect | 50.0 | Fills column with ammonium hydroxide. | Yes |
| 5 | 64 | 18 to Waste | 5.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 6 | 1 | Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 7 | 103 | Wait | 900.0 | Ammonium hydroxide cleaves oligo from support. | Yes |
| 8 | 36 | 10 to Collect | 23.0 | Ammon. hydrox. delivery to col. & collection vial. | Yes |
| 9 | 64 | 18 to Waste | 5.0 | 2nd rinse with acetonitrile. | Yes |
| 10 | 1 | Block Flush | 5.0 | 2nd block flush. | Yes |
| 11 | 103 | Wait | 900.0 | | Yes |
| 12 | 36 | 10 to Collect | 23.0 | | Yes |
| 13 | 64 | 18 to Waste | 5.0 | | Yes |
| 14 | 1 | Block Flush | 5.0 | | Yes |
| 15 | 103 | Wait | 900.0 | | Yes |
| 16 | 36 | 10 to Collect | 23.0 | | Yes |
| 17 | 64 | 18 to Waste | 5.0 | | Yes |
| 18 | 1 | Block Flush | 5.0 | | Yes |
| 19 | 103 | Wait | 900.0 | | Yes |
| 20 | 5 | Flush to Collect | 15.0 | Flushes ammonium hydroxide into collect. vial. | Yes |
| 21 | 36 | 10 to Collect | 50.0 | | Yes |
| 22 | 5 | Flush to Collect | 15.0 | | Yes |
| 23 | 1 | Block Flush | 4.0 | | Yes |
| 24 | 64 | 18 to Waste | 7.0 | | Yes |
| 25 | 42 | 18 to Column | 90.0 | | Yes |
| 26 | 2 | Reverse Flush | 120.0 | | Yes |
| 27 | 1 | Block Flush | 10.0 | | Yes |
| 28 | 100 | 10 Vent | 30.0 | | Yes |
| 29 | 107 | End | | | Yes |

# Table AI-29
# 394 End Procedure Listing

**Name : End RNA** **6:50:42P, 1/ 4/91**
**Steps: 34**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 2 | Reverse Flush | 60.0 | Flushes columns and column blocks in reverse. | Yes |
| 3 | 115 | Prep 10 | 15.0 | Prepares ammonium hydroxide for delivery. | Yes |
| 4 | 36 | 10 to Collect | 11.0 | Fills column with ammonium hydroxide. | Yes |
| 5 | 64 | 18 to Waste | 5.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 6 | 1 | Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 7 | 103 | Wait | 900.0 | Ammonium hydroxide cleaves oligo from support. | Yes |
| 8 | 103 | Wait | 900.0 | Ammonium hydroxide cleaves oligo from support. | Yes |
| 9 | 36 | 10 to Collect | 11.0 | Ammon. hydrox. delivery to col. & collection vial. | Yes |
| 10 | 64 | 18 to Waste | 5.0 | 2nd rinse with acetonitrile. | Yes |
| 11 | 1 | Block Flush | 5.0 | 2nd block flush. | Yes |
| 12 | 103 | Wait | 900.0 | | Yes |
| 13 | 103 | Wait | 900.0 | | Yes |
| 14 | 36 | 10 to Collect | 11.0 | | Yes |
| 15 | 64 | 18 to Waste | 5.0 | | Yes |
| 16 | 1 | Block Flush | 5.0 | | Yes |
| 17 | 103 | Wait | 900.0 | | Yes |
| 18 | 103 | Wait | 900.0 | | Yes |
| 19 | 36 | 10 to Collect | 11.0 | | Yes |
| 20 | 64 | 18 to Waste | 5.0 | | Yes |
| 21 | 1 | Block Flush | 5.0 | | Yes |
| 22 | 103 | Wait | 900.0 | | Yes |
| 23 | 103 | Wait | 900.0 | | Yes |
| 24 | 5 | Flush to Collect | 9.0 | Flushes ammonium hydroxide into collect. vial. | Yes |
| 25 | 36 | 10 to Collect | 9.0 | | Yes |
| 26 | 5 | Flush to Collect | 9.0 | | Yes |
| 27 | 2 | Reverse Flush | 60.0 | | Yes |
| 28 | 1 | Block Flush | 4.0 | | Yes |
| 29 | 64 | 18 to Waste | 5.0 | | Yes |
| 30 | 42 | 18 to Column | 30.0 | | Yes |
| 31 | 2 | Reverse Flush | 60.0 | | Yes |
| 32 | 1 | Block Flush | 10.0 | | Yes |
| 33 | 100 | 10 Vent | 30.0 | | Yes |
| 34 | 107 | End | | | Yes |

# Table AI-30
# 394 End Procedure Listing

**Name : End RNA 10** **6:51:22P, 1/ 4/91**
**Steps: 33**

| Step Numb. | Function # Name | Step Time | Step Active for Bases | Safe Step |
|---|---|---|---|---|
| 1 | 106 Begin | | | Yes |
| 2 | 2 Reverse Flush | 60.0 | Flushes columns and column blocks in reverse. | Yes |
| 3 | 115 Prep 10 | 15.0 | Prepares ammonium hydroxide for delivery. | Yes |
| 4 | 36 10 to Collect | 50.0 | Fills column with ammonium hydroxide. | Yes |
| 5 | 64 18 to Waste | 5.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 6 | 1 Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 7 | 103 Wait | 900.0 | Ammonium hydroxide cleaves oligo from support. | Yes |
| 8 | 103 Wait | 900.0 | Ammonium hydroxide cleaves oligo from support. | Yes |
| 9 | 36 10 to Collect | 23.0 | Ammon. hydrox. delivery to col. & collection vial. | Yes |
| 10 | 64 18 to Waste | 5.0 | 2nd rinse with acetonitrile. | Yes |
| 11 | 1 Block Flush | 5.0 | 2nd block flush. | Yes |
| 12 | 103 Wait | 900.0 | | Yes |
| 13 | 103 Wait | 900.0 | | Yes |
| 14 | 36 10 to Collect | 23.0 | | Yes |
| 15 | 64 18 to Waste | 5.0 | | Yes |
| 16 | 1 Block Flush | 5.0 | | Yes |
| 17 | 103 Wait | 900.0 | | Yes |
| 18 | 103 Wait | 900.0 | | Yes |
| 19 | 36 10 to Collect | 23.0 | | Yes |
| 20 | 64 18 to Waste | 5.0 | | Yes |
| 21 | 1 Block Flush | 5.0 | | Yes |
| 22 | 103 Wait | 900.0 | | Yes |
| 23 | 103 Wait | 900.0 | | Yes |
| 24 | 5 Flush to Collect | 15.0 | Flushes ammonium hydroxide into collect. vial. | Yes |
| 25 | 36 10 to Collect | 50.0 | | Yes |
| 26 | 5 Flush to Collect | 15.0 | | Yes |
| 27 | 1 Block Flush | 4.0 | | Yes |
| 28 | 64 18 to Waste | 7.0 | | Yes |
| 29 | 42 18 to Column | 90.0 | | Yes |
| 30 | 2 Reverse Flush | 120.0 | | Yes |
| 31 | 1 Block Flush | 10.0 | | Yes |
| 32 | 100 10 Vent | 30.0 | | Yes |
| 33 | 107 End | | | Yes |

# Table AI-31
# ABI Shut (Shut Down) Procedure

| Step Numb. | Function # | Function Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 15.0 | Old phosphoramidites and reagents in the delivery | Yes |
| 3 | 64 | 18 to Waste | 15.0 | lines are removed and forced back into the | Yes |
| 4 | 1 | Block Flush | 15.0 | reservoirs by an acetonitrile wash | Yes |
| 5 | 42 | 18 to Column | 30.0 | | Yes |
| 6 | 1 | Block Flush | 30.0 | | Yes |
| 7 | 5 | Flush to Collect | 15.0 | | Yes |
| 8 | 109 | Waste - Port | | | Yes |
| 9 | 42 | 18 to Column | 30.0 | | Yes |
| 10 | 3 | Trityl Flush | 30.0 | | Yes |
| 11 | 110 | Waste - Bottle | | | Yes\ |
| 12 | 42 | 18 to Column | 60.0 | | Yes |
| 13 | 2 | Reverse Flush | 60.0 | | Yes |
| 14 | 42 | 18 to Column | 60.0 | | Yes |
| 15 | 1 | Block Flush | 60.0 | | Yes |
| 16 | 2 | Reverse Flush | 60.0 | | Yes |
| 17 | 70 | 18 to A | 10.0 | | Yes |
| 18 | 71 | 18 to G | 10.0 | | Yes |
| 19 | 72 | 18 to C | 10.0 | | Yes |
| 20 | 73 | 18 to T | 10.0 | | Yes |
| 21 | 74 | 18 to 5 | 10.0 | | Yes |
| 22 | 75 | 18 to 6 | 10.0 | | Yes |
| 23 | 76 | 18 to 7 | 10.0 | | Yes |
| 24 | 77 | 18 to 8 | 10.0 | | Yes |
| 25 | 78 | 18 to Tet | 10.0 | | Yes |
| 26 | 79 | 18 to 10 | 10.0 | | Yes |
| 27 | 82 | 18 to 11 + 12 | 20.0 | | Yes |
| 28 | 85 | 18 to 14 + 15 | 20.0 | | Yes |
| 29 | 6 | Flush to A | 60.0 | The lines are cleared by an argon flush to | Yes |
| 30 | 7 | Flush to G | 60.0 | each bottle. | Yes |
| 31 | 8 | Flush to C | 60.0 | | Yes |
| 32 | 9 | Flush to T | 60.0 | | Yes |
| 33 | 10 | Flush to 5 | 60.0 | | Yes |
| 34 | 11 | Flush to 6 | 60.0 | | Yes |
| 35 | 12 | Flush to 7 | 60.0 | | Yes |
| 36 | 13 | Flush to 8 | 60.0 | | Yes |
| 37 | 15 | Flush to Tet | 60.0 | | Yes |
| 38 | 16 | Flush to 10 | 60.0 | | Yes |
| 39 | 17 | Flush to 11+12 | 60.0 | | Yes |
| 40 | 18 | Flush to 14+15 | 60.0 | | Yes |
| 41 | 1 | Block Flush | 15.0 | | Yes |
| 42 | 107 | End | | | Yes |

# Table AI-32
# 392/394 Bottle Change Procedure Listing
# (Bottle 1 – all instruments)

**Name : Bot01 ABI** **4:24:31A, 1/ 8/90**
**Steps: 11**

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 3 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 4 | 70 | 18 to A | 3.0 | Washes old reagent into reservoir. | Yes |
| 5 | 6 | Flush to A | 10.0 | Flushes acetonitrile into reservoir. | Yes |
| 6 | 104 | Interrupt | | User installs new bottle. | Yes |
| 7 | 6 | Flush to A | 5.0 | Headspace purged with argon. | Yes |
| 8 | 50 | A to Waste | 2.0 | Line filled with fresh reagent. | Yes |
| 9 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks. | Yes |
| 10 | 1 | Block Flush | 5.0 | Flushes column and reagent blocks. | Yes |
| 11 | 107 | End | | | Yes |

# Table AI-33
# 392/394 Bottle Change Procedure Listing
# (Bottle 2 – all instruments)

**Name : Bot02 ABI** **4:25:31A, 1/ 8/90**
**Steps: 11**

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 3 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 4 | 71 | 18 to G | 3.0 | Washes old reagent into reservoir. | Yes |
| 5 | 7 | Flush to G | 10.0 | Flushes acetonitrile into reservoir. | Yes |
| 6 | 104 | Interrupt | | User installs new bottle. | Yes |
| 7 | 7 | Flush to G | 5.0 | Headspace purged with argon. | Yes |
| 8 | 51 | G to Waste | 2.0 | Line filled with fresh reagent. | Yes |
| 9 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks. | Yes |
| 10 | 1 | Block Flush | 5.0 | Flushes column and reagent blocks. | Yes |
| 11 | 107 | End | | | Yes |

# Table AI-34
# 392/394 Bottle Change Procedure Listing
# (Bottle 3 – all instruments)

**Name : Bot03 ABI** **4:26:06A, 1/ 8/90**
**Steps: 11**

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 3 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 4 | 72 | 18 to C | 3.0 | Washes old reagent into reservoir. | Yes |
| 5 | 8 | Flush to C | 10.0 | Flushes acetonitrile into reservoir. | Yes |
| 6 | 104 | Interrupt | | User installs new bottle. | Yes |
| 7 | 8 | Flush to C | 5.0 | Headspace purged with argon. | Yes |
| 8 | 52 | C to Waste | 2.0 | Line filled with fresh reagent. | Yes |
| 9 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks. | Yes |
| 10 | 1 | Block Flush | 5.0 | Flushes column and reagent blocks. | Yes |
| 11 | 107 | End | | | Yes |

# Table AI-35
# 392/394 Bottle Change Procedure Listing
# (Bottle 4 – all instruments)

**Name : Bot04 ABI** **4:26:26A, 1/ 8/90**
**Steps: 11**

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 3 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 4 | 73 | 18 to T | 3.0 | Washes old reagent into reservoir. | Yes |
| 5 | 9 | Flush to T | 10.0 | Flushes acetonitrile into reservoir. | Yes |
| 6 | 104 | Interrupt | | User installs new bottle. | Yes |
| 7 | 9 | Flush to T | 5.0 | Headspace purged with argon. | Yes |
| 8 | 53 | T to Waste | 2.0 | Line filled with fresh reagent. | Yes |
| 9 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks. | Yes |
| 10 | 1 | Block Flush | 5.0 | Flushes column and reagent blocks. | Yes |
| 11 | 107 | End | | | Yes |

## Table AI-36
## 392/394 Bottle Change Procedure Listing
## (Bottle 5 – all instruments)

**Name : Bot05 ABI** **4:26:46A, 1/ 8/90**
**Steps: 11**

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 3 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 4 | 74 | 18 to 5 | 3.0 | Washes old reagent into reservoir. | Yes |
| 5 | 10 | Flush to 5 | 10.0 | Flushes acetonitrile into reservoir. | Yes |
| 6 | 104 | Interrupt | | User installs new bottle. | Yes |
| 7 | 10 | Flush to 5 | 5.0 | Headspace purged with argon. | Yes |
| 8 | 54 | 5 to Waste | 2.0 | Line filled with fresh reagent. | Yes |
| 9 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks. | Yes |
| 10 | 1 | Block Flush | 5.0 | Flushes column and reagent blocks. | Yes |
| 11 | 107 | End | | | Yes |

## Table AI-37
## 392/394 Bottle Change Procedure Listing
## (Bottle 6 – eight-position only)

**Name : Bot06 ABI** **4:27:08A, 1/ 8/90**
**Steps: 11**

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 3 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 4 | 75 | 18 to 6 | 3.0 | Washes old reagent into reservoir. | Yes |
| 5 | 11 | Flush to 6 | 10.0 | Flushes acetonitrile into reservoir. | Yes |
| 6 | 104 | Interrupt | | User installs new bottle. | Yes |
| 7 | 11 | Flush to 6 | 5.0 | Headspace purged with argon. | Yes |
| 8 | 55 | 6 to Waste | 2.0 | Line filled with fresh reagent. | Yes |
| 9 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks. | Yes |
| 10 | 1 | Block Flush | 5.0 | Flushes column and reagent blocks. | Yes |
| 11 | 107 | End | | | Yes |

## Table AI-38
## 392/394 Bottle Change Procedure Listing
## (Bottle 7 – eight-position only)

**Name : Bot07 ABI** **4:27:27A, 1/ 8/90**
**Steps: 11**

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 3 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 4 | 76 | 18 to 7 | 3.0 | Washes old reagent into reservoir. | Yes |
| 5 | 12 | Flush to 7 | 10.0 | Flushes acetonitrile into reservoir. | Yes |
| 6 | 104 | Interrupt | | User installs new bottle. | Yes |
| 7 | 12 | Flush to 7 | 5.0 | Headspace purged with argon. | Yes |
| 8 | 56 | 7 to Waste | 2.0 | Line filled with fresh reagent. | Yes |
| 9 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks. | Yes |
| 10 | 1 | Block Flush | 5.0 | Flushes column and reagent blocks. | Yes |
| 11 | 107 | End | | | Yes |

## Table AI-39
## 392/394 Bottle Change Procedure Listing
## (Bottle 8 – eight-position only)

**Name : Bot08 ABI** **4:28:07A, 1/ 8/90**
**Steps: 11**

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 3 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 4 | 77 | 18 to 8 | 3.0 | Washes old reagent into reservoir. | Yes |
| 5 | 13 | Flush to 8 | 10.0 | Flushes acetonitrile into reservoir. | Yes |
| 6 | 104 | Interrupt | | User installs new bottle. | Yes |
| 7 | 13 | Flush to 8 | 5.0 | Headspace purged with argon. | Yes |
| 8 | 57 | 8 to Waste | 2.0 | Line filled with fresh reagent. | Yes |
| 9 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks. | Yes |
| 10 | 1 | Block Flush | 5.0 | Flushes column and reagent blocks. | Yes |
| 11 | 107 | End | | | Yes |

## Table AI-40
## 392/394 Bottle Change Procedure Listing
## (Bottle 9 – all instruments)

**Name : Bot9 ABI** 4:28:32A, 1/ 8/90
**Steps: 11**

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 5.0 | Flushes column blocks and reagent blocks. | Yes |
| 3 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks with acetonitrile. | Yes |
| 4 | 78 | 18 to Tet | 3.0 | Washes old reagent into reservoir. | Yes |
| 5 | 15 | Flush to Tet | 10.0 | Flushes acetonitrile into reservoir. | Yes |
| 6 | 104 | Interrupt | | User installs new bottle. | Yes |
| 7 | 15 | Flush to Tet | 5.0 | Headspace purged with argon. | Yes |
| 8 | 58 | Tet to Waste | 2.0 | Line filled with fresh reagent. | Yes |
| 9 | 64 | 18 to Waste | 7.0 | Rinses reagent blocks. | Yes |
| 10 | 1 | Block Flush | 5.0 | Flushes column and reagent blocks. | Yes |
| 11 | 107 | End | | | Yes |

## Table AI-41
## 392/394 Bottle Change Procedure Listing
## (Bottle 10 – all instruments)

**Name : Bot10 ABI** 4:29:12A, 1/ 8/90
**Steps: 6**

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 103 | Wait | 1.0 | | Yes |
| 3 | 100 | 10 Vent | 5.0 | | Yes |
| 4 | 104 | Interrupt | | | Yes |
| 5 | 103 | Wait | 1.0 | | Yes |
| 6 | 107 | End | | | Yes |

## Table AI-42
## 392/394 Bottle Change Procedure Listing
## (Bottle 11 – all instruments)

**Name : Bot11 ABI** **4:29:33A, 1/ 8/90**
**Steps: 9**

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 5.0 | | Yes |
| 3 | 64 | 18 to Waste | 7.0 | | Yes |
| 4 | 104 | Interrupt | | | Yes |
| 5 | 102 | Cap Prep | 5.0 | | Yes |
| 6 | 60 | 11 to Waste | 5.0 | | Yes |
| 7 | 64 | 18 to Waste | 7.0 | | Yes |
| 8 | 1 | Block Flush | 5.0 | | Yes |
| 9 | 107 | End | | | Yes |

## Table AI-43
## 392/394 Bottle Change Procedure Listing
## (Bottle 12 – all instruments)

**Name : Bot12 ABI** **4:29:56A, 1/ 8/90**
**Steps: 9**

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 5.0 | | Yes |
| 3 | 64 | 18 to Waste | 7.0 | | Yes |
| 4 | 104 | Interrupt | | | Yes |
| 5 | 102 | Cap Prep | 5.0 | | Yes |
| 6 | 61 | 12 to Waste | 5.0 | | Yes |
| 7 | 64 | 18 to Waste | 7.0 | | Yes |
| 8 | 1 | Block Flush | 5.0 | | Yes |
| 9 | 107 | End | | | Yes |

## Table AI-44
## 392/394 Bottle Change Procedure Listing
## (Bottle 14 – all instruments)

**Name : Bot14 ABI** 4:30:20A, 1/ 8/90
**Steps: 8**

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 5.0 | | Yes |
| 3 | 64 | 18 to Waste | 7.0 | | Yes |
| 4 | 104 | Interrupt | | | Yes |
| 5 | 62 | 14 to Waste | 5.0 | | Yes |
| 6 | 64 | 18 to Waste | 7.0 | | Yes |
| 7 | 1 | Block Flush | 5.0 | | Yes |
| 8 | 107 | End | | | Yes |

## Table AI-45
## 392/394 Bottle Change Procedure Listing
## (Bottle 15 – all instruments)

**Name : Bot15 ABI** 4:30:46A, 1/ 8/90
**Steps: 8**

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 5.0 | | Yes |
| 3 | 64 | 18 to Waste | 7.0 | | Yes |
| 4 | 104 | Interrupt | | | Yes |
| 5 | 63 | 15 to Waste | 5.0 | | Yes |
| 6 | 64 | 18 to Waste | 7.0 | | Yes |
| 7 | 1 | Block Flush | 5.0 | | Yes |
| 8 | 107 | End | | | Yes |

## Table AI-46
## 392/394 Bottle Change Procedure Listing
## (Bottle 18 – all instruments)

**Name : Bot18 ABI** 4:31:10A, 1/ 8/90
**Steps: 7**

| Step Numb. | Function # | Name | Step Time | Description | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 1 | Block Flush | 5.0 | | Yes |
| 3 | 64 | 18 to Waste | 7.0 | | Yes |
| 4 | 104 | Interrupt | | | Yes |
| 5 | 64 | 18 to Waste | 7.0 | | Yes |
| 6 | 1 | Block Flush | 5.0 | | Yes |
| 7 | 107 | End | | | Yes |

## Table AI-47
## 392/394 0.25-gram Auto Dilute Procedure Listing
## (eight-position instrument)

**Name : Auto 0.25g** 10:24:28P, 1/ 4/91
**Steps: 59**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 64 | 18 to Waste | 5.0 | | Yes |
| 3 | 1 | Block Flush | 4.0 | | Yes |
| 4 | 14 | Flush to Bases | 10.0 | | Yes |
| 5 | 86 | 18 to Bases | 5.0 | | Yes |
| 6 | 104 | Interrupt | | | Yes |
| 7 | 170 | Dilute 'A' | | | Yes |
| 8 | 171 | Dilute 'G' | | | Yes |
| 9 | 172 | Dilute 'C' | | | Yes |
| 10 | 173 | Dilute 'T' | | | Yes |
| 11 | 174 | Dilute '5' | | | Yes |
| 12 | 175 | Dilute '6' | | | Yes |
| 13 | 176 | Dilute '7' | | | Yes |
| 14 | 177 | Dilute '8' | | | Yes |
| 15 | 1 | Block Flush | 4.0 | | Yes |
| 16 | 14 | Flush to Bases | 120.0 | | Yes |
| 17 | 114 | Vent Bases | 3.0 | | Yes |
| 18 | 101 | Phos Prep | 5.0 | | Yes |
| 19 | 150 | Bottle01 On | | | Yes |

# Table AI-47 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases | Safe Step |
|---|---|---|---|---|---|
| 20 | 50 | A to Waste | 2.0 | | Yes |
| 21 | 151 | Bottle01 Off | | | Yes |
| 22 | 152 | Bottle02 On | | | Yes |
| 23 | 51 | G to Waste | 2.0 | | Yes |
| 24 | 153 | Bottle02 Off | | | Yes |
| 25 | 154 | Bottle03 On | | | Yes |
| 26 | 52 | C to Waste | 2.0 | | Yes |
| 27 | 155 | Bottle03 Off | | | Yes |
| 28 | 156 | Bottle04 On | | | Yes |
| 29 | 53 | T to Waste | 2.0 | | Yes |
| 30 | 157 | Bottle04 Off | | | Yes |
| 31 | 158 | Bottle05 On | | | Yes |
| 32 | 54 | 5 to Waste | 2.0 | | Yes |
| 33 | 159 | Bottle05 Off | | | Yes |
| 34 | 160 | Bottle06 On | | | Yes |
| 35 | 55 | 6 to Waste | 2.0 | | Yes |
| 36 | 161 | Bottle06 Off | | | Yes |
| 37 | 162 | Bottle07 On | | | Yes |
| 38 | 56 | 7 to Waste | 2.0 | | Yes |
| 39 | 163 | Bottle07 Off | | | Yes |
| 40 | 164 | Bottle08 On | | | Yes |
| 41 | 57 | 8 to Waste | 2.0 | | Yes |
| 42 | 165 | Bottle08 Off | | | Yes |
| 43 | 64 | 18 to Waste | 7.0 | | Yes |
| 44 | 1 | Block Flush | 5.0 | | Yes |
| 45 | 107 | End | | | Yes |

# Table AI-48
# 392/394 0.5-gram Auto Dilute Procedure Listing
# (eight-position instrument)

**Name : Auto 0.5g** **10:23:36P, 1/ 4/91**
**Steps: 53**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 64 | 18 to Waste | 5.0 | | Yes |
| 3 | 1 | Block Flush | 4.0 | | Yes |
| 4 | 14 | Flush to Bases | 10.0 | | Yes |
| 5 | 86 | 18 to Bases | 5.0 | | Yes |
| 6 | 104 | Interrupt | | | Yes |
| 7 | 170 | Dilute 'A' | | | Yes |
| 8 | 170 | Dilute 'A' | | | Yes |
| 9 | 171 | Dilute 'G' | | | Yes |
| 10 | 171 | Dilute 'G' | | | Yes |
| 11 | 172 | Dilute 'C' | | | Yes |
| 12 | 172 | Dilute 'C' | | | Yes |
| 13 | 173 | Dilute 'T' | | | Yes |
| 14 | 173 | Dilute 'T' | | | Yes |
| 15 | 174 | Dilute '5' | | | Yes |
| 16 | 174 | Dilute '5' | | | Yes |
| 17 | 175 | Dilute '6' | | | Yes |
| 18 | 175 | Dilute '6' | | | Yes |
| 19 | 176 | Dilute '7' | | | Yes |
| 20 | 176 | Dilute '7' | | | Yes |
| 21 | 177 | Dilute '8' | | | Yes |
| 22 | 177 | Dilute '8' | | | Yes |
| 23 | 1 | Block Flush | 4.0 | | Yes |
| 24 | 14 | Flush to Bases | 120.0 | | Yes |
| 25 | 114 | Vent Bases | 3.0 | | Yes |
| 26 | 101 | Phos Prep | 5.0 | | Yes |
| 27 | 150 | Bottle01 On | | | Yes |
| 28 | 50 | A to Waste | 2.0 | | Yes |
| 29 | 151 | Bottle01 Off | | | Yes |
| 30 | 152 | Bottle02 On | | | Yes |
| 31 | 51 | G to Waste | 2.0 | | Yes |
| 32 | 153 | Bottle02 Off | | | Yes |
| 33 | 154 | Bottle03 On | | | Yes |
| 34 | 52 | C to Waste | 2.0 | | Yes |
| 35 | 155 | Bottle03 Off | | | Yes |
| 36 | 156 | Bottle04 On | | | Yes |
| 37 | 53 | T to Waste | 2.0 | | Yes |

# Table AI-48 (cont,)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases | Safe Step |
|---|---|---|---|---|---|
| 38 | 157 | Bottle04 Off | | | Yes |
| 39 | 158 | Bottle05 On | | | Yes |
| 40 | 54 | 5 to Waste | 2.0 | | Yes |
| 41 | 159 | Bottle05 Off | | | Yes |
| 42 | 160 | Bottle06 On | | | Yes |
| 43 | 55 | 6 to Waste | 2.0 | | Yes |
| 44 | 161 | Bottle06 Off | | | Yes |
| 45 | 162 | Bottle07 On | | | Yes |
| 46 | 56 | 7 to Waste | 2.0 | | Yes |
| 47 | 163 | Bottle07 Off | | | Yes |
| 48 | 164 | Bottle08 On | | | Yes |
| 49 | 57 | 8 to Waste | 2.0 | | Yes |
| 50 | 165 | Bottle08 Off | | | Yes |
| 51 | 64 | 18 to Waste | 7.0 | | Yes |
| 52 | 1 | Block Flush | 5.0 | | Yes |
| 53 | 107 | End | | | Yes |

# Table AI-49
# 392/394 1-gram Auto Dilute Procedure Listing
# (eight-position instrument)

**Name : Auto 1.0g** **10:22:29P, 1/ 4/91**
**Steps: 69**

| Step Numb. | Function # | Name | Step Time | Step Active for Bases | Safe Step |
|---|---|---|---|---|---|
| 1 | 106 | Begin | | | Yes |
| 2 | 64 | 18 to Waste | 5.0 | | Yes |
| 3 | 1 | Block Flush | 4.0 | | Yes |
| 4 | 14 | Flush to Bases | 10.0 | | Yes |
| 5 | 86 | 18 to Bases | 5.0 | | Yes |
| 6 | 104 | Interrupt | | | Yes |
| 7 | 170 | Dilute 'A' | | | Yes |
| 8 | 170 | Dilute 'A' | | | Yes |
| 9 | 170 | Dilute 'A' | | | Yes |
| 10 | 170 | Dilute 'A' | | | Yes |
| 11 | 171 | Dilute 'G' | | | Yes |
| 12 | 171 | Dilute 'G' | | | Yes |
| 13 | 171 | Dilute 'G' | | | Yes |
| 14 | 171 | Dilute 'G' | | | Yes |
| 15 | 172 | Dilute 'C' | | | Yes |
| 16 | 172 | Dilute 'C' | | | Yes |
| 17 | 172 | Dilute 'C' | | | Yes |
| 18 | 172 | Dilute 'C' | | | Yes |
| 19 | 173 | Dilute 'T' | | | Yes |
| 20 | 173 | Dilute 'T' | | | Yes |
| 21 | 173 | Dilute 'T' | | | Yes |
| 22 | 173 | Dilute 'T' | | | Yes |
| 23 | 174 | Dilute '5' | | | Yes |
| 24 | 174 | Dilute '5' | | | Yes |
| 25 | 174 | Dilute '5' | | | Yes |
| 26 | 174 | Dilute '5' | | | Yes |
| 27 | 175 | Dilute '6' | | | Yes |
| 28 | 175 | Dilute '6' | | | Yes |
| 29 | 175 | Dilute '6' | | | Yes |
| 30 | 175 | Dilute '6' | | | Yes |
| 31 | 176 | Dilute '7' | | | Yes |
| 32 | 176 | Dilute '7' | | | Yes |
| 33 | 176 | Dilute '7' | | | Yes |
| 34 | 176 | Dilute '7' | | | Yes |
| 35 | 177 | Dilute '8' | | | Yes |
| 36 | 177 | Dilute '8' | | | Yes |
| 37 | 177 | Dilute '8' | | | Yes |
| 38 | 177 | Dilute '8' | | | Yes |
| 39 | 1 | Block Flush | 4.0 | | Yes |
| 40 | 14 | Flush to Bases | 120.0 | | Yes |
| 41 | 114 | Vent Bases | 3.0 | | Yes |
| 42 | 101 | Phos Prep | 5.0 | | Yes |

# Table AI-49 (cont.)

| Step Numb. | Function # | Name | Step Time | Step Active for Bases | Safe Step |
|---|---|---|---|---|---|
| 43 | 150 | Bottle01 On | | | Yes |
| 44 | 50 | A to Waste | 2.0 | | Yes |
| 45 | 151 | Bottle01 Off | | | Yes |
| 46 | 152 | Bottle02 On | | | Yes |
| 47 | 51 | G to Waste | 2.0 | | Yes |
| 48 | 153 | Bottle02 Off | | | Yes |
| 49 | 154 | Bottle03 On | | | Yes |
| 50 | 52 | C to Waste | 2.0 | | Yes |
| 51 | 155 | Bottle03 Off | | | Yes |
| 52 | 156 | Bottle04 On | | | Yes |
| 53 | 53 | T to Waste | 2.0 | | Yes |
| 54 | 157 | Bottle04 Off | | | Yes |
| 55 | 158 | Bottle05 On | | | Yes |
| 56 | 54 | 5 to Waste | 2.0 | | Yes |
| 57 | 159 | Bottle05 Off | | | Yes |
| 58 | 160 | Bottle06 On | | | Yes |
| 59 | 55 | 6 to Waste | 2.0 | | Yes |
| 60 | 161 | Bottle06 Off | | | Yes |
| 61 | 162 | Bottle07 On | | | Yes |
| 62 | 56 | 7 to Waste | 2.0 | | Yes |
| 63 | 163 | Bottle07 Off | | | Yes |
| 64 | 164 | Bottle08 On | | | Yes |
| 65 | 57 | 8 to Waste | 2.0 | | Yes |
| 66 | 165 | Bottle08 Off | | | Yes |
| 67 | 64 | 18 to Waste | 7.0 | | Yes |
| 68 | 1 | Block Flush | 5.0 | | Yes |
| 69 | 107 | End | | | Yes |

# *Appendix II*

# 392 Synthesizer Schematics
# 2 Column, 5 Base
# 2 Column, 8 Base
# 394 Synthesizer Schematics
# 4 Column, 5 Base
# 4 Column, 8 Base

NOTES: △ = 1/4-28 BARB

GAS INLET

602202

FILTER 10 mic

602201

VACUUM PUMP ASSY 002698

VAC. 1-7

602200 REG 1 — 6.5" — 602200 REG 2 — 3.5" — 602200 REG 3 — 6.5"

MAN. C 17.0"

MAN. A — MAN. B

45 P BASE — 46 V BASE — 47 P NH4OH — 48 V NH4OH — 49 P TET — 55 EXTRA

51 P I2 — 52 P TCA — 53 P CAP — 54 P CH3CN — 50 EXTRA — 44 EXTRA

602180 40.0" 1.6mm

P1 P2

WASTE BOTTLE 10L 140040 — 602544 — WASTE MANIFOLD — PLUGGED W2, W3 — TO VENT — W1–W8 — 602686

225062 21.0" .187I.D.

602179 27.0" .8mm — 602181 24.0" .8mm — 602299 PVC 6.0"

REG 3 — MANIFOLD C — COL. 1 602177 17.0" — COL. 2 602178 17.0"

602189

35 GAS — 34 PRESS — 33 CH3CN — 32 LP GAS — 31 TET — 30 5 — 29 T — 28 C — 27 G — 26 A

602251 REAR — VAC 6 17.0" — R-11 — 7.0" .5mm — 602198

602251 FRONT — F-11 — VAC 5 17.5" — 9" .5mm CLEAR — 602198 (2x)

25 WASTE — 24 NH4OH — 23 I2 — 22 TCA — 21 CAP2 — 20 CAP1 — 19 600191 — 18 600191 — 17 — 16 COL1

W6 33.0"

602189 (5x)

602183 A — 602183 G — 602183 C — 602183 T — 602183 5

7.0" — 1.5"

MANIFOLD A — MANIFOLD B — 10" — P BASE 13.5" — V BASE 5.5"

602187 250ml 11 CAP1 — 602187 250ml 12 CAP2 — 602185 500ml 14 TCA — 602188 250ml 15 I2

602184 250ml 9 TET — 602186 250ml 10 NH4OH

602192 7.0" (2x) — 1 — 2 — 1.5" — 0.5" — W1 9.0" — 7.0"

602198 (2x) — COL. 1 — COL. 2 — 11.5" .5mm CLEAR

602199 (2x) — VAC 4 18.5" — VAC 3 14.5" — 602258 A-5 — 602258 B-5

COLUMN 1 — W5 18.5" — MAN. C — WASTE 3 — PORT1 2 — VAL1 1 — O GAS LOWER

COLUMN 2 — W4 23.0" — MAN. C — WASTE 7 — PORT2 6 — VAL2 5 — GAS 4

602195 (2x) 22.0" .8mm

TRITYL OUTPUT — 602182 (2x) 30.0" .8mm — FRACTION COLLECTOR — AUXILLARY A B — P D — 18 — 602458 — 4L — 18 CH3CN

602194 19.5" — 20.0" — 20.0" — 3.0" — 3.0" — 2.0" — 2.0" — 23.0" — 5.0" — 13.0" — 27.0" — 24.0" — 10.0" — 19.5" — 19.50" — 11.0" — 5.0" — 5.0" — 8.0" — 8.0" — 4.0" — 1.5" — 16.0" — 16.0" — 22.0" — 26.0"

| REV | DESCRIPTION | APPROVED | DATE |
|---|---|---|---|
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| B | REDRAWN PER ECO#3796 | KN | 8.2.90 |
| C | REVISE PER ECO# 4289 | | |

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| ITEM | QTY | MATERIAL-DESCRIPTION | MAT'L-PART NO |
|---|---|---|---|
| | | | |

| DRAWN BY | DATE | | |
|---|---|---|---|
| J. B. ENGLETON | 2/27/90 | | |
| CHECKED | | | |
| ERIC NORDMAN | 3.22.90 | | |
| RELEASE TO PROD | | 3.22.90 | |
| SUPERVISOR'S DWG | | | |

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PLUMBING DIAGRAM 392, 2 COL. 5 BASE

PART NUMBER 901238

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| C | REVISE PER ECO# 4289 | | |

P BASE 13.5" V BASE 5.5" 10"
MANIFOLD A
MANIFOLD B
8 602183 8
7 602183 7
6 602183 6
5 602183 5
4 602183 T
3 602183 C
2 602183 G
1 602183 A
7.0"
1.5"
602189 (8X)

2.5" 7.0" 0.5" 1.5" W1 9.0"
2
1
602192 7.0" (2X)
602195 (2X) 22.0" .8mm
7 WASTE 6 PORT2 5 VIAL2 4 GAS 602258 B-5 VAC 3 14.5"
W4 23.0"
COLUMN 2
MAN. C
3 WASTE 2 PORT1 1 VIAL1 0 GAS 602258 A-5 VAC 4 18.5" LOWER
W5 18.5
COLUMN 1
MAN. C
602199 (2X) 11.5" .5mm CLEAR
COL 2
COL 1
602198 (2X) 9" .5mm CLEAR
TRITYL OUTPUT 1 2 3 4
30.0" .8mm 602182 (2X)
FRACTION COLLECTOR
18 P D AUXILLARY A B
602458 4L
18 $CH_3CN$

26.0" 22.0" 16.0" 16.0" 8.0" 1.5"
15 602188 250ml $I_2$
14 602185 500ml TCA
12 602187 250ml CAP2
11 602187 250ml CAP1
10 602186 250ml $NH_4OH$
9 602184 250ml TET
8.0" 8.0" 4.0"
602189
25 WASTE 24 $NH_4OH$ 23 $I_2$ 22 TCA 21 CAP2 20 CAP1 19 600191 18 600191 17 COL 2 16 COL1 602251 F-11 VAC 5 17.5" FRONT
W6 33.0"
602196 7.0" .5mm
35 34 TET 33 B 32 7 31 6 30 5 29 T 28 C 27 G 26 A R-11 602251 VAC 6 17.0" REAR
5.0" 5.0" 11.0"
20.50" 19.50"
5.0" .8mm 602197
VAC 7 5.0" E-5 602258 43 LPGAS 42 $CH_3CN$ 41 PRESS 40 GAS
602299 PVC 6.00"
602181 24.0" .8mm
602179 27.0" .8mm
225062 21.0" .187" I.D.
COL 1 602177 13.0" REG 3 MANIFOLD C COL 2 602178 17.0"

10.0" V VACUUM PUMP ASSY 002698 P VAC. 1-7
602201
24.0" 27.0" REG 1 602200 5.0"
13.00" REG 2 602200 3.50"
23.0" FILTER 10 mic
602202 GAS INLET
MAN C. 17.0" REG 3 602200 6.5"
19.5" 20.0" 2.0" 2.0" 3.0" 3.0"
MAN. A MAN. B
44 EXTRA 45 P BASE 46 V BASE W7 37.0" 47 P $NH_4OH$ 48 V $NH_4OH$ W8 40.0" 49 P TET
50 EXTRA 51 P $I_2$ 52 P. TCA 53 P CAP 54 P $CH_3CN$ 55 EXTRA
602180 40.0" 1.6mm
P1 P2

602686 W1-W8 W2, W3 PLUGGED WASTE MANIFOLD TO VENT 602544 10L 140040 WASTE BOTTLE

NOTES: △ = 1/4-28 BARB

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DATE
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PLUMBING DIAGRAM
392, 2 COL, 8 BASE

Applied Biosystems
PART NUMBER 901240
D - 901240 - 1

NOTES: △ = 1/4-28 BARB

GAS INLET

602202

FILTER 10 mic

602201

VACUUM PUMP ASSY 002698

VAC. 1-7

10.0"

23.0"

602200 REG 1

602200 REG 2

602200 REG 3

MAN. C 17.0"

6.5"

3.5"

5.0"

13.0"

27.0"

24.0"

20.0"

19.5"

3.0"

2.0"

602299 PVC 6.00"

602179 27.0" .8mm

602181 24.0" .8mm

225062 21.0" .187"I.D.

602180 40.0" 1.6mm

602178 COL. 2 17.0"

602177 COL. 1 17.0"

602178 COL. 4 17.0"

602177 COL. 3 13.0"

REG 3 MANIFOLD C

602251 REAR

602198 7.0" .5mm

602251 FRONT

602198 (4x) .5mm CLEAR

602251 VAC. 5 17.5"

602199 (4x) 11.5" .5mm CLEAR

602195 (4x) 22.0" .8mm

602182 (4x) 30.0" .8mm

602189 (5x)

602192 (4) 1.5"

602244 WASTE MANIFOLD

602244 10L 140040 WASTE BOTTLE

602686 W1-WB

TO VENT

TRNTL OUTPUT

AUXILLARY

FRACTION COLLECTOR

602458 4L

18 $CH_3CN$

| ITEM | QTY | MATERIAL-DESCRIPTION | MAT'L-PART NO |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |

| | | | | | |
|---|---|---|---|---|---|
| | | J. B. ENGLETON | 2/27/90 | | |
| | | ERIC NORDMAN | 3.22.90 | PLUMBING DIAGRAM 394, 4 COL, 5 BASE | |
| | | RELEASE TO PROD | 3.22.90 | | |
| | | SUPERSEDES DWG | | SCALE FULL | |

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XXX = ± .005
ANGLES = 1 DEG
DIMENSIONS ARE IN INCHES

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| C | REVISE PER ECO# 4289 | | |

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NOTES: △ = 1/4-28 BARB

GAS INLET

| ITEM | QTY | MATERIAL - DESCRIPTION | MAT'L - PART NO |
|---|---|---|---|
| | | | |

| | | | |
|---|---|---|---|
| DRAWN BY | J. B. ENGLETON | 2/27/90 | |
| ENGINEER | ERIC NORDMAN | 2.22.90 | |
| RELEASE TO PROD | | 2.22.90 | |
| SCALE | FULL | SHEET | |

PLUMBING DIAGRAM
394, 4 COL, 8 BASE

Applied Biosystems

PART NUMBER 901241

D - 901241 -1

| REV | DESCRIPTION | APPROVED | DATE |
|---|---|---|---|
| C | REVISE PER ECO# 4289 | KN | 8/2/90 |
| B | REDRAWN PER ECO#3796 | KN | |
| A | AS ISSUED | KN | 2.22.90 |

10 - 901241 - 1

# *Appendix III*

# Flow Tests PressureTests

This appendix describes the flow tests and pressure tests in the self-testing module of the Model 392/394. It provides information on how to set up and conduct these tests.


Introduction ..... App. III-2
Access to Flow and Pressure Tests ..... App. III-2
Flow Rate Tests ..... App. III-3
Drip Test ..... App. III-4
Set Regulator 2 ..... App. III-4
Set Regulator 1 ..... App. III-5
Set Regulator 3 ..... App. III-5
Flow to Column ..... App. III-6
Flow Through Column ..... App. III-7
Flow to Phosphoramidites (A, G, C, T, and 5-8) ..... App. III-8
Flow from Each Bottle to Column 1 ..... App. III-10
Pressure Tests ..... App. III-11
Test 0 ..... App. III-12
Test 1 ..... App. III-12
Tests 2 Through 8 ..... App. III-12
Tests 9 Through 13 ..... App. III-14

# Introduction

Model 392/394 synthesizers perform several types of self-tests, including flow and pressure tests. The flow tests allow you to measure flow rates through all essential delivery lines in the instrument—to the reagent bottles, to the columns, and through the columns. The pressure tests enable you to detect leaks (if any) in various parts of the system.

Although these tests are performed automatically after you press the appropriate function keys on the instrument, you must make certain preparations for each test and participate to some extent. For example, in flow tests, you must be prepared to catch and weigh the reagents flowing from columns or bottle positions. In pressure tests, you must install dry bottles and plug the appropriate outlets in the area being tested. This appendix gives procedures for carrying out the tests, including the preparatory steps and other steps that you must perform.

## Access to Flow and Pressure Tests

To reach the instrument's Self Test menu, press the *Self Test* soft key on page 3 of the Main Menu. Page 1 of the Self Test menu will appear. Then press the *Next Page* soft key to display page 2 (Figure III-1 ). You will see soft keys leading to the pressure and flow tests. To reach the menu for selecting pressure tests, press *Pressure Test.* To reach the menu for selecting flow tests, press *Flow Rate Test.*

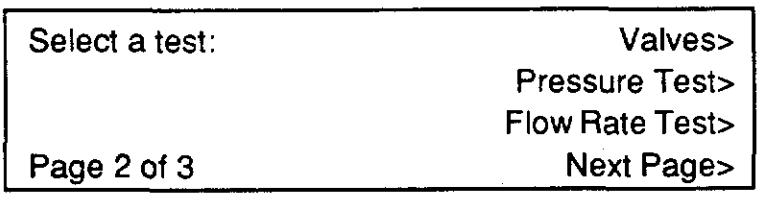



Figure III-1 . Self Test menu, page 2

# Flow Rate Tests

The Flow Rate Test menu (Figure III-2 ) enables you to select from a series of flow tests available in the system. When this menu is displayed, the cursor rests on the current test name in the lower left corner. To scroll through the available tests and select a different test, press the **Prev.** or **Next** key.

Two soft keys are on the menu: *Set Dilute Flow* and *Start*. *Set Dilute Flow* is discussed under "Flow to Phosphoramidites (A, G, C, T, and 5-8)," later in this appendix. The *Start* key initiates the selected flow test. After the test is started, the soft keys on the menu change to *Int Immed*, *Hold*, and *Jump*.

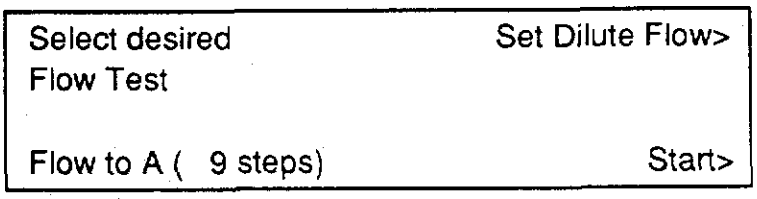



Figure III-2 . Flow Rate Test menu

During the course of the flow tests, an Interrupt function will automatically stop the test to let you tare a balance (i.e., weigh an empty collection vessel) or to weigh reagent collected at an outlet. When such an interrupt occurs, the soft keys on the Flow Rate Test menu automatically change to *Resume*, *Abort*, and *Jump*. When you are ready to continue the test, you press the *Resume* key. (The interrupt, abort, or jump commands work in the same way as for syntheses. For more information, see "Interrupting, Holding, or Jumping" in Section 3.)

During the flow tests, it might be necessary to adjust one of the instrument's pressure regulators. For access to them, remove the left side panel of the instrument. You will see three regulators, each with a pressure gauge and a black adjustment knob. Regulator 1 is the uppermost; Regulator 2 is in the middle; and Regulator 3 is the lowest. The three regulators pressurize the following bottles:

| | |
|---|---|
| Regulator 1: | Bottles 1-9 |
| Regulator 2: | Bottles 11-18 |
| Regulator 3: | Bottle 10 |

To increase the pressure, turn the black knob clockwise. To decrease the pressure, turn the knob counterclockwise while venting one of the regulator's bottles by removing it.

Procedures for the most frequently used flow tests are given in the following subsections.

## Drip Test

In the drip test, acetonitrile from bottle 18 is flushed sequentially through each bottle position and column. This test verifies that the instrument is correctly plumbed.

1. Connect a large bottle of acetonitrile to the pressure/delivery port on the side of the instrument.
2. Set pressure regulator 1 to approximately 5 psi. Set regulator 2 to approximately 6.5 psi. Set regulator 3 to approximately 12-13 psi.
3. Put beakers in position to catch the acetonitrile drip from the delivery line of each bottle position (1 through 5 or 8, 9 through 12, and 15). The delivery line is the longest of the two lines to each position. (The other line is a pressure/vent line.)
4. Select "Drip Test" on the Flow Rate Test menu, and press the *Start* soft key.

While the test is running, acetonitrile should drip from each delivery line in turn, beginning at bottle position 1 and ending with the waste port. Note whether the dripping occurs in sequential order. Also, make sure that the acetonitrile is coming from the delivery lines and not the pressure/vent line. If the drip sequence is out of order or if the drip comes from the wrong line, the instrument's plumbing is incorrect. In this case, contact the local ABI service representative.

## Set Regulator 2

The flow test "Set Regulator 2" determines whether the flow controlled by regulator 2 is within the specified limits (1.35-1.44 grams/30 seconds). This is tested by delivering acetonitrile from bottle 14 to all the columns.

1. Place a bottle of acetonitrile on position 14.
2. Attach measuring lines (ABI Part No. 602278) to each column. Put the other end of the line from column 1 in a beaker that has been placed on a balance capable of weighing to 0.01 gram.
3. Select "Set Reg2" on the Flow Rate Test menu, and press the *Start* soft key. The measuring line from column 1 fills, and the test is automatically interrupted.
4. Press the Tare key on the balance (or weigh the beaker and record its weight). If you removed the measuring line, place it back into the beaker.
5. Press the *Resume* soft key to continue the test. Acetonitrile flows from position 14 to the column and into the beaker for 30 seconds. Then the test is again interrupted automatically.
6. Weigh the beaker containing the acetonitrile and record the net weight of the acetonitrile.
7. Place the measuring line from column 2 into an empty beaker and press *Resume*. Repeat the procedure for column 2 and for the other columns (if any) on the instrument.

If the amount of acetonitrile from any column weighs less than 1.35 grams, adjust regulator 2 to increase the pressure. If the acetonitrile weighs more than 1.44 grams, adjust regulator 2 to decrease the pressure. If you decrease the pressure, vent bottle 14 to let the pressure inside the bottle equilibrate. Then close the bottle and deliver acetonitrile to a column for 30 seconds before remeasuring the flow.

Note that the difference between the flow amounts from each column should not exceed 0.04 gram. If the difference is greater, replace the point restrictors with a new set. If differences greater than 0.04 gram persist, call the local ABI service representative.

## Set Regulator 1

The flow test "Set Regulator 1" determines whether the flow controlled by regulator 1 is within the specified limits (1.18-1.22 gram/30 seconds). This is tested by delivering acetonitrile from bottle 1 to column 1. (This test delivers acetonitrile only to column 1; it is assumed that the "Set Regulator 2" test has been conducted and that flows to the other columns are satisfactory.)

1. Place a bottle of acetonitrile on position 1.
2. Attach a measuring line (ABI Part No. 602278) to column 1. Put the other end of the line in a beaker that has been placed on a balance capable of weighing to 0.01 gram.
3. Select "Set Reg1" on the Flow Rate Test menu, and press the *Start* soft key. The measuring line fills, and the test is automatically interrupted.
4. Press the Tare key on the balance (or weigh the beaker and record its weight). If you removed the measuring line, place it back into the beaker.
5. Press the *Resume* soft key to continue the test. Acetonitrile flows from position 1 to the column and into the beaker for 30 seconds. Then the test is again interrupted automatically.
6. Weigh the beaker containing the acetonitrile and record the net weight of the acetonitrile.

If the acetonitrile from the column weighs less than 1.18 gram, adjust regulator 1 to increase the pressure. If the acetonitrile weighs more than 1.22 gram, adjust regulator 1 to decrease the pressure. If you decrease the pressure, vent the acetonitrile bottle in position 1 to let the pressure inside the bottle equilibrate. Then close the bottle and deliver acetonitrile to column 1 for 30 seconds before remeasuring the flow.

## Set Regulator 3

The flow test "Set Regulator 3" determines whether the flow controlled by regulator 3 is within the specified limits (1.95-2.10 grams/30 seconds). This is tested by delivering acetonitrile from bottle 10 to column 1. (This test delivers acetonitrile only to column 1; it is assumed that the "Set Regulator 2" test has been conducted and that flows to the other columns are satisfactory.)

1. Place a bottle of acetonitrile on position 10.
2. Attach a measuring line (ABI Part No. 602278) to column 1. Put the other end of the line in a beaker that has been placed on a balance capable of weighing to 0.01 gram.
3. Select "Set Reg3" on the Flow Rate Test menu, and press the *Start* soft key. The measuring line fills, and the test is automatically interrupted.
4. Press the Tare key on the balance (or weigh the beaker and record its weight). If you removed the measuring line, place it back into the beaker.
5. Press the *Resume* soft key to continue the test. Acetonitrile flows from position 10 to the column and into the beaker for 30 seconds. Then the test is again interrupted automatically.
6. Weigh the beaker containing the acetonitrile and record the net weight of the acetonitrile.

If the acetonitrile from the column weighs less than 1.95 grams, adjust regulator 3 to increase the pressure. If the acetonitrile weighs more than 2.10 grams, adjust regulator 3 to decrease the pressure. If you decrease the pressure, vent the acetonitrile bottle in position 10 to let the pressure inside the bottle equilibrate. Then close the bottle and deliver acetonitrile to column 1 for 30 seconds before remeasuring the flow.

## Flow to Column

The flow test "Flow to Column" measures the flow from bottles 2-9, 11-15, and 18 to column 1. The flow rate specifications are as follows:

| **Bottle #** | **Grams/30 sec.** |
|---|---|
| 2-9 | 1.18-1.22 |
| 11, 12 | 1.30-1.46 |
| 14 | 1.35-1.44 |
| 15, 18 | 1.30-1.44 |

(This test delivers acetonitrile only to column 1; it is assumed that the "Set Regulator 2" test has been conducted and that flows to the other columns are satisfactory.)

1. Place bottles of acetonitrile on positions 2 through 9, 11 through 15, and 18.
2. Attach a measuring line (ABI Part No. 602278) to column 1. Put the other end of the line in a beaker that has been placed on a balance capable of weighing to 0.01 gram.
3. Select "Flw to Col" on the Flow Rate Test menu, and press the *Start* soft key. The measuring line fills with acetonitrile from bottle 2. This priming step lasts for 25 seconds, and then the test is automatically interrupted.
4. Press the Tare key on the balance (or weigh the beaker and record its weight). If you removed the measuring line, place it back into the beaker.

5. Press the *Resume* soft key to continue the test. Acetonitrile flows from position 2 to the column and into the beaker for 30 seconds. Then the test is again interrupted automatically.

6. Weigh the beaker containing the acetonitrile and record the net weight of the acetonitrile.

7. Empty the beaker and place it back onto the balance (or use a dry beaker). Place the measuring line in the beaker.

8. Press *Resume*. The measuring line will fill with acetonitrile from the next bottle position for 25 seconds and then the test will be interrupted.

9. Repeat Steps 4 through 8 until you have weighed acetonitrile from all remaining bottle positions in the group being tested. The steps in the test are identical for all bottle positions except that bottles 9 and 18 will deliver acetonitrile for 60 seconds instead of 25 seconds to prime the lines.

## Flow Through Column

The flow test "Flow Through Column" measures the flow from each column to the waste manifold, to the trityl ports, and to the DNA collection vials. The specifications are:

| | |
|---|---|
| Flow rate to the waste manifold: | 1.14-1.34 grams/30 seconds |
| Flow rate to the trityl ports: | 1.15-1.31 grams/30 seconds |
| Flow rate to the DNA collection vials: | 2.60-3.10 grams/30 seconds |

1. Place bottles of acetonitrile on positions 10 and 14.

2. Put column connectors on all columns.

3. Put trityl lines on all trityl ports.

4. Put DNA collection vials on all DNA collection positions.

5. Disconnect the waste line.

6. Select "Flw thru Col" on the Flow Rate Test menu.

7. Hold a beaker directly below the waste manifold and press the *Start* soft key. This starts a priming step. Acetonitrile from bottle 14 is delivered through column 1 to the waste manifold for 25 seconds. Then the test is automatically interrupted.

8. Place the beaker on a balance and press the Tare key (or weigh the beaker and record its weight).

9. Place the beaker back under the waste manifold and press the *Resume* soft key to continue the test. Acetonitrile flows from bottle 14 through column 1 and then to waste for 30 seconds. Then the test is again interrupted automatically.

10. Weigh the beaker containing the acetonitrile and record the net weight of the acetonitrile.

11. Empty the beaker, hold it under the waste manifold, and press the *Resume* soft key to begin another priming step. Acetonitrile will now flow through column 2 to waste for 25 seconds. Then the test is automatically interrupted. Repeat the taring, filling, and weighing steps (8 through 10) for column 2.

12. Repeat for columns 3 and 4 (if present).

13. During the interrupt period after weighing the acetonitrile from the last column, place the trityl lines into an empty beaker.

14. Press *Resume*. This starts another priming step. Acetonitrile from bottle 14 is delivered again through column 1 to the trityl ports for 25 seconds. Then the test is automatically interrupted.

15. Tare the balance using the beaker.

16. Place the trityl lines back into the beaker and press the *Resume* soft key to continue the test. Acetonitrile flows from bottle 14 through column 1 and then to the trityl ports for 30 seconds. Then the test is again interrupted.

17. Weigh the beaker containing the acetonitrile and record the net weight of the acetonitrile.

18. Empty the beaker, place the trityl lines in it, and press the *Resume* soft key to begin another priming step. Acetonitrile will now flow through column 2 to the trityl ports for 25 seconds. Then the test is automatically interrupted. Repeat the taring, filling, and weighing steps (15 through 17) for column 2.

19. Repeat for columns 3 and 4 (if present).

20. After weighing the acetonitrile from the last column, press *Resume*. This starts another priming step. Acetonitrile from bottle 10 is delivered again through column 1 to the DNA collect vial for 5 seconds. Then the test is automatically interrupted.

21. Remove the vial and use it to tare the balance.

22. Replace the vial on the instrument and press the *Resume* soft key to continue the test. Acetonitrile flows from bottle 10 through column 1 and then to the vial for 30 seconds. The test is again interrupted.

23. Weigh the vial and record the net weight of the acetonitrile.

24. Press the *Resume* soft key to begin another priming step. Acetonitrile will now flow through column 2 to the DNA collection vial for 5 seconds. Then the test is automatically interrupted. Repeat the taring, filling, and weighing steps (21 through 23) for column 2.

25. Repeat for columns 3 and 4 (if present).

## Flow to Phosphoramidites (A, G, C, T, and 5-8)

A separate test is available to measure the flow of acetonitrile from bottle 18 to each phosporamidite bottle position, for example "Flow to A," "Flow to G," "Flow to 6," and so on. You perform these tests in order to calibrate the auto-dilution procedures.

The following procedure applies in general to all of these flow tests.

1. Install an empty bottle on the instrument at the phosphoramidite position being tested (1 through 8).
2. Install a bottle of acetonitrile at position 18.
3. Select "Flw to *x*" on the Flow Rate Test menu, where *x* is the phosphoramidite A, G, C, T, or 5-8. Then press the *Start* soft key. The flow route is primed for 5 seconds, and then the test is automatically interrupted.
4. Remove the phosphoramidite bottle and empty into waste.
5. Place the bottle on a balance capable of weighing to 0.01 gram and press the Tare key (or weigh the bottle and record its weight). Put the bottle back into position on the instrument.
6. Press the *Resume* soft key to continue the test. Acetonitrile flows from bottle 18 to the phosphoramidite bottle for 30 seconds. Then the test is again interrupted automatically.
7. Remove the phosphoramidite bottle and weigh it again with the acetonitrile. Record the net weight of the acetonitrile.

After obtaining the flow rate of acetonitrile to the bottle positions, you are ready to calibrate the auto-dilution procedure. To do so, press the *Set Dilute Flow* soft key on the Flow Rate Test menu. You will see the Auto Dilute Calibration menu (Figure III-3 ).

| | |
|---|---|
| Bot #1: 4.92 gm/30 sec. | #2: 4.91 gm/30 sec. |
| #3: 4.93 gm/30 sec. | #4: 4.92 gm/30 sec. |
| #5: 4.92 gm/30 sec. | #6: 4.91 gm/30 sec. |
| #7: 4.93 gm/30 sec. | #8: 4.92 gm/30 sec. |

Figure III-3 . Auto Dilute Calibration menu

The cursor rests on the first value for grams/30 sec (following "Bot #1:"). Use the numeric keypad to enter the flow rate obtained from the "Flow to A" test. Then move the cursor to the Bottle #2 value and enter the results from the "Flow to B" test. Enter the results from the other flow tests after the appropriate bottle numbers. The software uses the data entered through this menu to determine the correct acetonitrile delivery times for the ABI Auto Dilute procedures.

## Flow from Each Bottle to Column 1

Flow tests are available to measure the flow of acetonitrile from each bottle position to column 1. These tests are named B 01->Col1, B 02->Col1, ... B 18->Col1. The specifications are:

| Bottle # | Grams/30 sec |
|---|---|
| 1-9 : | 1.18-1.22 |
| 10 | 1.95-2.10 |
| 11, 12 | 1.30-1.46 |
| 14 | 1.35-1.44 |
| B15, 18 | 1.30-1.44 |

The following is a general procedure for all of these tests.

1. Install a bottle of acetonitrile on the instrument at the bottle position being tested.
2. Attach a measuring line (ABI Part No. 602278) to column 1. Place the free end of the line in a beaker that has been placed on a balance capable of weighing to 0.01 gram.
3. Select "B *xx*->Col1" on the Flow Rate Test menu, where *xx* is the bottle position. Then press the *Start* soft key. The flow route is primed for a period that varies with the bottle position, and then the test is automatically interrupted.
4. Discard the acetonitrile in the beaker and replace on the balance.
5. Press the Tare key (or weigh the beaker and record its weight). Put the beaker back into position on the instrument.
6. Press the *Resume* soft key to continue the test. Acetonitrile flows from the bottle to the column for 30 seconds. Then the test is again interrupted automatically.
7. Weigh the beaker with the acetonitrile. Record the net weight of the acetonitrile. Discard the acetonitrile.
8. Press the *Resume* soft key to continue the test. More acetonitrile flows from the bottle to the column (for 30 seconds). The test is again interrupted automatically.
9. Weigh the beaker with the acetonitrile. Record the net weight of the acetonitrile. This last step verifies the first weighing.

# Pressure Tests

Pressure tests are conducted to check the instrument's plumbing for leaks, before chemicals are installed. They are performed as part of installation and when a leak is suspected.

---

***CAUTION*** *Do not run pressure tests with chemicals on the instrument. Damage to the pressure transducer may result.*

---

Before running pressure tests, you must put clean, dry bottles on all the bottle positions (including 18). Then activate the "Block Flush" function (#1) for 30 seconds. For information on activating a function see "Main Menu Option: Manual Control," in Section 3.

The Pressure Test menu (Figure III-4 ) allows you to select a range of pressure tests. Thirteen are available, numbered 1 through 13. The first line of this menu contains the statement "$x_1$ thru $x_2$," where $x_1$ is the number of the first test to be performed and $x_2$ is the number of the last. You can enter numbers from the numeric keypad to specify each end of the range.

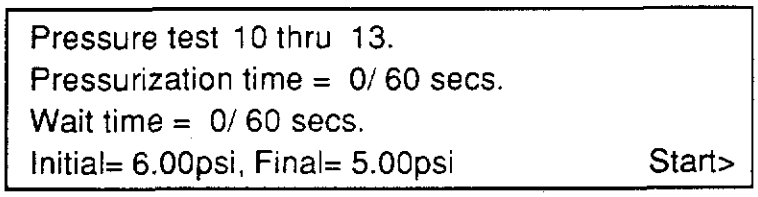



Figure III-4 . Pressure Test menu

The second line of the Pressure Test menu shows the standard pressurization time for the first test in the range selected. During this period, a valve opens to pressurize the area of the instrument being tested. At the conclusion of this period, the valve closes and the initial pressure is measured and displayed on line 4. After a wait period, displayed on line 3, the final pressure is measured and shown on line 4.

You begin the pressure tests by pressing the *Start* soft key. The tests are performed automatically. If the difference between the initial and final pressure is within the specification, the word PASS will appear on the display when all the tests in the selected range are completed. If the pressure difference falls outside the specification on a given test, testing will stop, whether or not all the tests in the selected range have been completed. The word FAIL will appear on the display.

In addition to the automatic tests, 1 through 13, you can conduct a manual pressure test, Test 0, which cannot be selected on the Pressure Test menu. All of these tests are described next. At the end of this appendix, you will find a series of diagrams that show the areas of the instrument that are pressurized by Tests 1 through 13. These areas are marked by dashed lines. If a leak is found, the diagrams will help you to determine its location.

## Test 0

Test 0 verifies the tightness of the gas inlet and regulator system. This test requires that the left side panel of the instrument be removed.

1. Set the argon tank regulator to 60.0 psi.
2. Pressurize for 15 seconds.
3. Shut off the tank regulator and note the initial pressure for the three instrument regulators.
4. In 5 minutes, note the final pressure for all regulators. The specification is 3 psi in 5 minutes (i.e., the difference between the initial and final pressure should not exceed 3 psi).

## Test 1

Test 1 verifies the tightness of the valve blocks and columns. The valves on for this test are:

| | |
|---|---|
| 8-base instruments: 16, 17, 18, 19, 35, 40, and 41 | (40 is the pressurizing valve) |
| 5-base instruments: 16, 17, 18, 19, 34, and 35 | (35 is the pressurizing valve) |

The specification is 0.06 psi in 20 seconds, maximum.

1. Install column connectors (ABI Part No. 225049) on all columns.
2. On the first line of the Pressure Test menu, enter 1 as the first and last test in the range of tests to be performed.
3. Press *Start* to begin the test.

## Tests 2 Through 8

Tests 2 through 8 are conducted together in a series. They verify the tightness of the phosphoramidite bottles, bottles 9-12 and 14-15, and the trityl and bottle 18 lines. Information about these tests is tabulated next.

| Test | What Is Tested | Valves On 8-Base | Valves On 5-Base | Specification (max. psi/sec) | Press. Time (sec) |
|---|---|---|---|---|---|
| Test 2 | Bot. 1-8 | 26,27,35,(40),41 | 26,27,(35),*34 | 0.06/30 | 10 |
| Test 3 | Bot. 9 | 34,35,(40),41 | 31,(35),34 | 0.06/50 | 120 |
| Test 4 | Bot. 10 | 24,35,41, (47) | 24,34,(47) | 0.06/50 | 30 |
| Test 5 | Bot. 11-12 | 20,21,35,41,(53) | 20,21,34,(53) | 0.06/100 | 30 |
| Test 6 | Bot. 14 | 22,35,41,(52) | 22,34,(52) | 0.06/100 | 30 |
| Test 7 | Bot. 15 | 23,35,41,(51) | 23,34,(51) | 0.06/50 | 30 |
| Test 8 | Trityl lines, bottle 18 lines | 2,6,10,14,16,17,18, 19,35,41,42,(54) | 2,6,10,14,16, 17,18,19,33, 34,(54) | 0.06/40 | 10 |

* The valves in parentheses are the pressurizing valves.

1. Install dry bottles at positions 1-12, 14, and 15.
2. Plug all trityl ports with trityl plugs (ABI Part No. 110019).
3. Plug the pressure and delivery ports for bottle 18 with trityl plugs.
4. On the first line of the Pressure Test menu, enter 2 as the first and 8 as the last in the range of tests to be performed.
5. Press *Start* to begin the tests.

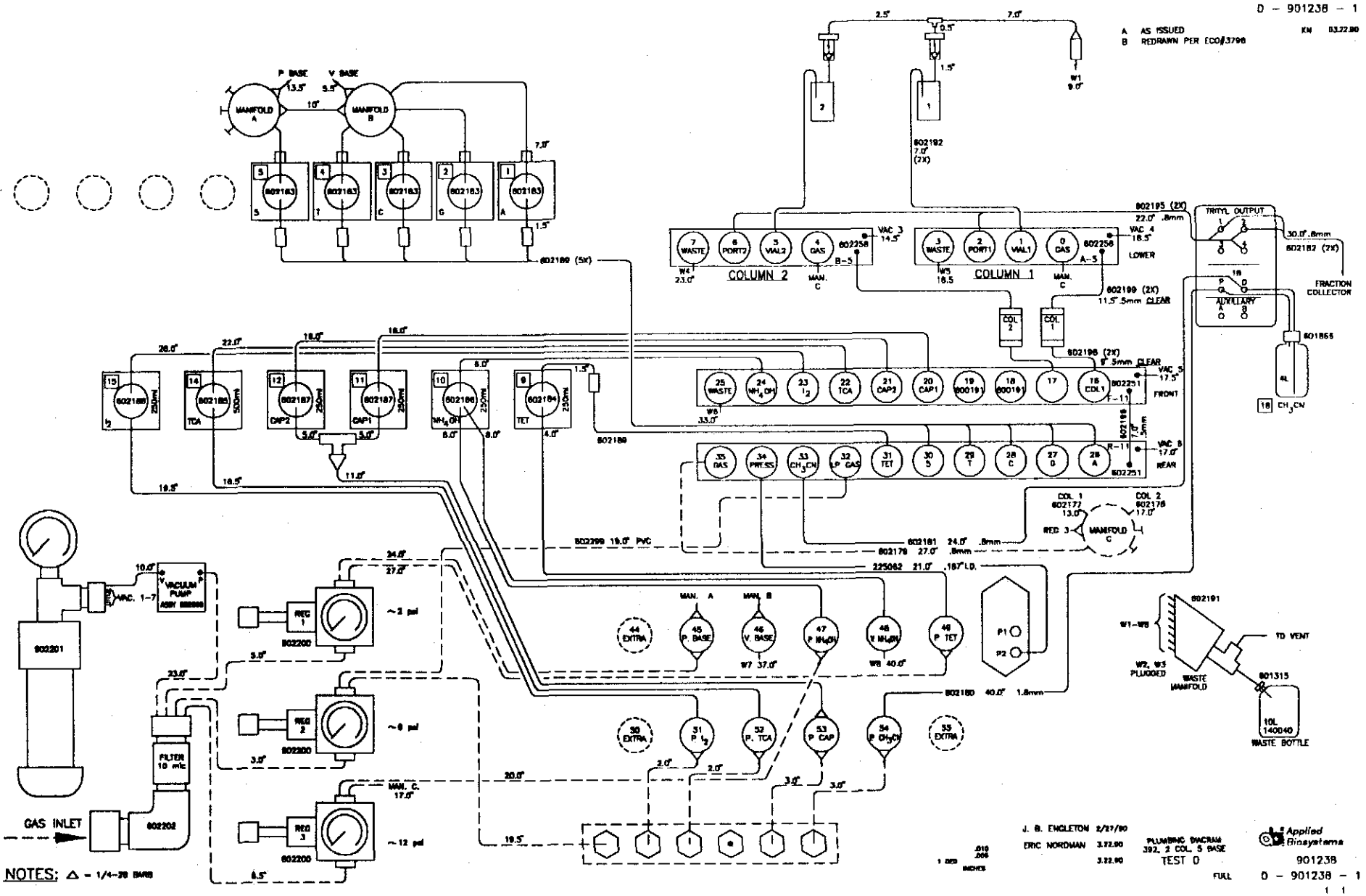
## Tests 9 Through 13

Tests 9 through 13 are conducted together in a series. Test 9 verifies the tightness of the waste system. Tests 10 through 13 are performed when the instrument is installed. They provide an overall verification of tightness for the valve blocks, columns, all bottles, the trityl lines, and the waste system. Information about these tests is tabulated below.

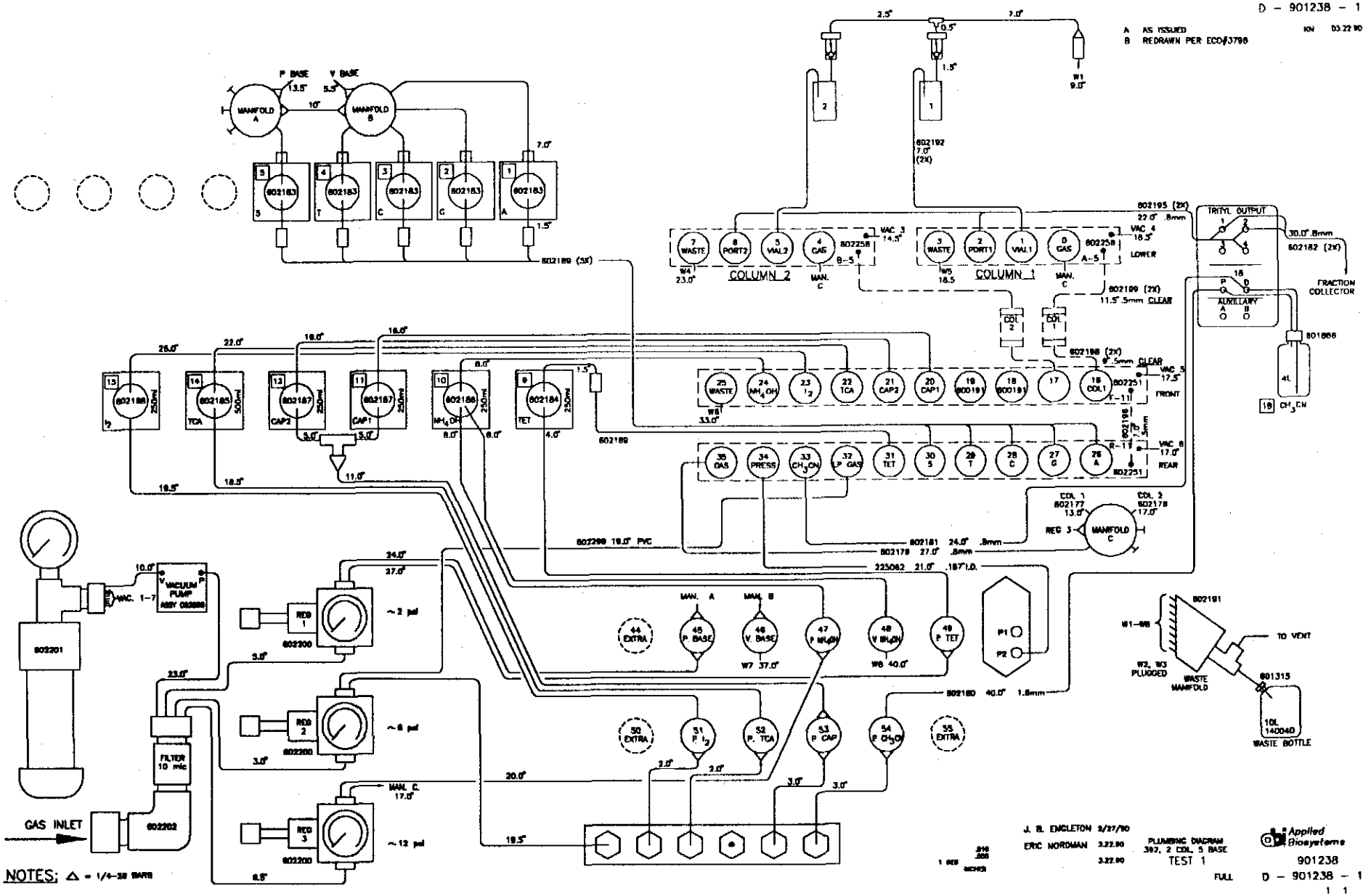
| Test | What Is Tested | Valves On | | Specification (max. psi/sec) | Press. Time (sec) |
|---|---|---|---|---|---|
| | | 8-Base | 5-Base | | |
| Test 9 | Waste system | 1,5,9,13,16,17,18,19, 25,35,41,(43) | 1,5,9,13,16, 17,18,19,25, (32),*34 | 0.06/10 | 10 |
| Test 10 | Valve blocks, columns | 16,17,18,19,35,(40), 41 | 16,17,18,19, 34,(35) | 0.06/10 | 10 |
| Test 11 | Bot. 1-9, Trityl lines | 2,6,10,14,16,17,18,19, 26,27,34,35,(40),41 | 2,6,10,14,16, 17,18,19,26, 27,34,(35) | 0.06/20 | 120 |
| Test 12 | Bot. 10-12, 14-15, 18 | 20,21,22,23,24,35, 41,42,(51,52,53,54) | 20,21,22,23, 24,33,34, (51,52,53,54) | 0.06/300 | 120 |
| Test 13 | Waste system | 1,5,9,13,16,17,18, 19,25,35,41,(43) | 1,5,9,13,16, 17,18,19,25, (32),34 | 0.06/5 | 10 |

* The valves in parentheses are the pressurizing valves.

1. Install plugs (ABI Part No. 110200) at the waste and vent line connections.
2. On the first line of the Pressure Test menu, enter 9 as the first and 13 as the last in the range of tests to be performed.
3. Press *Start* to begin the tests.


D - 901238 - 1
A AS ISSUED
B REDRAWN PER ECO#3796
COLUMN 2
COLUMN 1
FRACTION COLLECTOR
WASTE BOTTLE
GAS INLET
NOTES: △ = 1/4-28 BARB
J. B. ENGLETON 2/27/90
ERIC NORDMAN 3.22.90
PLUMBING DIAGRAM 392, 2 COL. 5 BASE TEST D
Applied Biosystems
901238
FULL
D - 901238 - 1

A AS ISSUED KN 03.22.90
B REDRAWN PER ECO#3798



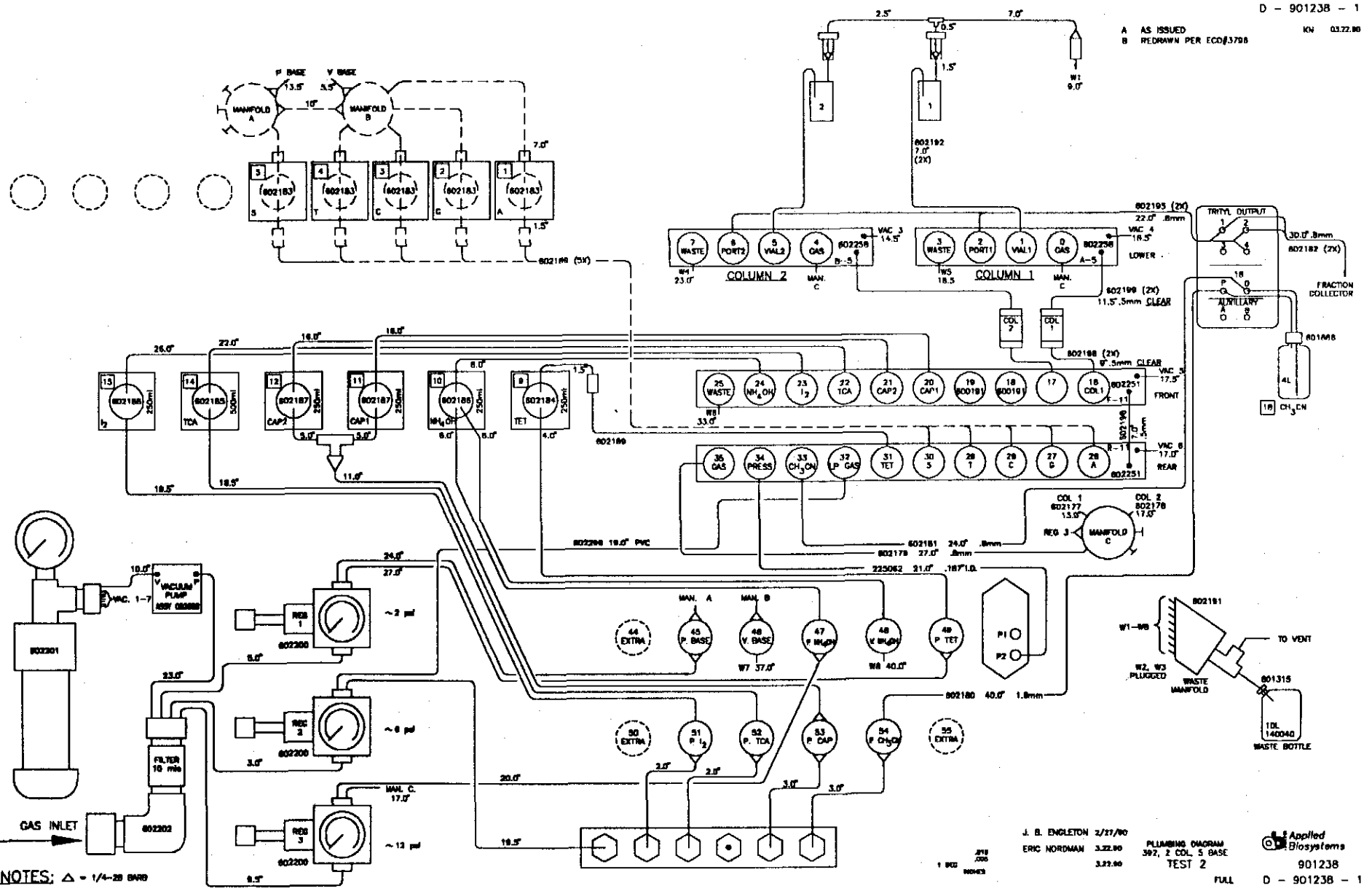

NOTES: △ = 1/4-28 SWR

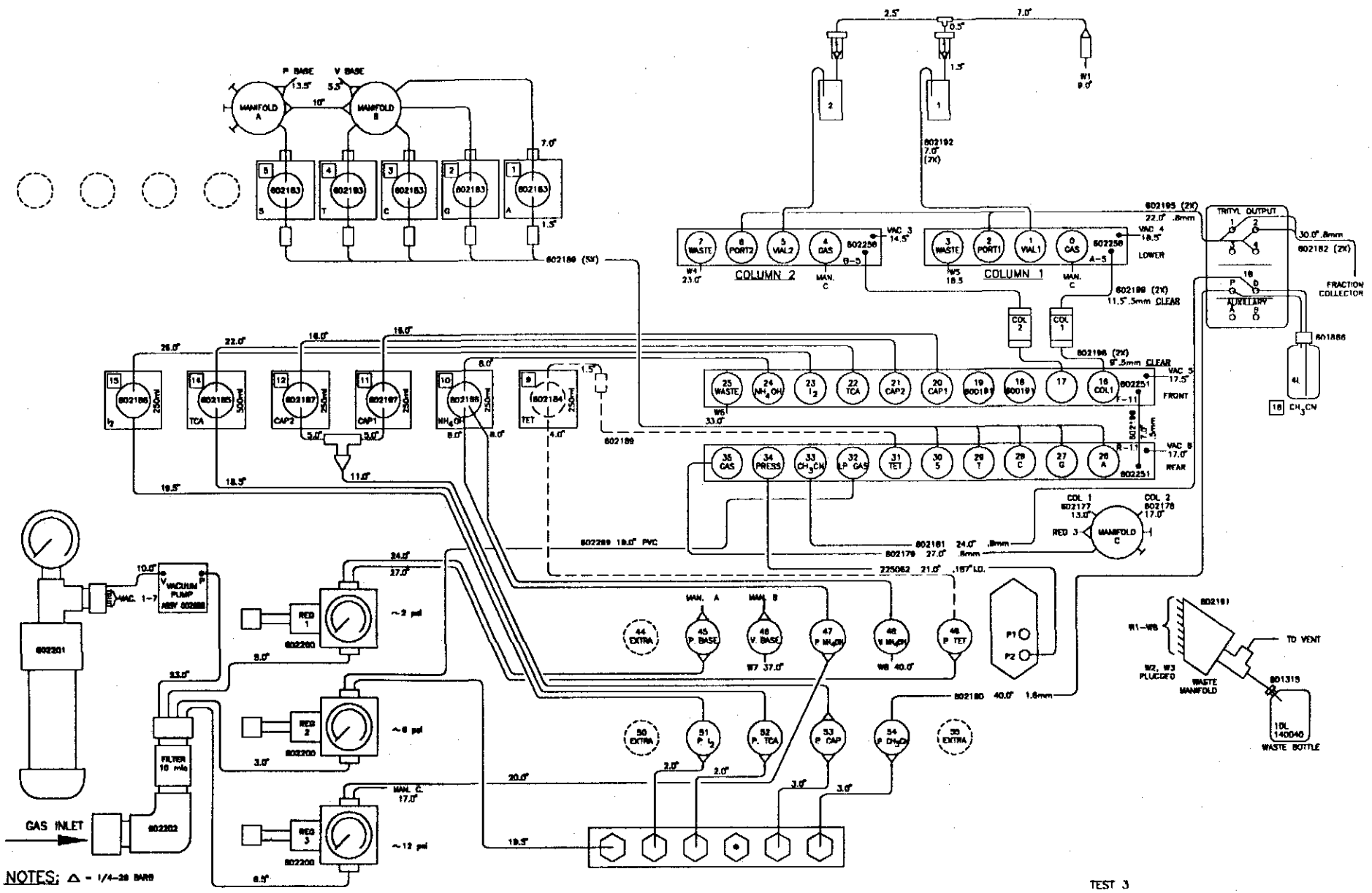
J. B. ENGLETON 3/27/90
ERIC NORDMAN 3.22.90
3.22.90

PLUMBING DIAGRAM
392, 2 COL, 5 BASE
TEST 1

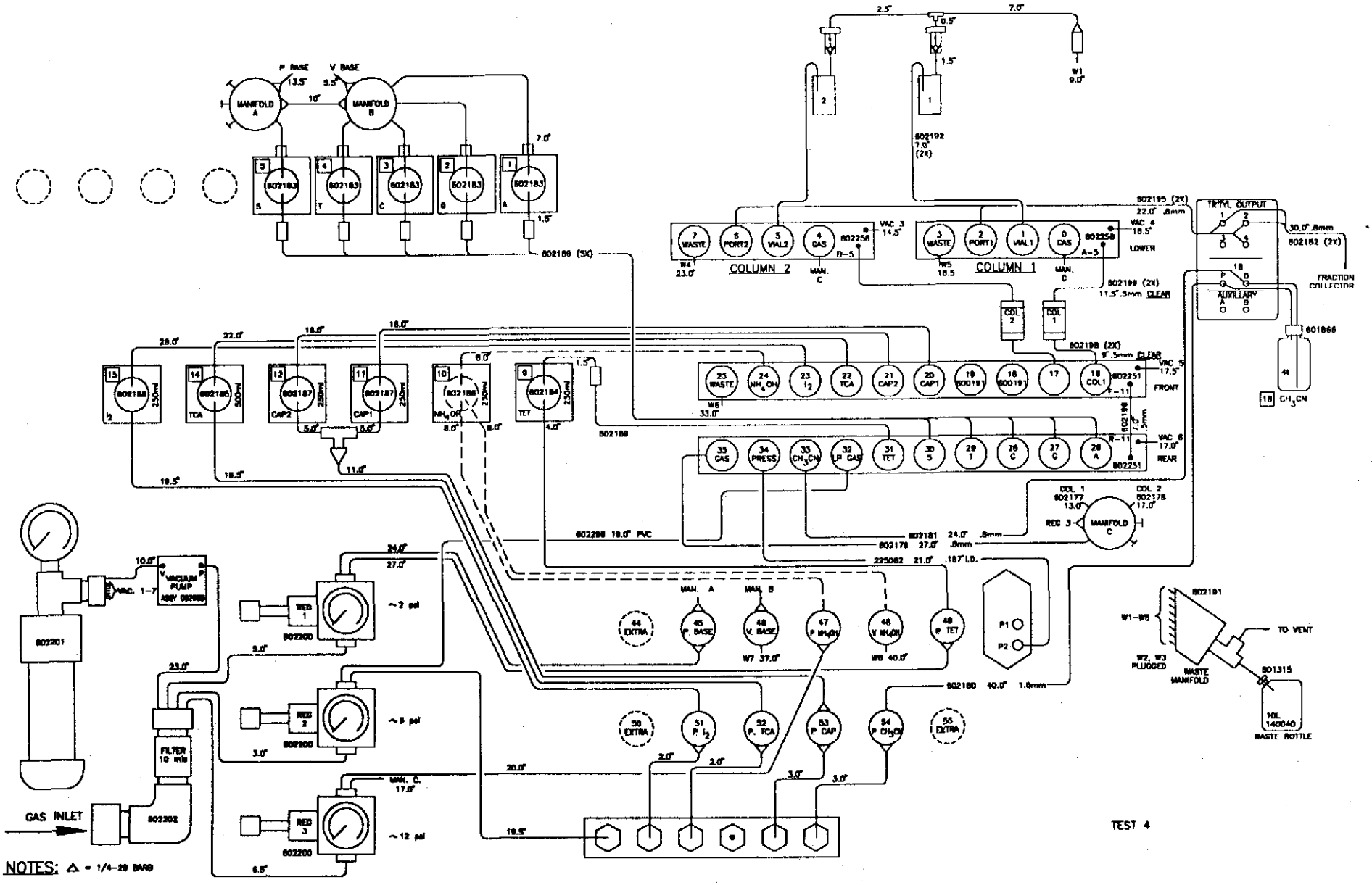
FULL

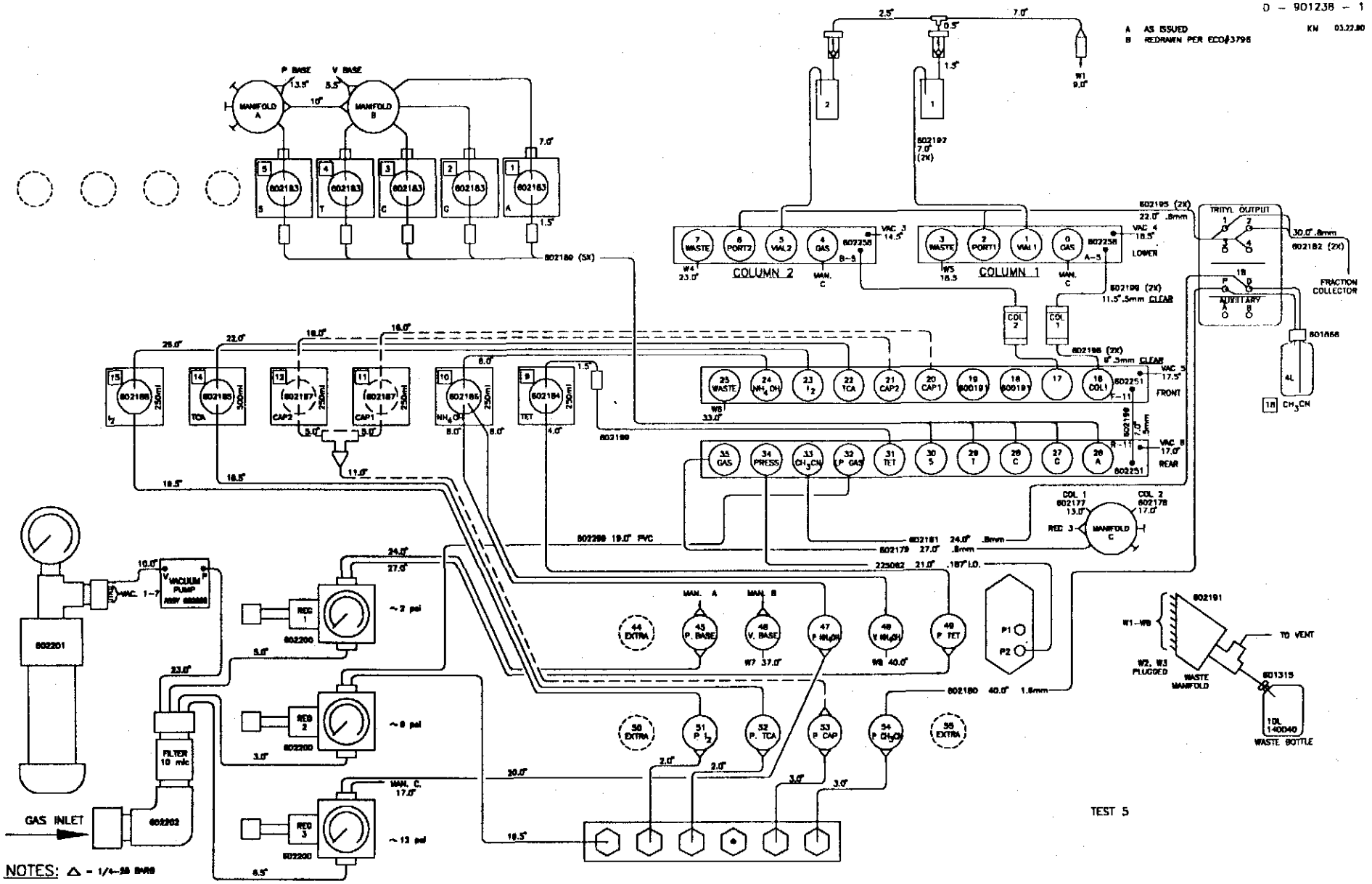
Applied Biosystems
901238
D - 901238 - 1
1 1


D - 901238 - 1
A AS ISSUED
B REDRAWN PER ECO#3798
KN 03.22.90
P BASE
V BASE
MANIFOLD A
MANIFOLD B
802183
802192 7.0" (2X)
802195 (2X) 22.0" .8mm
802196 (5X)
802199 (2X) 11.5" .5mm CLEAR
802198 (2X) 9" .5mm CLEAR
802256
802251
COLUMN 2
COLUMN 1
LOWER
FRONT
REAR
TRITYL OUTPUT
30.0" .8mm
802182 (2X)
FRACTION COLLECTOR
AUXILIARY
801666
4L
CH3CN
802185
802186
802187
802184
802189
802188 19.0" PVC
802181 24.0" .8mm
802179 27.0" .8mm
225062 21.0" .187"I.D.
802180 40.0" 1.8mm
802191
TO VENT
WASTE MANIFOLD
801315
10L 140040
WASTE BOTTLE
802301
VACUUM PUMP
802300
802200
802202
FILTER 10 mic
GAS INLET
~2 psi
~6 psi
~13 psi
NOTES: △ = 1/4-28 BARB
J. B. ENGLETON 2/27/90
ERIC NORDMAN 3.22.90
3.22.90
PLUMBING DIAGRAM
392, 2 COL, 5 BASE
TEST 2
FULL
Applied Biosystems
901238
D - 901238 - 1

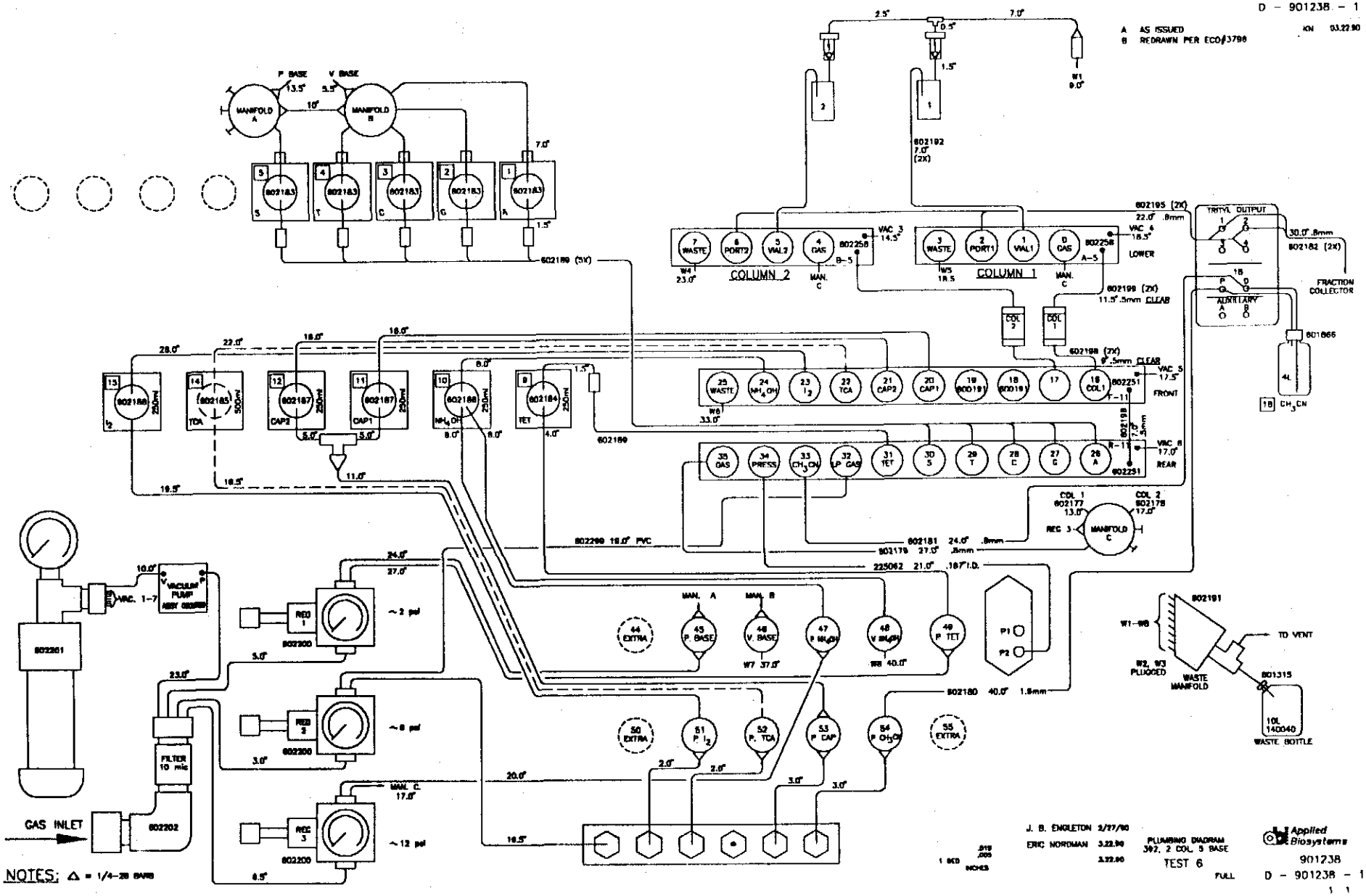



NOTES: △ = 1/4-28 BARB


COLUMN 2
COLUMN 1
TEST 4
GAS INLET
NOTES: △ = 1/4-20 BARB
FRONT
REAR
LOWER
AUXILIARY
TRITYL OUTPUT
FRACTION COLLECTOR
TO VENT
WASTE BOTTLE
WASTE MANIFOLD
MANIFOLD A
MANIFOLD B
MANIFOLD C
VACUUM PUMP ASSY 000000
FILTER 10 mic
P BASE
V BASE
P ½
P TCA
P CAP
P TET
P1
P2
W1
W2, W3 PLUGGED
W4 23.0"
W5 18.5
W6 33.0"
W7 37.0"
W8 40.0"
802192 7.0" (2X)
802195 (2X) 22.0" .8mm
802182 (2X) 30.0" .8mm
802198 (2X) 11.5" .3mm CLEAR
802199 (2X) 9" .5mm CLEAR
802186 (5X)
802188 19.5"
802298 18.0" PVC
802181 24.0" .8mm
802179 27.0" .8mm
225062 21.0" .187" I.D.
802180 40.0" 1.6mm
802191
801315
10L 140040
601866
4L
18 CH3CN
~2 psi
~5 psi
~12 psi
802201
802202
802300
802251
802258
802177 13.0"
802178 17.0"
VAC. 1-7
VAC. 3 14.5"
VAC. 4 18.5"
VAC. 5 17.5"
VAC. 6 17.0"
MAN. C 17.0"
App. III-19


D - 901238 - 1
A AS ISSUED KN 03.22.90
B REDRAWN PER ECO#3798
COLUMN 2
COLUMN 1
LOWER
FRONT
REAR
TRITYL OUTPUT
AUXILIARY
FRACTION COLLECTOR
MANIFOLD A
MANIFOLD B
MANIFOLD C
VACUUM PUMP
GAS INLET
TO VENT
WASTE MANIFOLD
WASTE BOTTLE
TEST 5
NOTES: △ - 1/4-28 BARB

A AS ISSUED KN 03.22.90
B REDRAWN PER ECO#3780

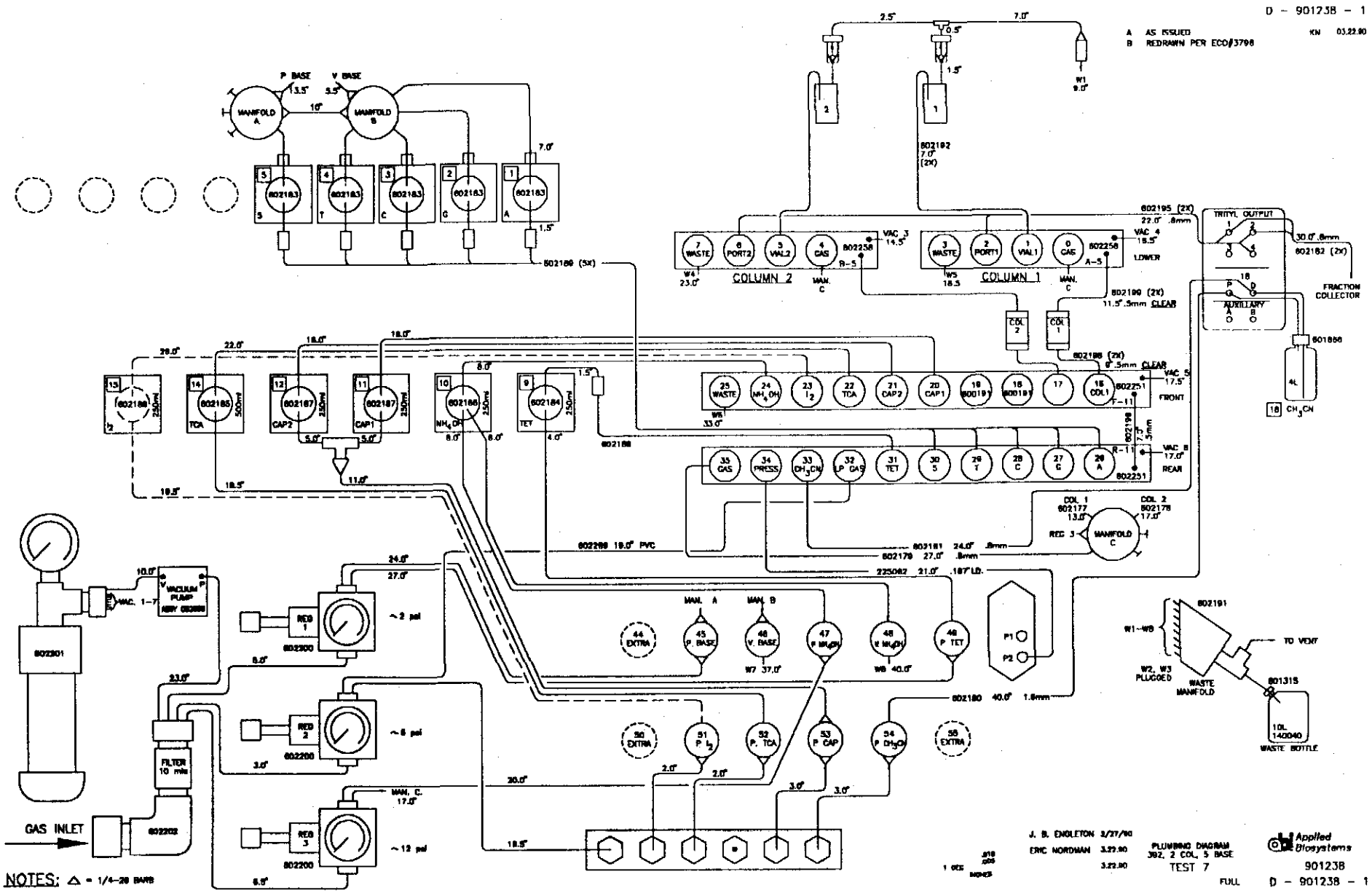


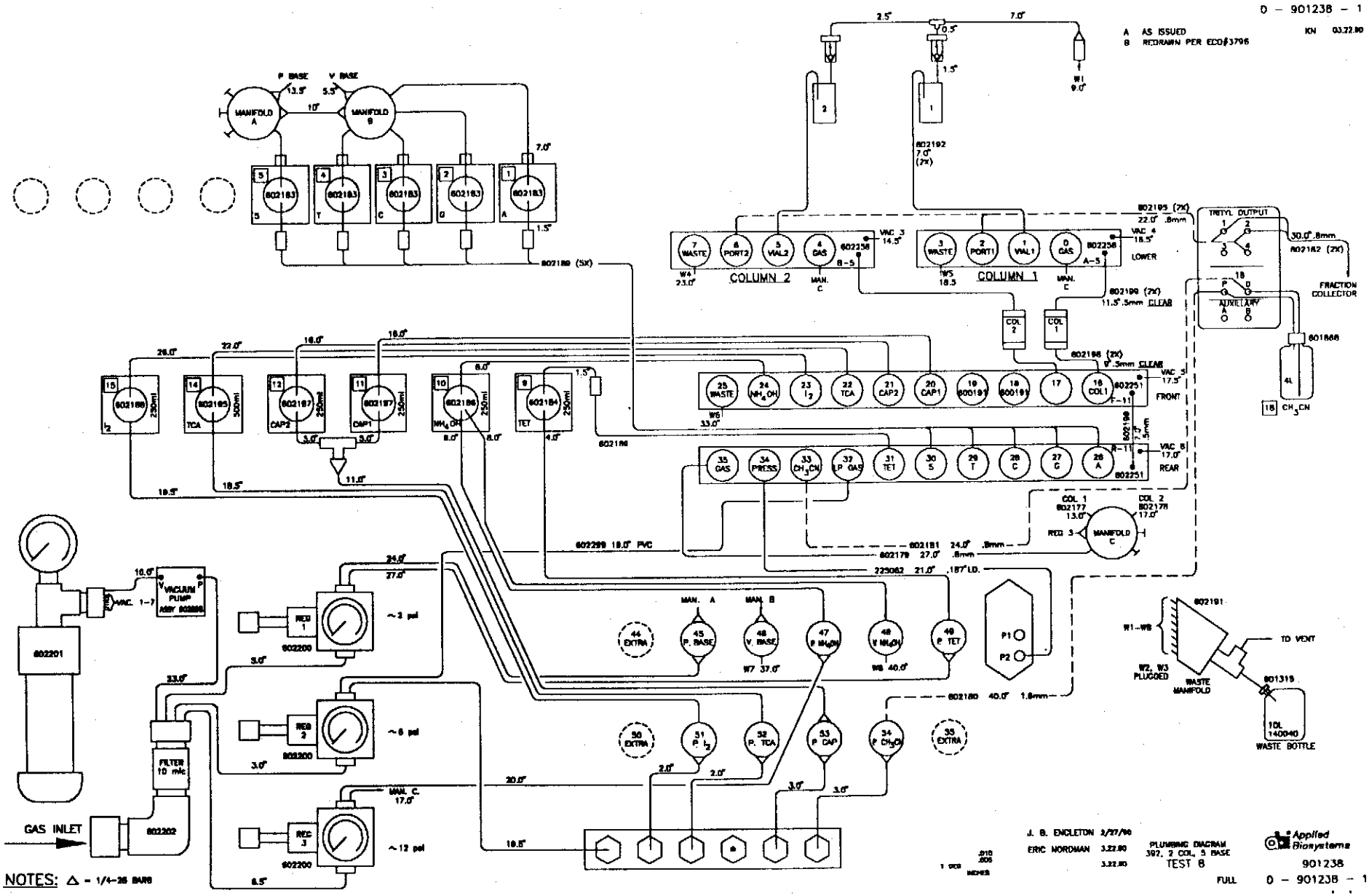

NOTES: △ = 1/4-28 SAMS

J. B. ENGLETON 3/27/90
ERIC NORDMAN 3.22.90
3.22.90

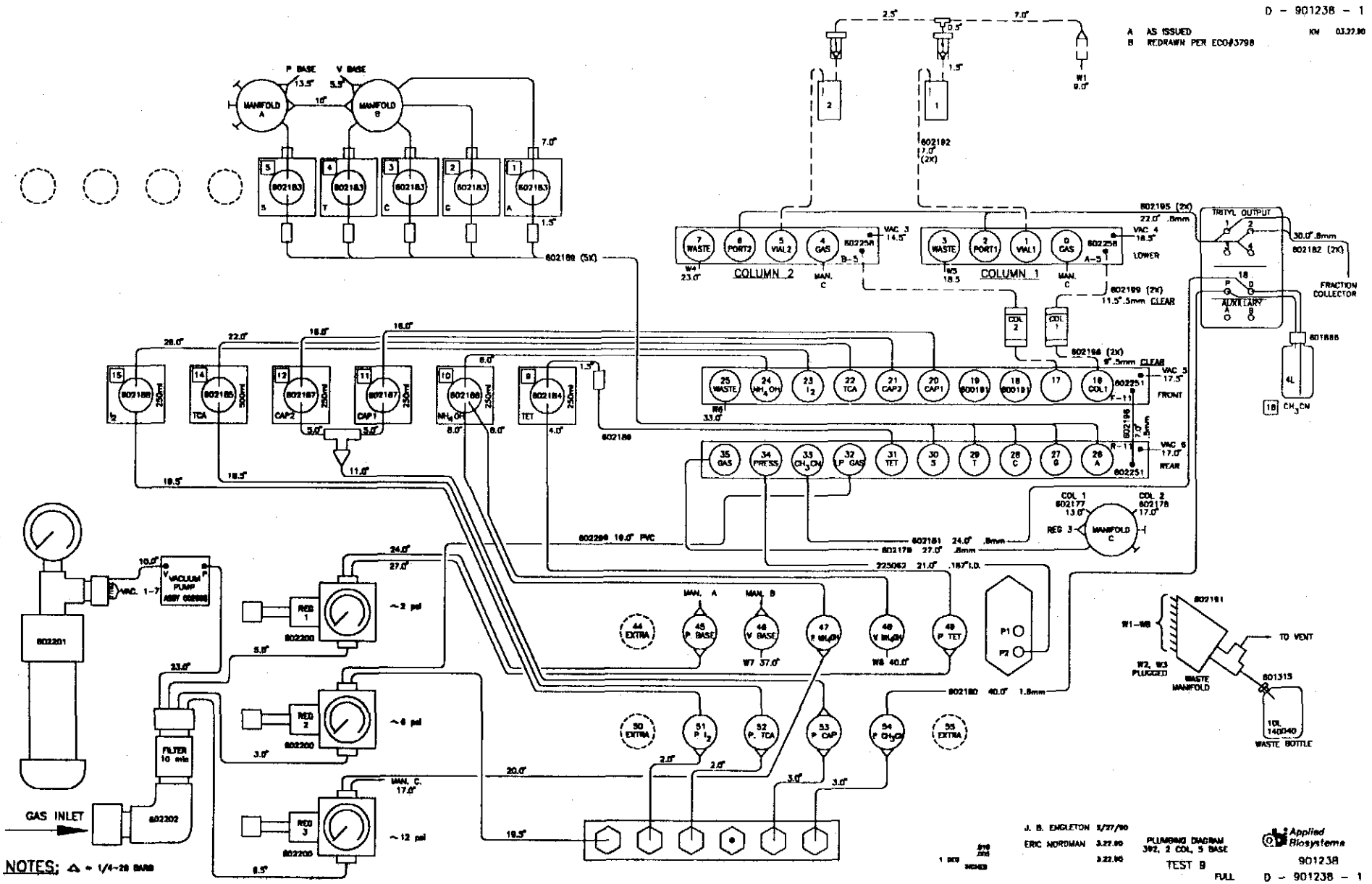
PLUMBING DIAGRAM
392, 2 COL 5 BASE
TEST 6
FULL

Applied Biosystems
901238
D - 901238 - 1
1 1

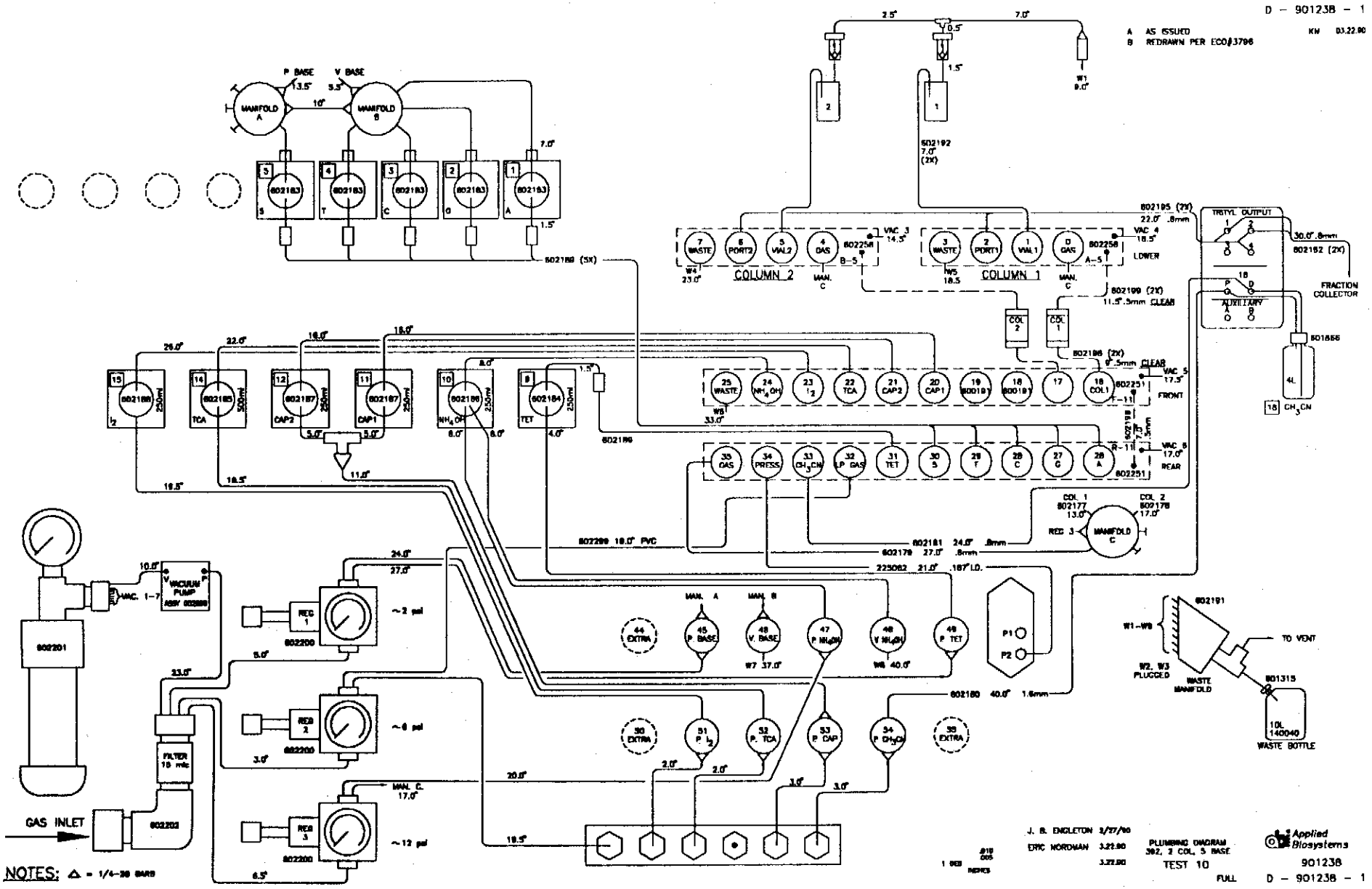

D - 901238 - 1
A AS ISSUED KN 03.22.90
B REDRAWN PER ECO#3798
P BASE
V BASE
MANIFOLD A
MANIFOLD B
802183
802192 7.0" (2X)
802195 (2X) 22.0" .8mm
802196 (5X)
802258
COLUMN 2
COLUMN 1
LOWER
802196 (2X) 11.5" .5mm CLEAR
TRITYL OUTPUT
30.0" .8mm 802182 (2X)
FRACTION COLLECTOR
AUXILIARY
801886
4L
18 CH3CN
802196 (2X) 9" .5mm CLEAR
802251
FRONT
REAR
802198 7.5" 3mm
802188
802185 500ml
802187 250ml
802186 250ml
802184 250ml
802299 19.0" PVC
802181 24.0" .8mm
802179 27.0" .8mm
225062 21.0" .187" I.D.
COL 1 802177 13.0"
COL 2 802178 17.0"
MANIFOLD C
802301
VACUUM PUMP
FILTER 10 mic
802202
GAS INLET
802300
802200
802180 40.0" 1.6mm
802191
TO VENT
WASTE MANIFOLD
801315
10L 140040
WASTE BOTTLE
J. B. ENGLETON 3/27/90
ERIC NORDMAN 3.22.90
3.22.90
PLUMBING DIAGRAM
392, 2 COL, 5 BASE
TEST 7
FULL
Applied Biosystems
901238
D - 901238 - 1
NOTES: △ = 1/4-28 BARB




NOTES: △ = 1/4-28 BARB




NOTES; △ = 1/4-28 BARB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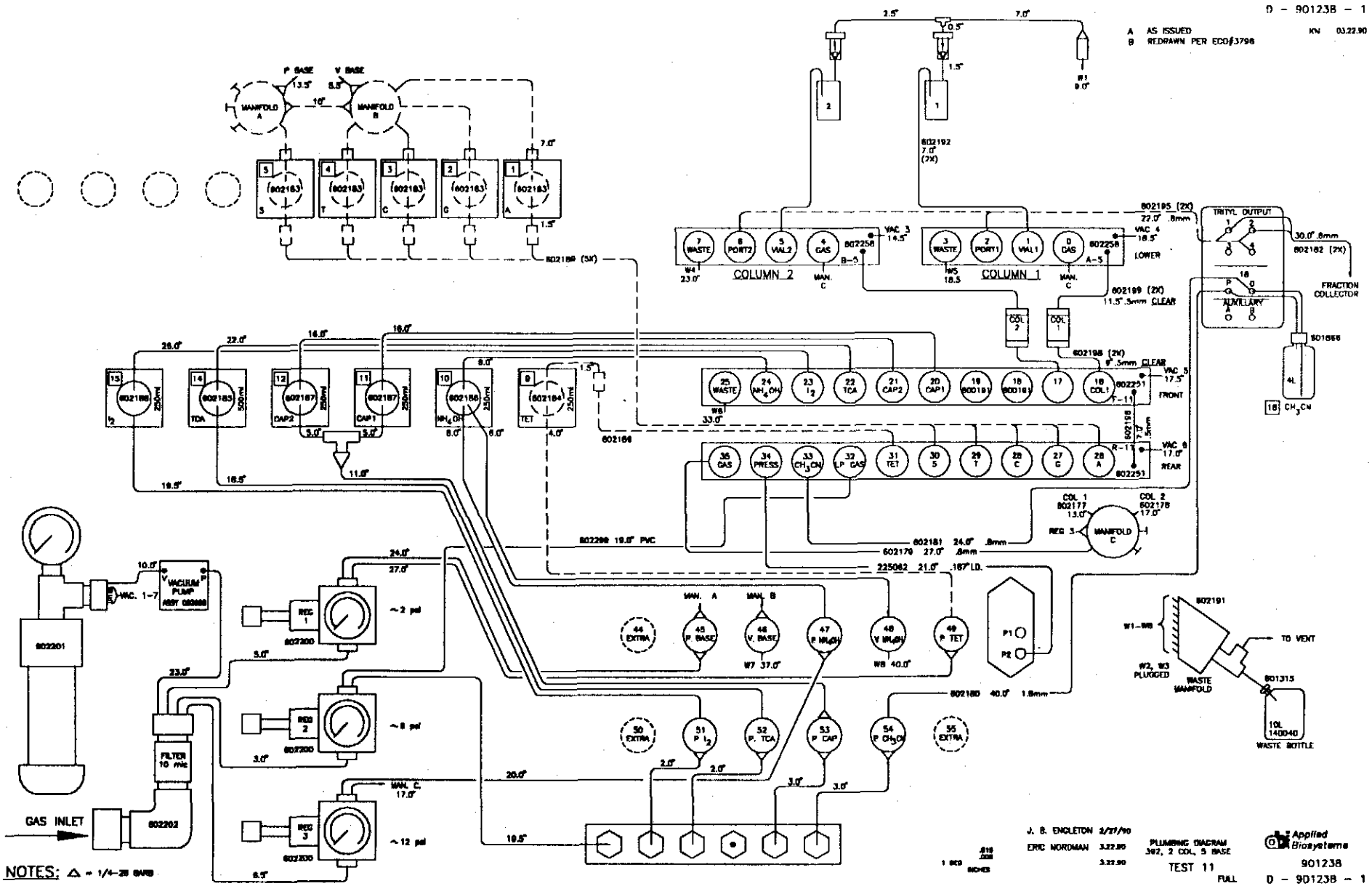
A AS ISSUED
B REDRAWN PER ECO#3796
KM 03.22.90
P BASE
V BASE
MANIFOLD A
MANIFOLD B
802183
802188 (5X)
802192 7.0" (2X)
802195 (2X) 22.0" .8mm
802199 (2X) 11.5" .5mm CLEAR
802186 (2X) 9" .5mm CLEAR
COLUMN 2
COLUMN 1
VAC 3 14.5"
VAC 4 18.5"
LOWER
VAC 5 17.5"
FRONT
VAC 6 17.0"
REAR
TRITYL OUTPUT
AUXILIARY
FRACTION COLLECTOR
30.0" .8mm
802182 (2X)
801858
4L
CH3CN
802185 250mm
802187 250mm
802188 250mm
802184 250mm
802189 500mm
802186
802251
802177 13.0"
802178 17.0"
MANIFOLD C
802179 27.0" .8mm
802181 24.0" .8mm
802288 18.0" PVC
225062 21.0" .187" I.D.
802180 40.0" 1.6mm
802191
TO VENT
W2, W3 PLUGGED
WASTE MANIFOLD
801315
10L 140040
WASTE BOTTLE
802201
VACUUM PUMP
MVC. 1-7
REG 1
REG 2
REG 3
802200
~2 psi
~6 psi
~12 psi
FILTER 10 mic
802202
GAS INLET
NOTES: △ = 1/4-28 BARB
J. B. ENGLETON 3/27/90
ERIC NORDMAN 3.22.90
3.22.90
PLUMBING DIAGRAM 392, 2 COL, 5 BASE
TEST 10
FULL
Applied Biosystems
901238
D - 901238 - 1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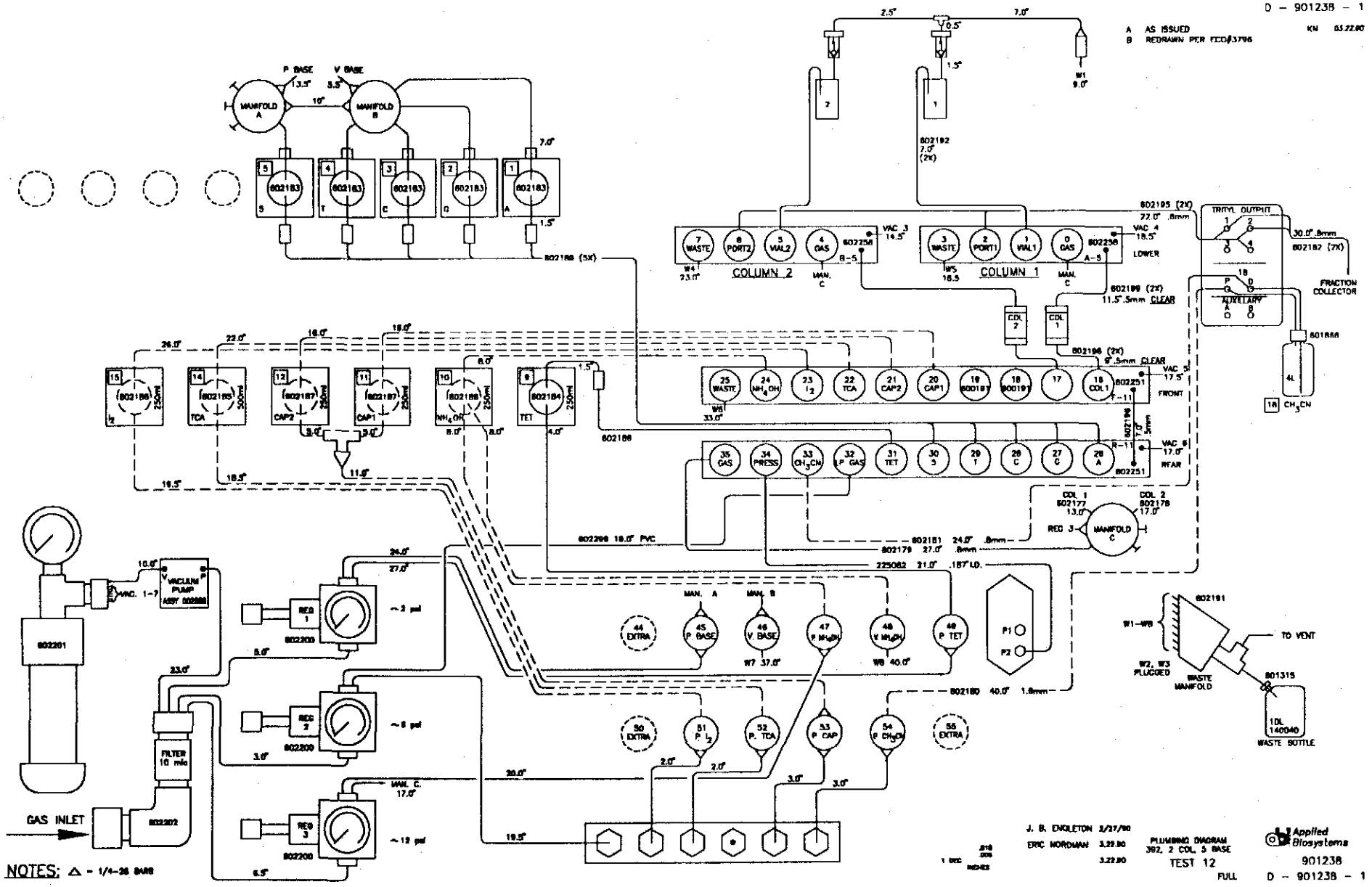
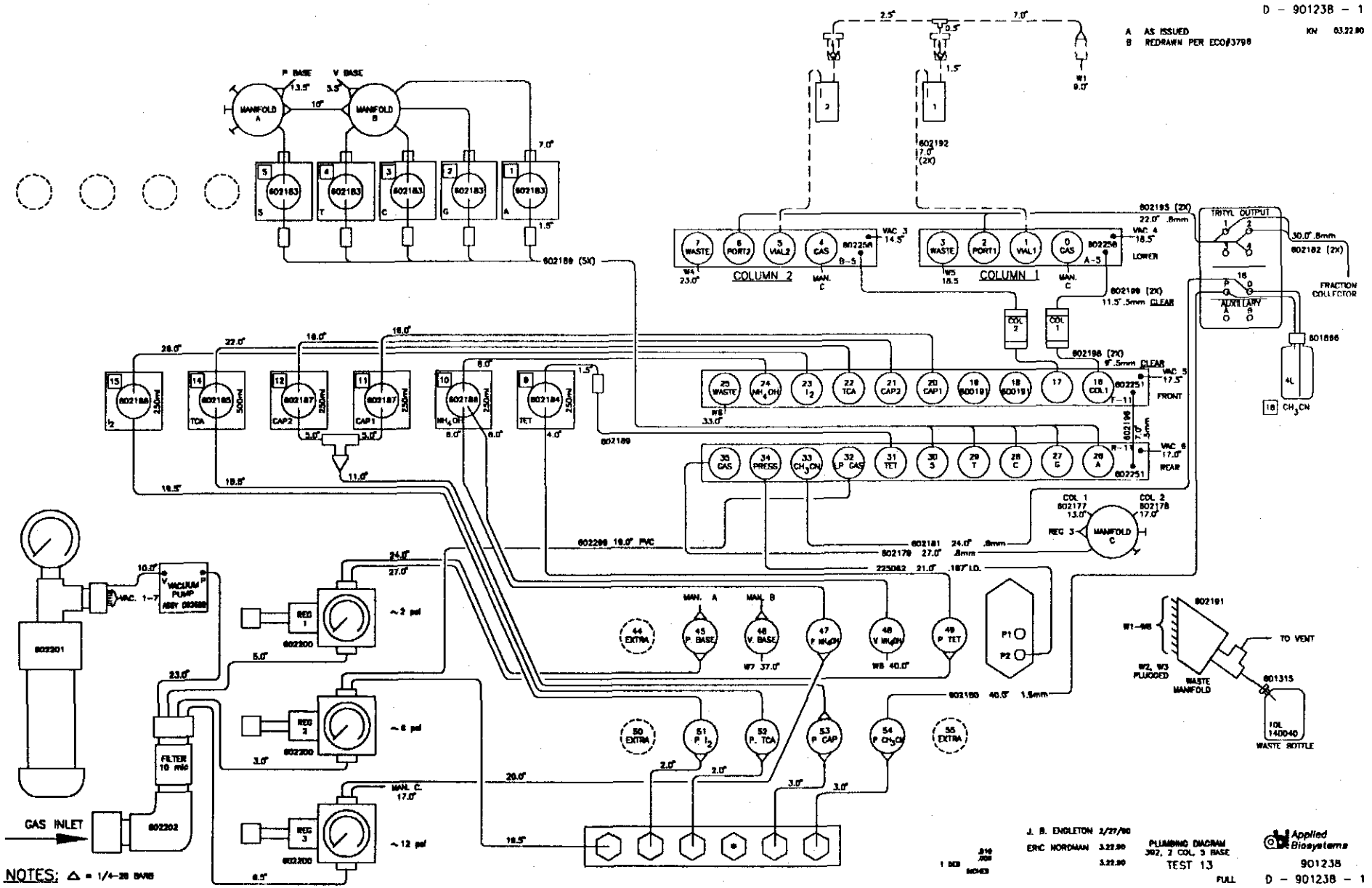
A AS ISSUED
B REDRAWN PER ECO#3796
KN 03.22.90
COLUMN 2
COLUMN 1
LOWER
FRONT
REAR
TRITYL OUTPUT
FRACTION COLLECTOR
AUXILIARY
GAS INLET
VACUUM PUMP
FILTER 10 mic
WASTE MANIFOLD
WASTE BOTTLE
TO VENT
NOTES: △ = 1/4-28 BARB
J. B. ENGLETON 2/27/90
ERIC NORDMAN 3.22.90
PLUMBING DIAGRAM
302, 2 COL, 5 BASE
TEST 11
FULL
Applied Biosystems
901238
D - 901238 - 1




NOTES: △ = 1/4-28 SAE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A AS ISSUED
B REDRAWN PER ECO#3798
KN 03.22.90
COLUMN 2
COLUMN 1
FRONT
REAR
GAS INLET
NOTES: △ = 1/4-28 BARB
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ERIC NORDMAN 3.22.90
3.22.90
PLUMBING DIAGRAM
392, 2 COL 3 BASE
TEST 13
FULL
Applied Biosystems
901238
D - 901238 - 1

# *Appendix IV*

# 392/394 Illustrated Parts List

# Model 392/394 DNA Synthesizer

## Illustrated Parts List

*May 1991*

Applied Biosystems

Model 392/394 DNA Synthesizer
ILLUSTRATED PARTS LIST
May 1991

| Photo Reference Number | Part Number | Description |
|---|---|---|
| 1 | 003440 | Tubing grommet |
| 2 | 003203 | Lower column luer |
| Not visible: | 003202 | Column bulkhead |
| | 602190 | Glass restrictor kit |
| 3 | 602192 | Collection vial cap assy |
| Not visible: | 140004 | 4 mL collection vial |
| | 221019 | 0.364 ID O-ring |
| 4 | 602199 | Column tube assy |
| 5 | 000592 | Upper column luer |
| 6 | 003444 | Drip tray, 2 column |
| | 003209 | Drip tray, 4 column |
| 7 | 003208 | Drip tray, bottle |
| 8 | 200082 | Slide knob, black |
| 9 | 602157 | Keyboard/display assy |
| 10 | 003207 | Upper bezel |
| 11 | 003206 | Lower bezel |
| 12 | 003432 | Dress panel, 5 bottle |
| | 003194 | Dress panel, 8 bottle |
| 13 | 003429 | 392 nameplate |
| | 003430 | 394 nameplate |
| 14 | 600569 | Power cable assy |
| 15 | 003204 | Trityl manifold |
| 16 | 602191 | Waste manifold assy |
| 17 | 200934 | Waste manifold tube |
| 18 | 110207 | Waste manifold plug |
| 19 | 602170 | 50 pin 36" twist pair ribbon cables |
| 20 | 110019 | 1/4-28 plug |
| 21 | 160005 | Pressure regulator |
| Not visible: | 602200 | Regulator assy with gauge and fittings |
| 22 | 222012 | Grommet |
| 23 | 002132 | Waste line plug |
| 24 | 160010 | Gauge, 0-15 psi |
| 25 | 225064 | PE/PVC tubing (low pressure side) |
| | 225043 | Polyurethane tubing (high pressure side) |
| 26 | 600344 | Angar valves |
| 27 | 003210 | Drip tray, valve |
| 28 | 225062 | Tygon tubing, 3/16" ID |
| 29 | 003205 | Manifold, 6 port |
| 30 (Not visible) | 003513 | Acrylic valve shield |
| 31 | 110181 | 1/4-28 barbed fitting |
| 32 | 100380 | Pressure transducer (permanently mounted on Distribution PCB 2, P/N 602194) |
| 33 | 200815 | Hose clamp |
| 34 | 602251 | ABI 11 port valve block |

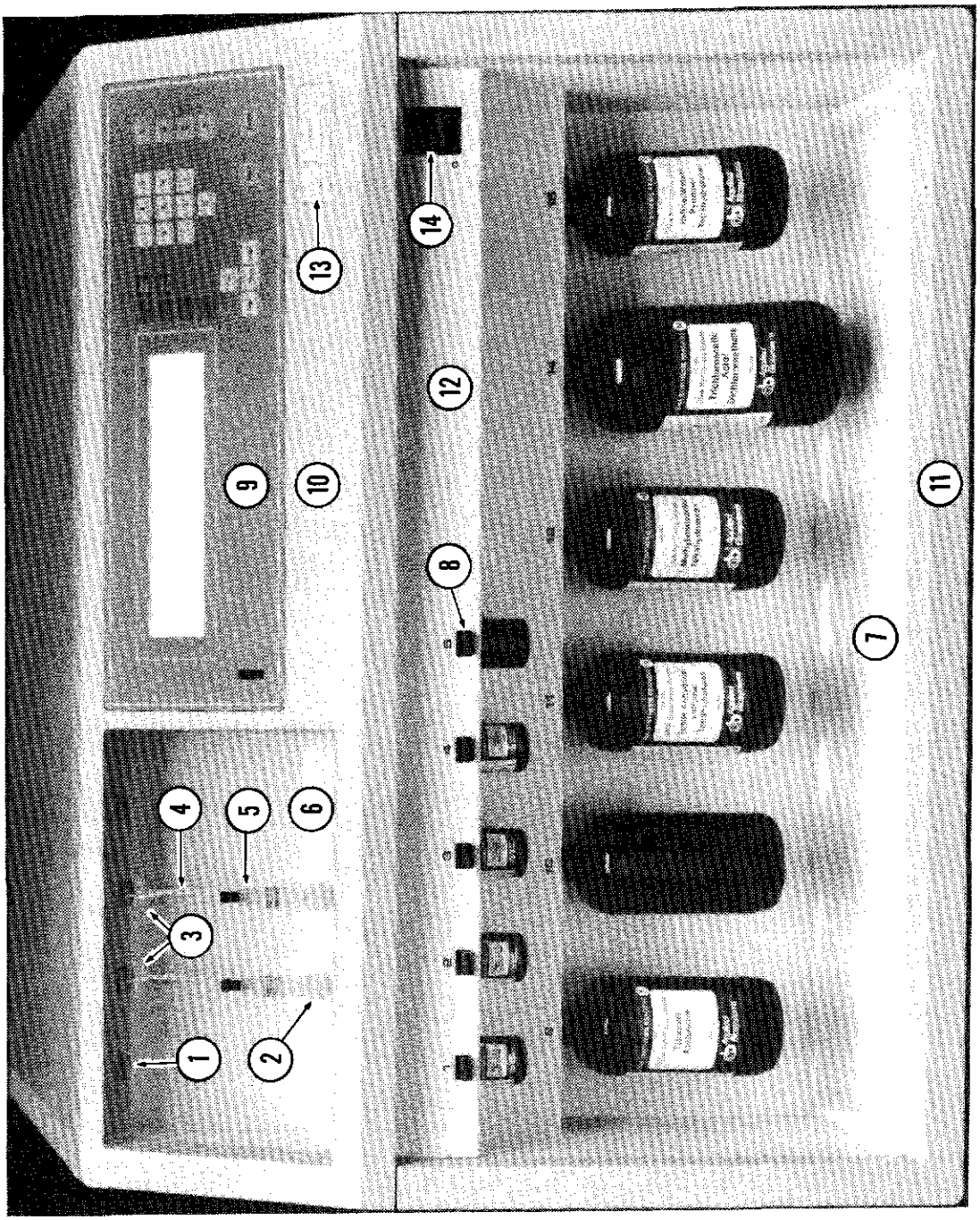
| Photo Reference Number | Part Number | Description |
|---|---|---|
| 35 | 000878 | Teflon manifold (B) |
| 35a | | (A) |
| 35b | | (C) |
| 36 | 110070 | 1/4-28 coupling |
| 37 | 602706 | Version 1.01 Melcard |
| 38 | 602253 | Fraction collector cable assy |
| | 253079 | Black 10 position terminal strip |
| 39 | 602165 | Function PCB |
| 40 | 200208 | Nylon standoff |
| 41 | 295060 | 220/240V fuse holder |
| | 295061 | 100/120V fuse holder |
| 42 | 200839 | Voltage configuration module |
| 43 | 602171 | 50 pin 18" twist pair ribbon cable |
| 44 | 602168 | Power supply assy |
| 45 | 001208 | Ratcheting receptacle housing |
| Not visible: | 003558 | 8 oz. receptacle |
| | 003559 | 16 oz. receptacle |
| | 003560 | Receptacle lid |
| | 002571 | Spring washer |
| | 602184 | Tetrazole insert |
| | 602186 | NH4OH insert |
| | 602187 | NMI insert |
| | 602187 | Acetic anhydride insert |
| | 602185 | TCA insert |
| | 602188 | Iodine insert |
| | 602189 | Matched impedance line |
| 46 | 200451 | Wire/tubing clip |
| 47 | 600192 | Receptacle housing |
| Not visible: | 602183 | Inserts 1-8 |
| | 602189 | Matched impedance lines |
| 48 | 110130 | 1/4-28 Teflon tee |
| 49 | 100087 | 4" muffin fan |
| Not visible: | 100095 | 4" fan filter |
| 50 | 130046 | EPR check valve |
| 51 | 002698 | Vacuum pump assy |
| 51a | | Vacuum switch |
| 51b | | Pressure switch |
| 51c | | Clippard valve |
| 52 (Not visible) | 602201 | Vacuum ballast assy |
| | 002155 | Ballast cylinder |
| | 110163 | Manifold, brass |
| | 160003 | Gauge, 30 Hg vacuum |
| | 110187 | 1/2"-1/4" fitting |
| | 110188 | Tee fitting |
| 53 | 200186 | 4" ribbon clamp |

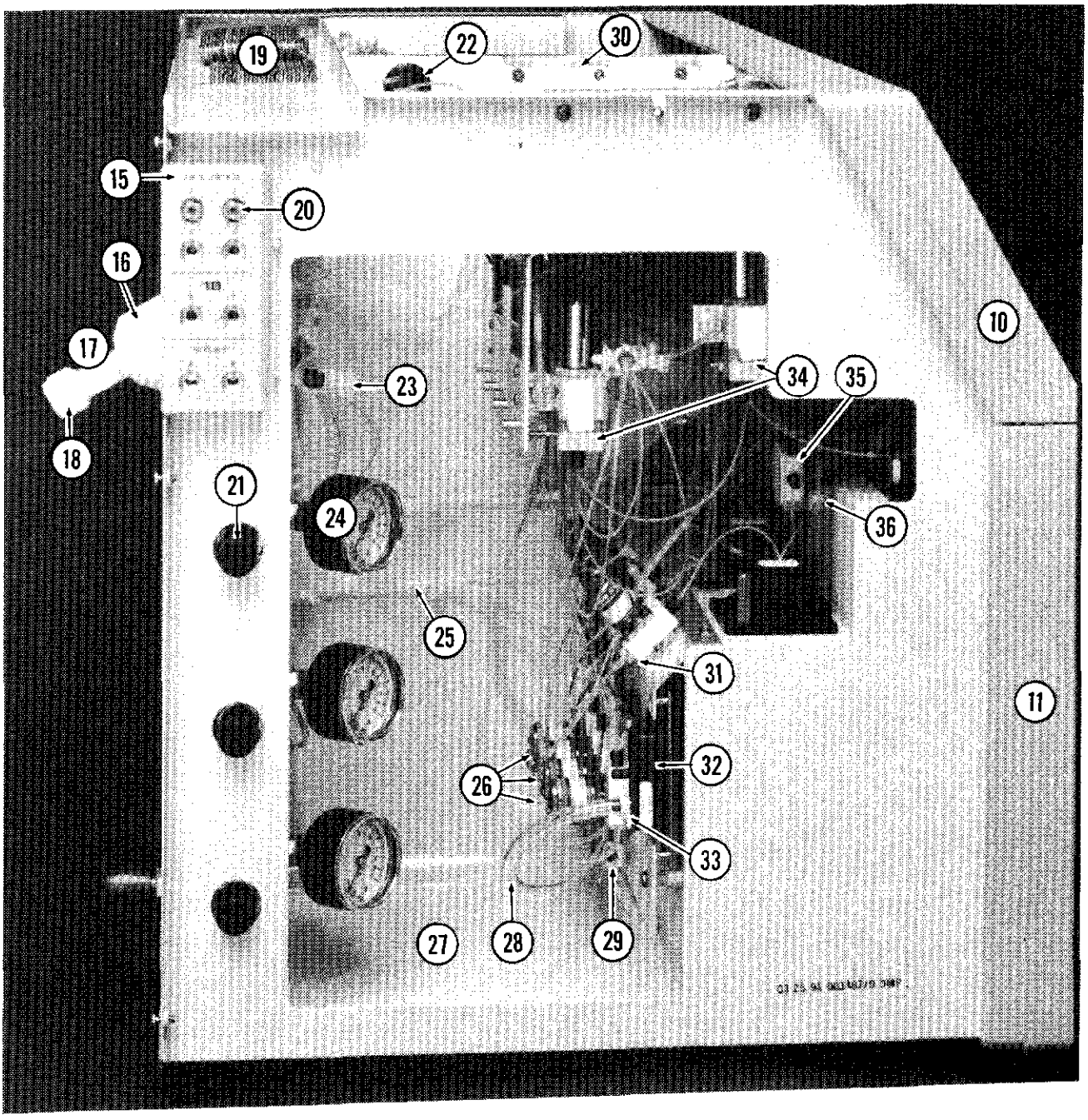
| Photo Reference Number | Part Number | Description |
|---|---|---|
| 54 | 602258 | ABI 5 port valve block |
| 55 | 003189 | Valve block mounting bracket |
| 56 | 602202 | Gas inlet assy |
| | 110158 | Gas inlet bulkhead (shown) |
| Not visible: | 110159 | Gas inlet brass manifold |
| | 110095 | Particle filter |
| | 110004 | 1/4"-1/4" elbow |
| 57 | 200440 | Tubing clamp |
| 58 | 110153 | Adaptor, brass, 1 barb |
| 59 | 110190 | Adaptor, brass, 2 barb |

Note: The following parts are not user accessible.

| | | |
|---|---|---|
| 60 | 608000 | Controller PCB |
| 61 | 602255 | Keyboard/display cable |
| 62 | 200838 | 3V lithium battery |
| 63 | 602167 | I/O Interface PCB |
| 64 | 291273 | 68000 CMOS microprocessor IC |
| 65 | 200864 | 8 position dipswitch |
| 66 | 291281 | Watchdog IC |
| 67 | 602549 | 68K configuration PROM |
| 68 | 291277 | Xilinx logic IC (2) |
| 69 | 291271 | 68901 multifunction peripheral IC |
| 70 | 602172 | 60 pin 6" ribbon cable |
| 71 | 252131 | 96 pin connector |
| 72 | 252133 | 50 pin Melcard connection |
| 73 | 291253 | 32K x 8 SRAM 100 ns IC |
| 74 | 291270 | Z8530 dual serial com controller |
| 75 | 291274 | 68440 DVAL DMA controller IC |
| 76 | 291267 | DS3691 RS422 line driver |
| 77 | 252135 | 8 pin mini din - Appletalk |
| 78 | 291275 | DS26C32 Quad line receiver |
| 79 | 252135 | 8 pin mini din - RS232 printer |
| 80 | 291229 | LT1081CN RS232 driver IC |
| 81 | 200164 | Reset switch (warm boot) |
| 82 | 602404 | Version 1.03 DTACK-IRG PAL |
| 83 | 602212 | Address decode PAL |

Model 392/394 DNA Synthesizer
ILLUSTRATED PARTS LIST
May 1991

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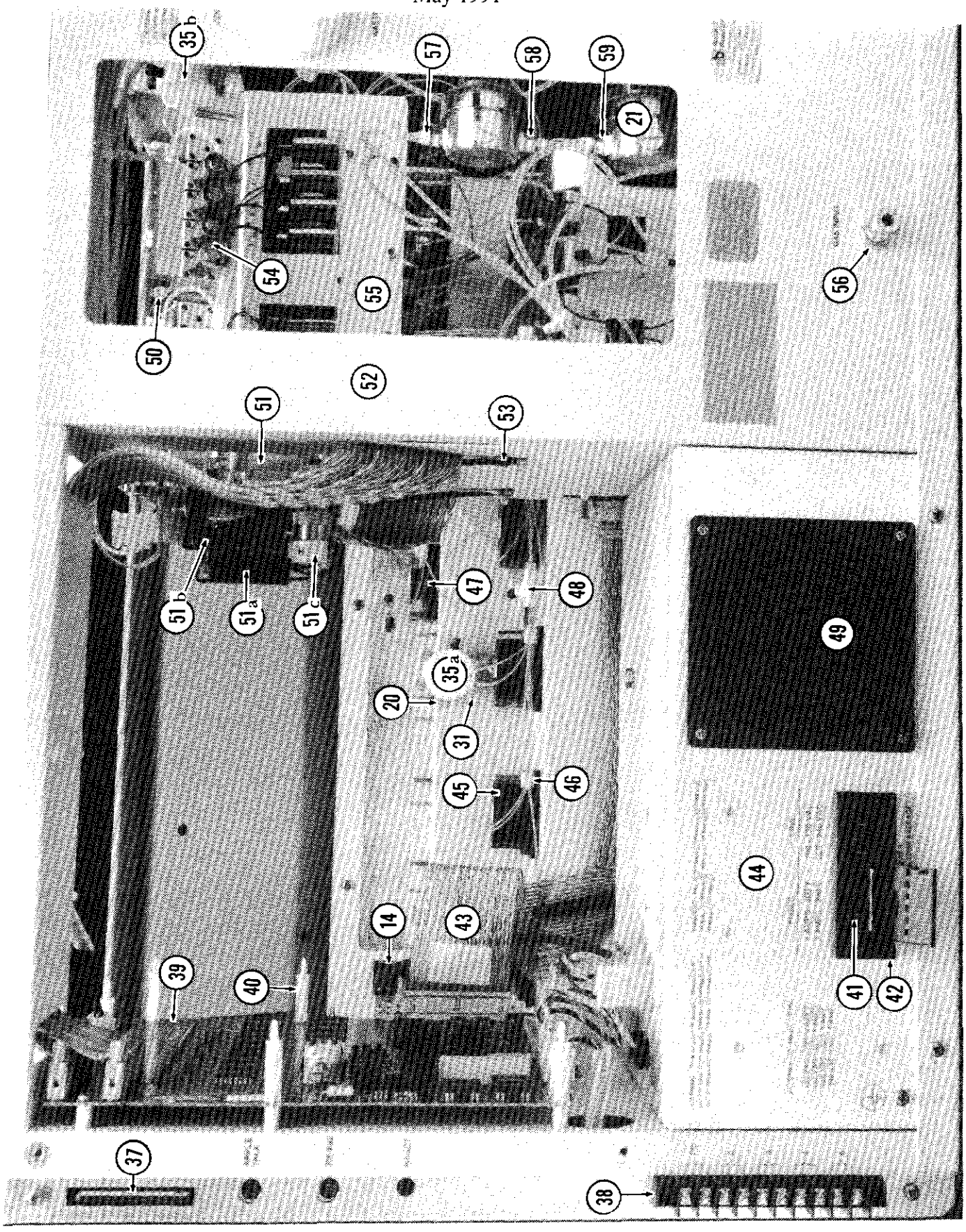
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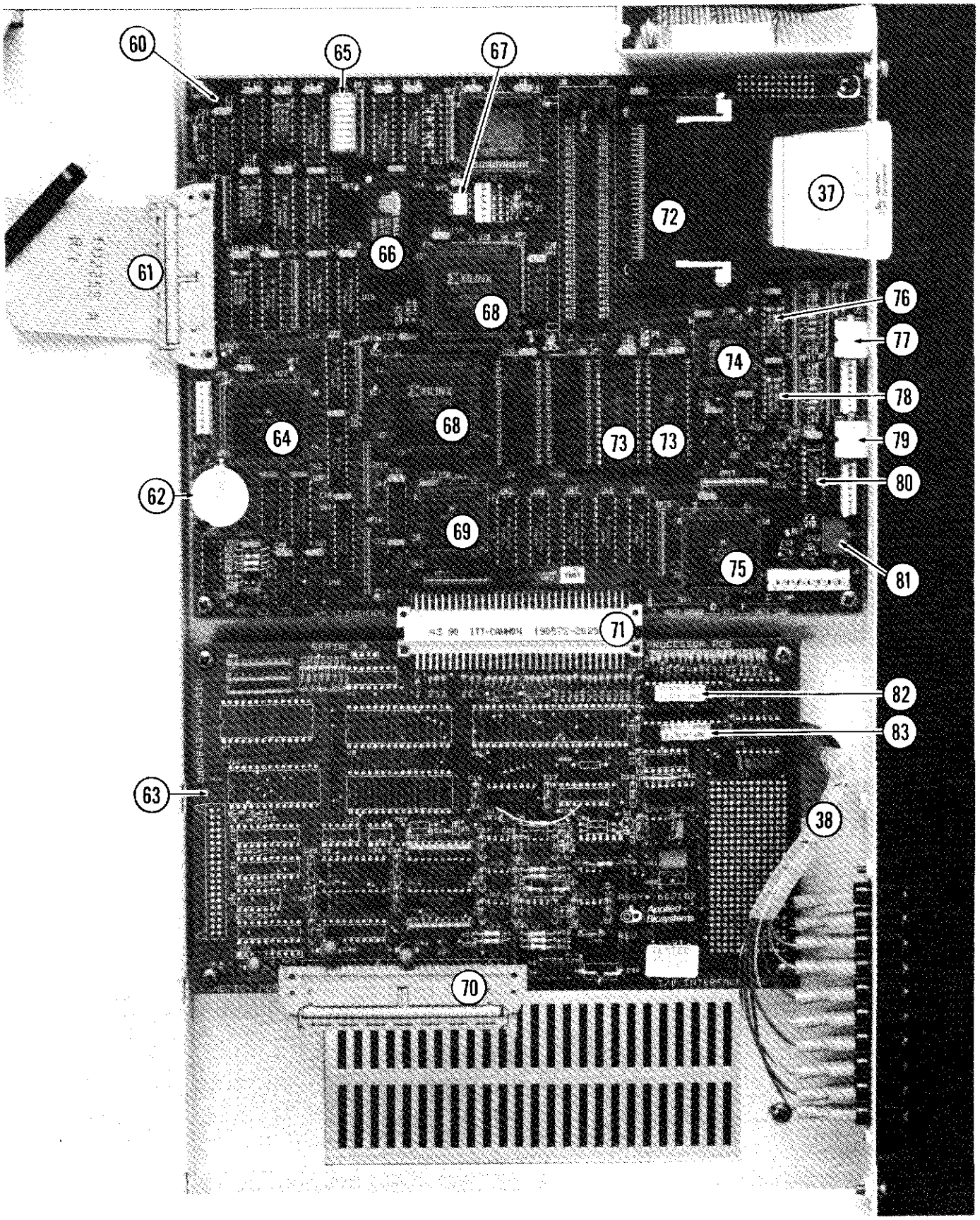
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*Appendix V*

# Warranty
# 392 and 394 DNA/RNA Synthesizer Pre-Installation Manual

Applied Biosystems, Inc. ("ABI") warrants to the Customer that, for a period ending on the earlier of one year from completion of installation or 15 months from the date of shipment to the Customer (the "Warranty Period"), the ABI Model 392/394 purchased by the customer (the "Instrument") will be free from defects in material and workmanship, and will perform in accordance with the performance specifications contained in the applicable ABI product literature (the "Specifications").

This Warranty does not apply to the Instrument's valves, reagent lines, or performance, unless the Customer uses only reagents and solvents supplied by ABI or expressly recommended by ABI, or to any damages caused by reagents or solvents not supplied by ABI, even though recommended by ABI. This Warranty does not extend to any Instrument or part thereof that has been subjected to misuse, neglect or accident, that has been modified or repaired by anyone other than ABI or that has not been used in accordance with the instructions contained in the Instrument Operator's Manual. Nor does this Warranty cover any Customer–installable consumable parts for the Instrument that are listed in the Instrument Operator's Manual.

ABI's obligation under this warranty is limited to repairs or replacements that ABI deems necessary to correct covered defects or failures of which ABI is notified prior to expiration of the Warranty Period. All repairs and replacements under this Warranty shall be performed by ABI on-site at the Customer's location at ABI's expense.

No agent, employee, or representative of ABI has any authority to bind ABI to any affirmation, representation, or warranty concerning the Instrument that is not contained in the printed product literature or this Warranty Statement. Any such affirmation, representation or warranty made by any agent, employee, or representative of ABI shall not be binding on ABI.

ABI shall not be liable for any incidental, special, or consequential loss, damage or expense directly or indirectly arising from the use of the Instrument. ABI makes no warranty whatsoever in regard to products or parts furnished by third parties; such products or parts will be subject to the warranties, if any, of their respective manufacturers.

This Warranty is limited to the original Customer and is not transferable.

THIS WARRANTY IS THE SOLE AND EXCLUSIVE WARRANTY AS TO THE INSTRUMENT AND IS EXPRESSLY IN LIEU OF ANY OTHER EXPRESS OR IMPLIED WARRANTIES, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE AND OF ANY OTHER OBLIGATION ON THE PART OF ABI.

# MODEL 392 and 394 DNA/RNA SYNTHESIZER
# PRE-INSTALLATION MANUAL

Part No. 901354, Rev. C
May 1991

Applied Biosystems

850 Lincoln Centre Drive, Foster City, California 94404 U.S.A.
Tel: (415) 570-6667 • Telex: 470052 APBIO UI • Fax: 415-572-2743

# MODEL 392 and 394 DNA/RNA SYNTHESIZER

## PRE-INSTALLATION MANUAL

Part No. 901354, Rev. C
May 1991

Applied Biosystems

850 Lincoln Centre Drive, Foster City, California 94404 U.S.A.
Tel: (415) 570-6667 • Telex: 470052 APBIO UI • Fax: 415-572-2743

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# SAFETY FIRST!

Safety is everyone's concern. Your laboratory has specific safety practices and policies designed to protect laboratory personnel from potential hazards, both obvious and hidden. If your instrument is to be installed by Applied Biosystems, please note that the ABI service engineer assigned to your laboratory has been trained to safely install your new ABI instrument and to comply with your safety rules. We require our engineers to take the necessary time to familiarize themselves with the locations of potential hazards, exits and safety equipment, and steps to be taken during an evacuation or other emergency.

We request that a representative from your laboratory be in the vicinity and available to our engineer at all times while on-site. We believe, as a minimum, available safety equipment should consist of a fire extinguisher (Halon™), eye wash, safety shower, eye and hand protection, adequate ventilation, and protection from sources of radiation (lasers, radioisotopes, contaminated equipment, radioactive wastes, etc.) that may be present in the area where an ABI service engineer will be working.

We make this request to ensure that both your laboratory personnel and our service personnel have a safe environment to work in during the installation and maintenance of your ABI instrument system.

# USER ATTENTION WORDS, MATERIAL SAFETY DATA SHEETS, AND WASTE PROFILES

## USER ATTENTION WORDS

Four "user attention words" appear in the text of all Applied Biosystems, Inc. documentation. Categorically, each word implies a particular level of observation or action as follows:

NOTE: This word is used to call attention to information.

IMPORTANT: This information is given because it is necessary for correct operation of the instrument.

CAUTION: This word informs the user that damage to the instrument could occur if the user does not comply with this information.

WARNING: Physical injury to the user or other persons could occur if these required precautions are not taken.

## MATERIAL SAFETY DATA SHEETS (MSDSs)

WARNING: Some chemicals used with this instrument are hazardous. Hazards are prominently displayed on the labels of all hazardous chemicals. In addition, material safety data sheets (MSDSs) are provided which give information on physical characteristics, hazards, precautions, first-aid, spill cleanup, and disposal procedures. The MSDSs are in the User's Safety Information section at the beginning of this manual. Please familiarize yourself with information in the MSDSs before handling the reagents or operating the instrument. Additional free copies of MSDSs are available from Applied Biosystems, Inc. on request.

## WASTE PROFILES

Some chemicals collected in the waste bottle after a normal synthesis cycle may be hazardous and/or require special handling. The waste profiles are in the User's Safety Information section at the beginning of this manual. They give percentage compositions of the reagents and inform the user about waste disposal (which must always be in accord with local, state and federal regulations). Additional free copies of the waste profiles are available from Applied Biosystems, Inc. upon request.

# ABBREVIATIONS

| | | |
|---|---|---|
| ABI | = | Applied Biosystems, Inc. |
| Btu/h | = | British thermal units/hour |
| CGA | = | Compressed Gas Association |
| cm | = | centimeter |
| DNA | = | deoxyribonucleic acid |
| EP | = | extended programmability |
| ea | = | each |
| fpm | = | feet per minute |
| ft | = | foot |
| g | = | gram |
| gal | = | gal |
| h | = | hour |
| HPLC | = | high performance liquid chromatography |
| Hz | = | hertz |
| i.d. | = | inside diameter |
| in. | = | inch |
| kg | = | kilogram |
| kPa | = | kiloPascal |
| kVA | = | kilovolt-amperes |
| L | = | liter |
| lb | = | pound |
| M | = | molar |
| m | = | meter |
| $m^3$ | = | cubic meters |
| mL | = | milliliter |
| mm | = | millimeter |
| mol. wt | = | molecular weight |
| MSDS | = | material safety data sheet |
| nm | = | nanometer |
| No. | = | number |
| o.d. | = | outside diameter |
| oz | = | ounce |
| par. | = | paragraph |
| PCR | = | polymerase chain reaction |
| ppm | = | parts per million |
| psi | = | pounds per square inch |
| V | = | volt |
| °C | = | degree Celsius |
| °F | = | degree Fahrenheit |
| µm | = | micron |
| µmol | = | micromole |

# MODEL 392/394 USER'S SAFETY INFORMATION

Part No. 901702, Rev. A
May 1991

Applied Biosystems

850 Lincoln Centre Drive, Foster City, California 94404 U.S.A.
Tel: (415) 570-6667 • Telex: 470052 APBIO UI • Fax: 415-572-2743

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# MODEL 392/394 USER'S SAFETY INFORMATION

## MODEL 392/394 SAFETY SYMBOLS DEFINED

WARNING: The symbols below are affixed to the Model 392/394. Read and understand the explanations of the symbols before you interact with the instrument in any way.

### Electrical

This symbol indicates the on position of the main power switch.

This symbol indicates the off position of the main power switch.

This protective grounding terminal must be connected to earth ground before any other electrical connections are made to the instrument.

This terminal either receives or delivers alternating current or voltage.

This terminal can receive or supply an alternating and a direct current or voltage.

This terminal may be connected to another instrument's signal ground reference. This is not a protective ground terminal.

For continued protection against the risk of fire, replace fuses only with Listed and Certified fuses of the specified type and rating as shown on the rear of the instrument.

## Hazardous Chemicals

This instrument contains hazardous chemicals in bottles, lines, and valve blocks. Do not operate or interact with the instrument until you have read all the Material Safety Data Sheets for chemicals associated with this instrument.

Contents of the numbered chemical bottles are as follows:

| Bottle | Volume, mL | Chemical |
|---|---|---|
| 1, 2, 3, and 4 | 30 | Anhydrous acetonitrile (for dissolving phosphoramidites |
| 9 | 45 | Tetrazole/acetonitrile |
| 11 | 45 | Acetic anhydride/lutidine/tetradydrofuran |
| 12 | 45 | 1-Methylimidazole/tetrahydrofuran |
| 14 | 450 | Trichloroacetic acid/dichlormethane |
| 15 | 200 | Iodine, water, pyridine, and tetrahydrofuran or tetraethylthiuram disulfide |

The Material Safety Data Sheets for hazardous chemicals supplied by Applied Biosystems, Inc. are presented on p. 7 through p. 35.

## Waste Output

This instrument produces hazardous liquid and vapor waste. Ensure that the waste container is correctly installed, as shown on pp. 5 and 6.

Ensure that harmful vapors are vented to a fume hood or similar facility by means of a vent line as shown on pp. 5 and 6.

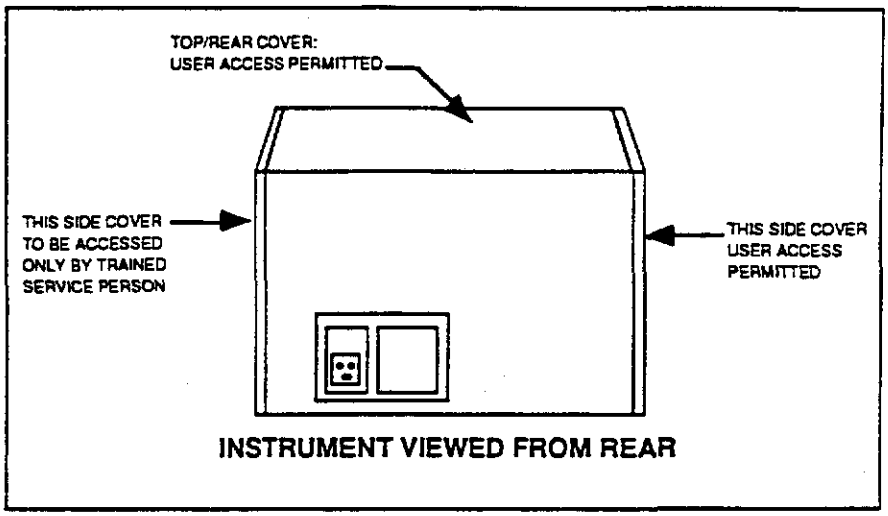
Do not handle or dispose of hazardous waste until you have read the Waste Profile on p. 36. The Waste Profile gives information on composition, physical data, reactivity, spill and leak cleanup, fire and explosion data, health hazards, waste disposal, special protective equipment, and special precautions. Dispose of hazardous waste in accord with all local, state, and federal regulations.

# SECONDARY CONTAINMENT

Provide secondary containment for all hazardous materials and waste.

# ACCESS

The figure below indicates instrument access:




**INSTRUMENT VIEWED FROM REAR**

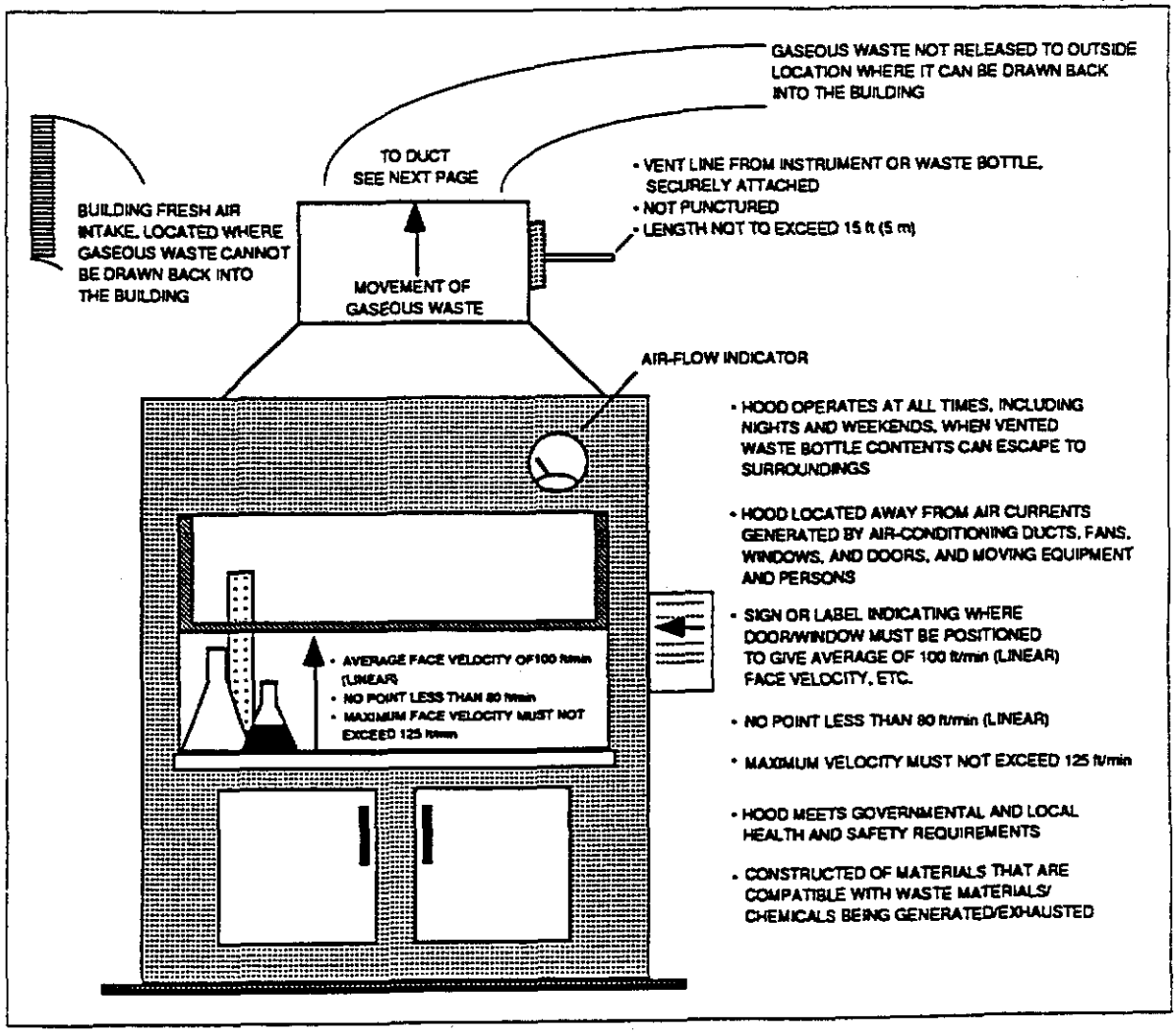
# LABORATORY VENTILATION REQUIREMENTS FOR APPLIED BIOSYSTEMS, INC. INSTRUMENTS

The information presented here reflects U.S. regulations and practices for venting all Applied Biosystems, Inc. instruments to a fume hood or to a duct.

> WARNING: Do not operate a vented instrument unless it is connected in accord with all the requirements presented here. The wastes produced by certain chemicals in Applied Biosystems instruments are hazardous and can cause injury, illness, or death. Handle all liquid, solid, and gaseous products of the instrument as potentially hazardous. Read all applicable material safety data sheets and waste profiles. Dispose of wastes in accord with all applicable health and safety regulations and laws. Always mix/prepare hazardous materials beneath a fume hood.

## Duct for Venting Gaseous Waste Produced by Instrument

*10/90*




- Periodically check and record face velocity (at least yearly).
- Periodically inspect and maintain exhaust system, including fans and motors (at least yearly).

# Duct for Venting Gaseous Waste Produced by Instrument

690

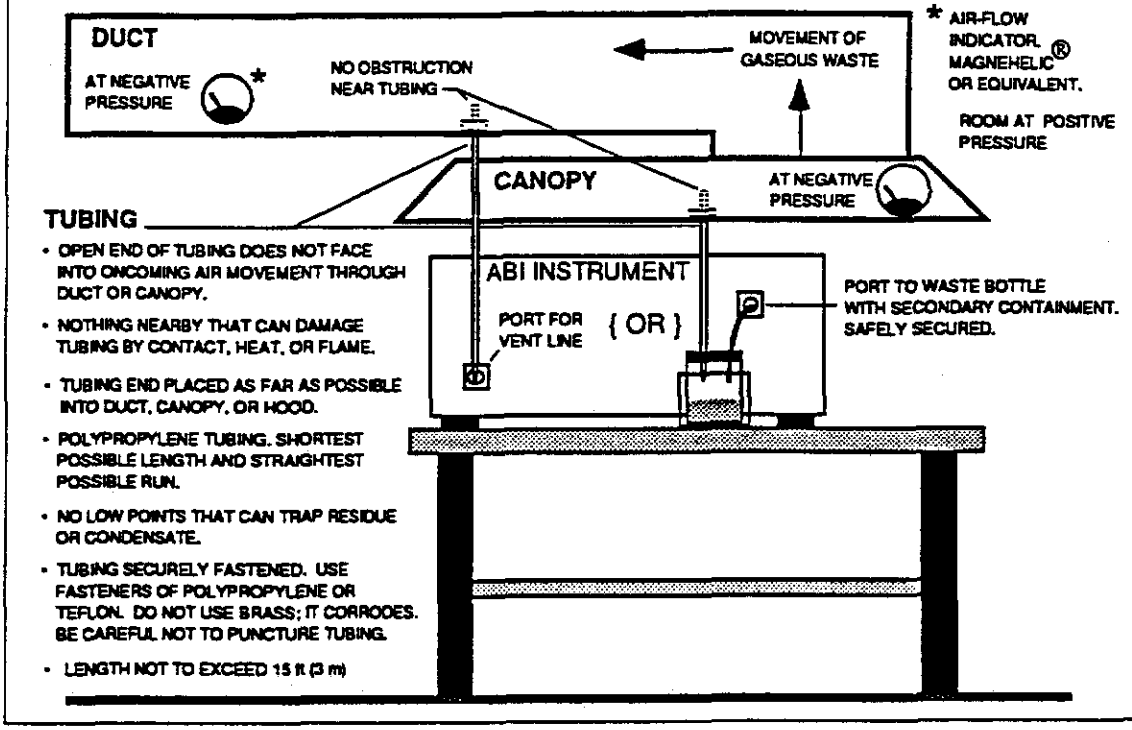
**DUCT-WORK**

- CONSTRUCTED OF MATERIALS COMPATIBLE WITH WASTE MATERIALS BEING GENERATED.
- DUCT EXHAUST SYSTEM OPERATES AT ALL TIMES, INCLUDING NIGHTS AND WEEKENDS, WHEN VENTED WASTE BOTTLE CONTENTS CAN ESCAPE TO SURROUNDINGS.
- NO STRONG OXIDIZERS, BASES, OR OTHER CHEMICALS THAT ARE INCOMPATIBLE WITH GASEOUS WASTE
- VAPOR OR GAS MOVEMENT USUALLY MEASURED AT 1000-2000 fpm*

* Velocities may be considerably less for nonmetal ducts.

**CANOPY**

- AIR EXHAUST OPERATES AT ALL TIMES, INCLUDING NIGHTS AND WEEKENDS, WHEN VENTED WASTE BOTTLE CONTENTS CAN ESCAPE TO SURROUNDINGS.
- DO NOT CONNECT TO A DUCTLESS HOOD OR SYSTEM THAT PURIFIES/FILTERS AIR AND RETURNS IT TO THE ROOM.




**TUBING**

- OPEN END OF TUBING DOES NOT FACE INTO ONCOMING AIR MOVEMENT THROUGH DUCT OR CANOPY.
- NOTHING NEARBY THAT CAN DAMAGE TUBING BY CONTACT, HEAT, OR FLAME.
- TUBING END PLACED AS FAR AS POSSIBLE INTO DUCT, CANOPY, OR HOOD.
- POLYPROPYLENE TUBING, SHORTEST POSSIBLE LENGTH AND STRAIGHTEST POSSIBLE RUN.
- NO LOW POINTS THAT CAN TRAP RESIDUE OR CONDENSATE.
- TUBING SECURELY FASTENED. USE FASTENERS OF POLYPROPYLENE OR TEFLON. DO NOT USE BRASS; IT CORRODES. BE CAREFUL NOT TO PUNCTURE TUBING.
- LENGTH NOT TO EXCEED 15 ft (3 m)

- Periodically inspect and maintain fans and motors (at least yearly).
- Visually inspect tubing periodically for (corrosion, if metal) breakage, crimping, or other damage. Replace the tubing if necessary.
- Remove buildup of condensate or particulates by flushing vent line with pure, dry pressurized gas. Handle and dispose of all wastes as if they were hazardous.
- Always wear a laboratory coat, eye protection, and gloves that resist waste chemicals when you work on waste bottles, exhaust lines/tubing, and liquid traps.

# MATERIAL SAFETY DATA SHEETS

## Acronyms Used

| | |
|---|---|
| ACGIH: | American Conference of Governmental Industrial Hygienisis |
| CAS#: | Chemical Abstract Service Reference Numbers for Specific Pure Chemicals |
| IDLH: | Immediate Danger to Life and Health |
| LEL: | Lower Explosion Limit |
| MSHA: | Mine Safety and Health Administration |
| NIOSH: | National Institute for Occupational Safety and Health |
| OSHA: | Occupational Safety and Health Administration |
| PEL: | Permissible Exposure Limit. The federal OSHA limit usually expressed as a TWA for an 8-hour work shift |
| PPM: | Parts Per Million |
| SCBA: | Self-Contained Breathing Apparatus |
| TLV: | Threshold Limit Value. The ACGIH recommended TWA, usually for an 8-hour work shift. |
| TWA: | Time Weighted Average |
| UEL: | Upper Explosive Limit |

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# MATERIAL SAFETY DATA SHEET

**EMERGENCY PHONE NUMBER** (USA) 415-570-6667, ext. 999 (UK) 0925-825650

## I. Identification

| | | | |
|---|---|---|---|
| PRODUCT NAME | Anhydrous Acetonitrile, 500 ml | PART NUMBER | 400262 |
| CHEMICAL NAME | Acetonitrile | CHEMICAL FAMILY | Nitrile |
| FORMULA | $CH_3CN$ | MOLECULAR WEIGHT | 41 |
| SYNONYMS | DNA Synthesis Reagents #13,16 & 18, methyl cyanide, ethanenitrile, cyanomethane | | |
| DEPARTMENT OF TRANSPORTATION | HAZARD CLASSIFICATION | 3, Flammable liquid, poisonous, DOT12A, UN 1648 | |
| | SHIPPING NAME | Methyl Cyanide, flammable liquid, poisonous | |

## II. Ingredients

| MATERIAL | % | TLV (Units) | PEL (Units) | CAS NUMBER |
|---|---|---|---|---|
| Acetonitrile | 100 | 40 ppm | 40 ppm | 75-05-8 |
| | | | | |
| | | | | |
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| | | | | |

## III. Physical Data

| | | | |
|---|---|---|---|
| BOILING POINT, 760 mmHg | 81.6°C (179°F) | FREEZING POINT | -45°C (-49°F) |
| SPECIFY GRAVITY ($H_2O$ = 1) | 0.787 | VAPOR PRESSURE, mmHg | 89 at 25°C |
| VAPOR DENSITY (Air = 1) | 1.3 | SOLUBILITY IN WATER, % BY WEIGHT | miscible |
| PERCENT VOLATILE BY VOLUME | 100% | EVAPORATION RATE (Butyl Acetate = 1) | 5.8 |
| APPEARANCE AND ODOR | Colorless liquid with a sharp ether-like odor | | |

## IV. Fire and Explosion Hazard Data

| | | | |
|---|---|---|---|
| FLASH POINT (method used) | 5.6°C (42°F) | | (closed cup) |
| FLAMMABLE LIMITS | Lower: 4.4% | Upper: | 16.0% |
| FIRE EXTINGUISHING MEDIA | Dry chemical, alcohol foam, carbon dioxide or Halon | | |
| SPECIAL FIRE FIGHTING PROCEDURES | Use self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. | | |
| UNUSUAL FIRE AND EXPLOSION HAZARDS | Water spray may be ineffective except in cooling containers exposed to fire. | | |

## V. Health Hazard Data

| | |
|---|---|
| EXPOSURE LIMITS | The current OSHA standard for acetonitrile exposure is 40 ppm in air averaged over 8 hours. See Note 1. |

### EFFECTS OF ACUTE OVEREXPOSURE

| | |
|---|---|
| SWALLOWING | High concentrations may cause nausea, vomiting, respiratory depression, weakness, chest or abdominal pain, vomiting of blood, convulsions, shock, unconsciousness and death. |
| SKIN | Exposure to the liquid may cause skin irritation. |
| INHALATION | May cause a slight flushing of the face, a feeling of chest tightness and irritation of the nose and throat. |
| EYES | May cause irritation. |

### EFFECTS OF CHRONIC OVEREXPOSURE

No chronic disease has been reported. May cause respiratory irritation.

### OTHER HEALTH HAZARDS

Points of systemic attack include kidneys, liver, lungs, skin, eyes, central nervous system and cardiovascular system. See Note 2.

| CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: | NTP No | IARC No | OSHA No |
|---|---|---|---|

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY CHEMICAL OVEREXPOSURE

A history of fainting spells or convulsive disorders may present an added risk to persons working with toxic nitriles.

### EMERGENCY AND FIRST AID PROCEDURES

| | |
|---|---|
| SWALLOWING | If conscious, give large quantities of water immediately and induce vomiting. Get medical attention immediately. Do not induce vomiting if unconscious. |
| SKIN | Remove contaminated clothing. Flush the contaminated area with large volumes of water for 15 minutes. Seek medical attention immediately. |
| INHALATION | Provide fresh air and rest. If breathing is difficult, provide oxygen and get medical attention immediately. |
| EYES | Flush eyes immediately with large amounts of water for 15 minutes. Get medical attention immediately. |

| | |
|---|---|
| NOTES TO PHYSICIAN | See Note 2. |

## VI. Reactivity Data

STABILITY:
STABLE [X]
UNSTABLE [ ]

CONDITIONS TO AVOID
Avoid contact with strong oxidizers.

INCOMPATIBILITY (materials to avoid) Contact with strong oxidizers may cause fire or explosion.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS Burning may release toxic vapors and gases, including carbon monoxide, oxides of nitrogen, and hydrogen cyanide.

HAZARDOUS POLYMERIZATION:
MAY OCCUR [ ]
WILL NOT OCCUR [X]

CONDITIONS TO AVOID
See Section IX.

## VII. Spill or Leak Procedures

STEPS TO BE TAKEN
Avoid inhalation and skin contact. Wear protective clothing. Remove all ignition sources. Ventilate area of spill or leak. See Note 3.

WASTE DISPOSAL METHOD This reagent should be disposed of as a regulated hazardous waste by a permitted hazardous waste management facility in accordance with federal, state, and local regulations.

## VIII. Special Protective Equipment

RESPIRATORY PROTECTION (Specify Type)
An MSHA- or NIOSH-approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for emergencies and high concentrations.

VENTILATION
Handle within a well-ventilated area. Minimize open exposure to air.

PROTECTIVE GLOVES
Butyl rubber or similar type resistant to solvents and petroleum products.

EYE PROTECTION
Safety glasses with side shields, monogoggles or face shield.

OTHER PROTECTIVE EQUIPMENT
As necessary to prevent skin contact.

## IX. Special Precautions

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE

Handle as a flammable liquid. Use with adequate ventilation. Do not breathe vapor. Store in a secure, well ventilated location suitable for flammable materials. Keep away from heat, sparks and flame.

OTHER PRECAUTIONS
Eye wash and safety shower should be in area of use. This reagent will attack many forms of rubber, plastics, coatings and finishes.

## X. Additional Information

Note 1: For acetonitrile the STEL is 60 ppm in air. The IDLH is 4,000 ppm. The $LD_{50}$ orally in rats is 3.8 g/kg. Prolonged exposure may cause irritation of the skin.

Note 2: In most cases of systemic acetonitrile poisoning there is a latent period of several hours between exposure and onset of symptoms. It has been thought that the delayed response is due to the slow release of cyanide.

Note 3: Small spills and leaks may be collected with absorbent towels or pads and removed to a well ventilated area away from ignition sources. Acetonitrile must not be allowed to enter confined spaces because of the possibility of explosion.

CAUTION!

THIS PRODUCT IS STRICTLY INTENDED FOR LABORATORY RESEARCH PURPOSES ONLY.
DISPOSE OF EMPTY BOTTLES PROPERLY.

Material Safety Data Sheet Number: 920262

PREPARED BY: B B Moon

Date Issued: 11/18/85

The opinions expressed herein are those of qualified experts within Applied Biosystems, Inc., information from our suppliers, and data presented in various technical publications. We believe that the information contained herein is current as of the date of this Material Safety Data Sheet. It is the user's responsibility to determine the suitability of this information for the adoption of safety precautions as may be necessary. We reserve the right to revise Material Safety Data Sheets from time to time as new technical information becomes available.

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# MATERIAL SAFETY DATA SHEET

**EMERGENCY PHONE NUMBER** (USA) 415-570-6667, ext. 999 (UK) 0925-825650

## I. Identification

| | | | |
|---|---|---|---|
| PRODUCT NAME | Tetrazole/Acetonitrile, 180 ml | PART NUMBER | 400606 |
| CHEMICAL NAME | N/A | CHEMICAL FAMILY | Mixture |
| FORMULA | NHN:NCH:N in $CH_3CN$ | MOLECULAR WEIGHT | N/A |
| SYNONYMS | DNA Synthesis Reagent #9, Activator, Tetrazole Reagent | | |
| DEPARTMENT OF TRANSPORTATION | HAZARD CLASSIFICATION | 3, Flammable liquid, poisonous, DOT12A, UN 1992 | |
| | SHIPPING NAME | Flammable liquid, poisonous, n.o.s. | |

## II. Ingredients

| MATERIAL | % | TLV (Units) | PEL (Units) | CAS NUMBER |
|---|---|---|---|---|
| Tetrazole | 4 | N/A | N/A | N/A |
| Acetonitrile | 96 | 40 ppm | 40 ppm | 75-05-8 |
| | | | | |
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| | | | | |
| | | | | |
| | | | | |

## III. Physical Data

| | | | |
|---|---|---|---|
| BOILING POINT, 760 mmHg | N/A | FREEZING POINT | N/A |
| SPECIFY GRAVITY ($H_2O$ = 1) | N/A | VAPOR PRESSURE, mmHg | N/A |
| VAPOR DENSITY (Air = 1) | 1.3 (acetonitrile) | SOLUBILITY IN WATER, % BY WEIGHT | 100% |
| PERCENT VOLATILE BY VOLUME | 96% | EVAPORATION RATE (Butyl Acetate = 1) | 5.8 |
| APPEARANCE AND ODOR | Colorless liquid with an ether-like odor | | |

## IV. Fire and Explosion Hazard Data

| | | | | |
|---|---|---|---|---|
| FLASH POINT (method used) | Acetonitrile only: 5.6°C (42°F) | | | (closed cup) |
| FLAMMABLE LIMITS | Lower: | 4.4% | Upper: | 16.0% |
| FIRE EXTINGUISHING MEDIA | Dry chemical, alcohol foam, carbon dioxide or Halon | | | |
| SPECIAL FIRE FIGHTING PROCEDURES | Use self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. | | | |
| UNUSUAL FIRE AND EXPLOSION HAZARDS | Water spray may be ineffective except in cooling containers exposed to fire. | | | |

## V. Health Hazard Data

| | |
|---|---|
| EXPOSURE LIMITS | The current OSHA standard for acetonitrile exposure is 40 ppm in air averaged over 8 hours. See Note 1. |

### EFFECTS OF ACUTE OVEREXPOSURE

| | |
|---|---|
| SWALLOWING | High concentrations may cause nausea, vomiting, respiratory depression, weakness, chest or abdominal pain, vomiting of blood, convulsions, shock, unconsciousness and death. |
| SKIN | Exposure to the liquid may cause irritation. |
| INHALATION | May cause a slight flushing of the face, a feeling of chest tightness and irritation of the nose and throat. |
| EYES | May cause irritation. |

### EFFECTS OF CHRONIC OVEREXPOSURE

No chronic disease has been reported for acetonitrile or tetrazole.

### OTHER HEALTH HAZARDS

Points of attack include kidneys, liver, lungs, skin, eyes, central nervous system and cardiovascular system. See Note 2.

| CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: | NTP No | IARC No | OSHA No |
|---|---|---|---|

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY CHEMICAL OVEREXPOSURE

A history of fainting spells or convulsive disorders may present an added risk to persons working with toxic nitriles.

### EMERGENCY AND FIRST AID PROCEDURES

| | |
|---|---|
| SWALLOWING | If conscious, give large volumes of water immediately and induce vomiting. Get medical attention immediately. Do not induce vomiting if unconscious. |
| SKIN | Remove contaminated clothing. Flush the contaminated area with large volumes of water for 15 minutes. Seek medical attention immediately. |
| INHALATION | Provide fresh air and rest. If breathing is difficult, provide oxygen and get medical attention immediately. |
| EYES | Flush eyes immediately with large amounts of water for at least 15 minutes. Seek medical attention immediately. |

| | |
|---|---|
| NOTES TO PHYSICIAN | See Note 2. |

## VI. Reactivity Data

| STABILITY: | CONDITIONS TO AVOID |
|---|---|
| STABLE [X] | Avoid contact with strong oxidizers, metals, or metal salts. Tetrazole may form explosive metal salts. |
| UNSTABLE [ ] | |

INCOMPATIBILITY (materials to avoid) Contact with strong oxidizers may cause fire or explosion.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS Burning may release toxic vapors and gases, including carbon monoxide, oxides of nitrogen, and hydrogen cyanide.

| HAZARDOUS POLYMERIZATION: | CONDITIONS TO AVOID |
|---|---|
| MAY OCCUR [ ] | See Section IX. |
| WILL NOT OCCUR [X] | |

## VII. Spill or Leak Procedures

STEPS TO BE TAKEN Avoid inhalation and skin contact. Wear protective clothing. Remove all ignition sources. Ventilate area of spill or leak. See Note 3.

WASTE DISPOSAL METHOD This reagent should be disposed of as a regulated hazardous waste by a permitted hazardous waste management facility in accordance with federal, state, and local regulations.

## VIII. Special Protective Equipment

| | |
|---|---|
| RESPIRATORY PROTECTION (Specify Type) | An MSHA- or NIOSH-approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for emergencies and high concentrations. |
| VENTILATION | Handle within a well-ventilated area. Minimize open exposure to air. |
| PROTECTIVE GLOVES | Butyl rubber or similar type resistant to solvents and petroleum products. |
| EYE PROTECTION | Safety glasses with side shields, monogoggles or face shield. |
| OTHER PROTECTIVE EQUIPMENT | As necessary to prevent skin contact. |

## IX. Special Precautions

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE

Handle as a flammable liquid. Use with adequate ventilation. Do not breathe vapor. Store in a secure, well ventilated location suitable for flammable materials. Keep away from heat, sparks and flame.

OTHER PRECAUTIONS Eye wash and safety shower should be in area of use. This reagent will attack many forms of rubber, plastics, coatings and finishes.

## X. Additional Information

Note 1: For acetonitrile the STEL is 60 ppm in air. The IDLH is 4,000 ppm. The $LD_{50}$ orally in rats is 3.8 g/kg. Prolonged exposure may cause irritation of the skin. No specific data is available for tetrazole.

Note 2: In most cases of systemic acetonitrile poisoning there is a latent period of several hours between exposure and onset of symptoms. It has been thought that the delayed response is due to the slow release of cyanide.

Note 3: Small spills and leaks may be collected with absorbent towels or pads and removed to a well ventilated area away from ignition sources. Acetonitrile must not be allowed to enter confined spaces because of the possibility of explosion.

**CAUTION!**

**THIS PRODUCT IS STRICTLY INTENDED FOR LABORATORY RESEARCH PURPOSES ONLY. DISPOSE OF EMPTY BOTTLES PROPERLY.**

Material Safety Data Sheet Number: 920606 PREPARED BY: R B Moon

Date Issued: 11/05/86

The opinions expressed herein are those of qualified experts within Applied Biosystems, Inc., information from our suppliers, and data presented in various technical publications. We believe that the information contained herein is current as of the date of this Material Safety Data Sheet. It is the user's responsibility to determine the suitability of this information for the adoption of safety precautions as may be necessary. We reserve the right to revise Material Safety Data Sheets from time to time as new technical information becomes available.

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Telex 781-02324174

# MATERIAL SAFETY DATA SHEET

**EMERGENCY PHONE NUMBER** (USA) 415-570-6667, ext. 999 (UK) 0925-825650

## I. Identification

| | | | |
|---|---|---|---|
| PRODUCT NAME | Acetic Anhydride/Lutidine/tetrahydrofuran, 180 ml | PART NUMBER | 400607 |
| CHEMICAL NAME | N/A | CHEMICAL FAMILY | Mixture |
| FORMULA | $(CH_3CO)_2O/(CH_3)_2C_5H_3N/C_4H_8O$ | MOLECULAR WEIGHT | N/A |
| SYNONYMS | DNA Synthesis Reagent #11, Cap A, Acetic Anhydride Reagent | | |
| DEPARTMENT OF TRANSPORTATION | HAZARD CLASSIFICATION | 3, Flammable liquid, corrosive, DOT12A, UN 2924 | |
| | SHIPPING NAME | Flammable liquid, corrosive, n.o.s. | |

## II. Ingredients

| MATERIAL | % | TLV (Units) | PEL (Units) | CAS NUMBER |
|---|---|---|---|---|
| Acetic anhydride | 10 | 5 ppm | 5 ppm | 108-24-7 |
| 2,6-Lutidine | 10 | N/A | N/A | 108-48-5 |
| Tetrahydrofuran (THF) | 80 | 200 ppm | 200 ppm | 109-99-9 |
| | | | | |
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| | | | | |
| | | | | |
| | | | | |

## III. Physical Data

| | | | |
|---|---|---|---|
| BOILING POINT, 760 mmHg | THF only: 66°C (151°F) | FREEZING POINT | N/A |
| SPECIFY GRAVITY ($H_2O$ = 1) | N/A | VAPOR PRESSURE, mmHg | N/A |
| VAPOR DENSITY (Air = 1) | 2.5 (THF) | SOLUBILITY IN WATER, % BY WEIGHT | 100% |
| PERCENT VOLATILE BY VOLUME | 100% | EVAPORATION RATE (Butyl Acetate = 1) | 800 (THF) |
| APPEARANCE AND ODOR | Colorless liquid with a sharp ether-like odor | | |

## IV. Fire and Explosion Hazard Data

| | | | |
|---|---|---|---|
| FLASH POINT (method used) | THF only: -14°C (6°F) | | (closed cup) |
| FLAMMABLE LIMITS | (THF) Lower: 1.8% | Upper: | 11.8% |
| FIRE EXTINGUISHING MEDIA | Dry chemical, alcohol foam, carbon dioxide or Halon | | |
| SPECIAL FIRE FIGHTING PROCEDURES | Use self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. | | |
| UNUSUAL FIRE AND EXPLOSION HAZARDS | Water spray may be ineffective. Reagent vapor may travel considerable distance to source of ignition. | | |

## V. Health Hazard Data

| | |
|---|---|
| EXPOSURE LIMITS | The current OSHA standard for THF exposure is 200 ppm averaged over 8 hours. See Note 1. |

### EFFECTS OF ACUTE OVEREXPOSURE

| | |
|---|---|
| SWALLOWING | Harmful if swallowed. May cause severe burns to throat and mucous membranes. May cause liver or kidney damage. |
| SKIN | Causes irritation. May cause severe burns if not washed away immediately. |
| INHALATION | Irritation of mucous membranes and upper respiratory tract. Exposure to high concentrations may cause severe damage to the lungs. |
| EYES | May cause serious burns. |

### EFFECTS OF CHRONIC OVEREXPOSURE

Liver and kidney damage have been reported in laboratory animals exposed to high concentrations of THF. Acetic anhydride vapor may cause chronic eye irritation.

### OTHER HEALTH HAZARDS

Points of attack include eyes, skin, respiratory system and central nervous system. Allergic sensitization of the skin may occur.

| CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: | NTP No | IARC No | OSHA No |
|---|---|---|---|

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY CHEMICAL OVEREXPOSURE

No specific information is available.

### EMERGENCY AND FIRST AID PROCEDURES

| | |
|---|---|
| SWALLOWING | If conscious, give large volumes of water immediately and induce vomiting. Get medical attention immediately. Do not induce vomiting if unconscious. |
| SKIN | Remove contaminated clothing. Flush the contaminated area with large volumes of water for 15 minutes. Seek medical attention immediately. |
| INHALATION | Provide fresh air and rest. If breathing is difficult, provide oxygen and get medical attention immediately. |
| EYES | Flush eyes immediately with large amounts of water for at least 15 minutes. Seek medical attention immediately. |

| | |
|---|---|
| NOTES TO PHYSICIAN | Special attention should be given to skin, eyes and respiratory tract. |

## VI. Reactivity Data

| STABILITY: | CONDITIONS TO AVOID |
|---|---|
| STABLE [X] | Contact with strong oxidizers may cause fire or explosion. |
| UNSTABLE [ ] | |

INCOMPATIBILITY (materials to avoid) Water, alcohols, strong aqueous bases, strong oxidizers.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS Burning may release toxic vapors and gases, including organic acids, carbon monoxide, and oxides of nitrogen.

| HAZARDOUS POLYMERIZATION: | CONDITIONS TO AVOID |
|---|---|
| MAY OCCUR [ ] | See Section IX. |
| WILL NOT OCCUR [X] | |

## VII. Spill or Leak Procedures

STEPS TO BE TAKEN Avoid inhalation and skin contact. Wear protective clothing. Remove all ignition sources. Ventilate area of spill or leak. See Note 2.

WASTE DISPOSAL METHOD This reagent should be disposed of as a regulated hazardous waste by a permitted hazardous waste management facility in accordance with federal, state, and local regulations.

## VIII. Special Protective Equipment

RESPIRATORY PROTECTION (Specify Type) An MSHA- or NIOSH-approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for emergencies and high concentrations.

| | |
|---|---|
| VENTILATION | Handle within a well-ventilated area. Minimize open exposure to air. |
| PROTECTIVE GLOVES | Butyl rubber or similar type resistant to THF. |
| EYE PROTECTION | Safety glasses with side shields, monogoggles or face shield. |
| OTHER PROTECTIVE EQUIPMENT | As necessary to prevent skin contact. |

## IX. Special Precautions

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE

Handle as a flammable liquid. Use with adequate ventilation. Do not breathe vapor. Store in a secure, well ventilated location suitable for flammable materials. Keep away from heat, sparks and flame.

OTHER PRECAUTIONS Eye wash and safety shower should be in area of use. This reagent will attack many forms of rubber, plastics, coatings and finishes. HANDLE WITH CARE!

## X. Additional Information

Note 1: For the THF the STEL is 250 ppm in air. The IDLH is 20,000 ppm. There is no STEL data for acetic anhydride; the IDHL level is 1000 ppm. There is no specific exposure limit data for 2,6-Lutidine.

Note 2: Small spills and leaks may be collected with absorbent towels or pads and removed to a well ventilated area away from ignition sources. This reagent must not be allowed to enter confined spaces because of the possibility of vapor explosion.

CAUTION!

THIS PRODUCT IS STRICTLY INTENDED FOR LABORATORY RESEARCH PURPOSES ONLY.
DISPOSE OF EMPTY BOTTLES PROPERLY.

Material Safety Data Sheet Number: 920607

PREPARED BY: B B Moon

Date Issued: 11/05/86

The opinions expressed herein are those of qualified experts within Applied Biosystems, Inc., information from our suppliers, and data presented in various technical publications. We believe that the information contained herein is current as of the date of this Material Safety Data Sheet. It is the user's responsibility to determine the suitability of this information for the adoption of safety precautions as may be necessary. We reserve the right to revise Material Safety Data Sheets from time to time as new technical information becomes available.

Applied Biosystems

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# MATERIAL SAFETY DATA SHEET

**EMERGENCY PHONE NUMBER** (USA) 415-6667, ext. 3333 (UK) 0925-825650

## I. Identification

| | | | |
|---|---|---|---|
| PRODUCT NAME | 1-Methylimidazole/Tetrahydrofuran, 180mL | PART NUMBER | 400785 |
| CHEMICAL NAME | 1-Methylimidazole in Tetrahydrofuran | CHEMICAL FAMILY | Mixture |
| FORMULA | $C_4H_6N_2$ in $C_4H_8O$ | MOLECULAR WEIGHT | N/A |
| SYNONYMS | DNA Synthesis Reagent #12, Cap B, N-Methylimidazole Reagent | | |
| DEPARTMENT OF TRANSPORTATION | HAZARD CLASSIFICATION: Flammable liquid<br>SHIPPING NAME: Tetrahydrofuran, UN2056 | | |

## II. Ingredients

| MATERIAL | % | TLV (Units) | PEL (Units) | CAS NUMBER |
|---|---|---|---|---|
| 1- Methylimidazole (NMI) | 16 | N/A | N/A | 616-47-7 |
| Tetrahydrofuran (THF) | 84 | 200ppm | 200ppm | 109-99-9 |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

## III. Physical Data

| | | | |
|---|---|---|---|
| BOILING POINT, 760 mmHg | THF only: 66°C (151°F) | FREEZING POINT | N/A |
| SPECIFY GRAVITY ($H_2O$ = 1) | N/A | VAPOR PRESSURE, mmHg | N/A |
| VAPOR DENSITY (Air = 1) | 2.5 (THF) | SOLUBILITY IN WATER, % BY WEIGHT | 100% |
| PERCENT VOLATILE BY VOLUME | 84% | EVAPORATION RATE (Butyl Acetate = 1) | 800 (THF) |
| APPEARANCE AND ODOR | Colorless liquid with an ether-like odor. | | |

## IV. Fire and Explosion Hazard Data

| | |
|---|---|
| FLASH POINT (method used) | THF only: -14°C (6°F) closed cup |
| FLAMMABLE LIMITS (THF) | Lower: 1.8% Upper: 11.8% |
| FIRE EXTINGUISHING MEDIA | Dry chemical, alcohol foam, carbon dioxide or Halon. |
| SPECIAL FIRE FIGHTING PROCEDURES | Use self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. See Note 1. |
| UNUSUAL FIRE AND EXPLOSION HAZARDS | Water spray may be ineffective except in cooling containers exposed to fire. |

## V. Health Hazard Data

| | |
|---|---|
| EXPOSURE LIMITS | The current OSHA standard for THF exposure is 200ppm averaged over 8 hours. There is no federal standard for NMI. See Note 2. |

### EFFECTS OF ACUTE OVEREXPOSURE

| | |
|---|---|
| SWALLOWING | Harmful if swallowed. High concentrations may cause nausea, dizziness, headaches, liver or kidney damage. |
| SKIN | Exposure to liquid may cause skin irritation. |
| INHALATION | May cause irritation of the upper respiratory tract, dizziness, nausea, headaches. |
| EYES | May cause irritation. |

### EFFECTS OF CHRONIC OVEREXPOSURE

Liver and kidney damage have been reported in laboratory animals exposed to high concentrations of THF.

### OTHER HEALTH HAZARDS

Points of attack include eyes, skin, respiratory system and central nervous system. Allergic sensitization of the skin may occur.

| CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: | NTP No | IARC No | OSHA No |
|---|---|---|---|

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY CHEMICAL OVEREXPOSURE

No specific information is available.

### EMERGENCY AND FIRST AID PROCEDURES

| | |
|---|---|
| SWALLOWING | If conscious, give large volumes of water immediately. Get medical attention. |
| SKIN | Remove contaminated clothing. Flush the contaminated area with water for 15 minutes. Seek medical attention. |
| INHALATION | Provide fresh air and rest. If breathing is difficult, provide oxygen and get medical attention immediately. |
| EYES | Flush eyes immediately with large amounts of water for at least 15 minutes. Seek medical attention immediately. |

NOTES TO PHYSICIAN

Special attention should be given to skin, eyes and respiratory tract.

## VI. Reactivity Data

STABILITY:
STABLE [X]
UNSTABLE [ ]

CONDITIONS TO AVOID
Avoid contact with strong acids or oxidizers.

INCOMPATIBILITY (materials to avoid) Contact with strong oxidizers may cause fire or explosion.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS Burning may release toxic vapors and gases, including carbon monoxide and oxides of nitrogen.

HAZARDOUS POLYMERIZATION:
MAY OCCUR [ ]
WILL NOT OCCUR [X]

CONDITIONS TO AVOID
See Section IX.

## VII. Spill or Leak Procedures

STEPS TO BE TAKEN Avoid inhalation and skin contact. Wear protective clothing. Remove all ignition sources. Ventilate area of spill or leak. See Note 3.

WASTE DISPOSAL METHOD This reagent should be disposed of as a regulated hazardous waste by a permitted hazardous waste management facility in accordance with federal, state, and local regulations.

## VIII. Special Protective Equipment

RESPIRATORY PROTECTION (Specify Type) An MSHA- or NIOSH-approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for emergency or high concentrations.

VENTILATION Handle within a well-ventilated area. Minimize open exposure to air.

PROTECTIVE GLOVES Latex or Neoprene rubber gloves are recommended.

EYE PROTECTION Safety glasses with side shields, monogoggles or face shield.

OTHER PROTECTIVE EQUIPMENT As necessary to prevent skin contact.

## IX. Special Precautions

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE

Handle as a flammable liquid. Use with adequate ventilation. Do not breathe vapor. store in a secure, well-ventilated location suitable for flammable materials. Keep away from heat, sparks and flame.

OTHER PRECAUTIONS Eye wash and safety shower should be in area of use. This reagent will attack many forms of rubber, plastics, coatings and finishes.

## X. Additional Information

NOTE 1: NFPA 704M rating: Health-2, Flammability-3, Reactivity-1.

NOTE 2: For THF, the STEL is 250ppm in air, and the IDLH is 20,000ppm. There is no specific information available for NMI.

NOTE 3: Small spills and leaks may be collected with absorbent towels or pads and removed to a well- ventilated area away from ignition sources. This reagent must not be allowed to enter confined spaces because of the possibility of vapor explosion.

**CAUTION!**

THIS PRODUCT IS STRICTLY INTENDED FOR LABORATORY RESEARCH PURPOSES ONLY.
DISPOSE OF EMPTY BOTTLES PROPERLY.

Material Safety Data Sheet Number: 920785 PREPARED BY: RB Moon

Date Issued: 11-2-87

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# MATERIAL SAFETY DATA SHEET

**EMERGENCY PHONE NUMBER** (USA) 415-570-6667, ext. 3333 (UK) 0925-825650

## I. Identification

| | | | |
|---|---|---|---|
| PRODUCT NAME | Iodine/Water/Pyridine/Tetrahydrofuran, 200 ml | PART NUMBER | 400753 |
| CHEMICAL NAME | N/A | CHEMICAL FAMILY | Mixture |
| FORMULA | $I_2/H_2O/C_5H_5N/C_4H_8O$ | MOLECULAR WEIGHT | N/A |
| SYNONYMS | DNA Synthesis Reagent #15, Iodine Oxidation Reagent | | |
| DEPARTMENT OF TRANSPORTATION | HAZARD CLASSIFICATION | 3, Tetrahydrofuran, UN2056 | |
| | SHIPPING NAME | Tetrahydrofuran | |

## II. Ingredients

| MATERIAL | % | TLV (Units) | PEL (Units) | CAS NUMBER |
|---|---|---|---|---|
| Iodine | 3 | 0.1 ppm | 0.1 ppm | 7553-56-2 |
| Water | 2 | --- | --- | --- |
| Pyridine | 20 | 5 ppm | 5 ppm | 110-86-1 |
| Tetrahydrofuran (THF) | 75 | 200 ppm | 200 ppm | 109-99-9 |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

## III. Physical Data

| | | | |
|---|---|---|---|
| BOILING POINT, 760 mmHg | THF only: 66°C (151°F) | FREEZING POINT | N/A |
| SPECIFY GRAVITY ($H_2O$ = 1) | N/A | VAPOR PRESSURE, mmHg | N/A |
| VAPOR DENSITY (Air = 1) | 2.5 (THF) | SOLUBILITY IN WATER, % BY WEIGHT | N/A |
| PERCENT VOLATILE BY VOLUME | 97% | EVAPORATION RATE (Butyl Acetate = 1) | 800 (THF) |
| APPEARANCE AND ODOR | Brown liquid with a pungent, disagreeable odor | | |

## IV. Fire and Explosion Hazard Data

| | | | | |
|---|---|---|---|---|
| FLASH POINT (method used) | THF only: -14°C (6°C) | | | (closed cup) |
| FLAMMABLE LIMITS | THF | Lower: 1.8% | Upper: | 11.8% |
| FIRE EXTINGUISHING MEDIA | Dry chemical, alcohol foam, carbon dioxide or Halon | | | |
| SPECIAL FIRE FIGHTING PROCEDURES | Use self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. See note 1. | | | |
| UNUSUAL FIRE AND EXPLOSION HAZARDS | Water spray may be ineffective except in cooling containers exposed to fire. | | | |

## V. Health Hazard Data

| | |
|---|---|
| EXPOSURE LIMITS | The current OSHA standards for THF and pyridine exposure are 200 ppm and 5 ppm, respectively, averaged over 8 hours. See Note 2. |

### EFFECTS OF ACUTE OVEREXPOSURE

| | |
|---|---|
| SWALLOWING | Harmful if swallowed. High concentrations may cause nausea, dizziness, headache, liver or kidney damage. |
| SKIN | Exposure to liquid may cause serious skin irritation. |
| INHALATION | May cause acute irritation of the upper respiratory tract, dizziness, nausea, headaches. |
| EYES | May cause severe irritation. |

### EFFECTS OF CHRONIC OVEREXPOSURE

Liver and kidney damage have been reported in laboratory animals exposed to high concentrations of THF. Liver and kidney injury have been reported in humans given pyridine as an oral medication.

### OTHER HEALTH HAZARDS

Points of attack include eyes, skin, respiratory system and central nervous system. Allergic sensitization of the skin may occur.

| CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: | NTP No | IARC No | OSHA No |
|---|---|---|---|

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY CHEMICAL OVEREXPOSURE

No specific information is available.

### EMERGENCY AND FIRST AID PROCEDURES

| | |
|---|---|
| SWALLOWING | If conscious, give large volumes of water immediately. Get medical attention. |
| SKIN | Remove contaminated clothing. Flush the contaminated area with large volumes of water for 15 minutes. Seek medical attention. |
| INHALATION | Provide fresh air and rest. If breathing is difficult, provide oxygen and get medical attention immediately. |
| EYES | Flush eyes immediately with large amounts of water for at least 15 minutes. Seek medical attention immediately. |

| | |
|---|---|
| NOTES TO PHYSICIAN | Special attention should be given to skin, eyes and respiratory tract. |

## VI. Reactivity Data

| STABILITY: | CONDITIONS TO AVOID |
|---|---|
| STABLE [X] | Avoid contact with strong oxidizers. |
| UNSTABLE [ ] | |

INCOMPATIBILITY (materials to avoid) Contact with strong oxidizers may cause fire or explosion.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS Burning may release toxic vapors and gases, including iodine, hydrogen iodide, carbon monoxide, oxides of nitrogen, and toxic cyanides.

| HAZARDOUS POLYMERIZATION: | CONDITIONS TO AVOID |
|---|---|
| MAY OCCUR [ ] | See Section IX. |
| WILL NOT OCCUR [X] | |

## VII. Spill or Leak Procedures

STEPS TO BE TAKEN Avoid inhalation and skin contact. Wear protective clothing. Remove all ignition sources. Ventilate area of spill or leak. See Note 3.

WASTE DISPOSAL METHOD This reagent should be disposed of as a regulated hazardous waste by a permitted hazardous waste management facility in accordance with federal, state, and local regulations.

## VIII. Special Protective Equipment

RESPIRATORY PROTECTION (Specify Type) An MSHA- or NIOSH-approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for emergencies and high concentrations.

| | |
|---|---|
| VENTILATION | Handle within a well-ventilated area. Minimize open exposure to air. |
| PROTECTIVE GLOVES | Neoprene or nitrile rubber gloves are recommended. |
| EYE PROTECTION | Safety glasses with side shields, monogoggles or face shield. |
| OTHER PROTECTIVE EQUIPMENT | As necessary to prevent skin contact. |

## IX. Special Precautions

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE

Handle as a flammable liquid. Use with adequate ventilation. Do not breathe vapor. Store in a secure location suitable for flammable materials. Keep away from heat, sparks and flame.

OTHER PRECAUTIONS Eye wash and safety shower should be in area of use. This reagent will attack many forms of rubber, plastics, coatings and finishes.

## X. Additional Information

Note 1: NFPA 704M rating: Health-2, Flammability-3, Reactivity-1.

Note 2: For THF the STEL is 250 ppm in air, and the IDLH is 20,000 ppm. For pyridine the STEL in air is 10 ppm and IDLH level is 3,600 ppm. There is no STEL data for iodine.

Note 3: Small spills and leaks may be collected with absorbent towels or pads and removed to a well ventilated area away from ignition sources. This reagent must not be allowed to enter confined spaces because of the possibility of vapor explosion.

CAUTION!

THIS PRODUCT IS STRICTLY INTENDED FOR LABORATORY RESEARCH PURPOSES ONLY.
DISPOSE OF EMPTY BOTTLES PROPERLY

Material Safety Data Sheet Number: 920753

PREPARED BY: B B Moon

Date Issued: 9-9-87

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# MATERIAL SAFETY DATA SHEET

**EMERGENCY PHONE NUMBER** (USA) 415-570-6667, ext. 999 (UK) 0925-825650

## I. Identification

| | | | |
|---|---|---|---|
| PRODUCT NAME | Trichloroacetic Acid/Dichloromethane, 450 ml | PART NUMBER | 400236 |
| CHEMICAL NAME | N/A | CHEMICAL FAMILY | Mixture |
| FORMULA | $CCl_3COOH$ in $CH_2Cl_2$ | MOLECULAR WEIGHT | N/A |
| SYNONYMS | DNA Synthesis Reagent #14, TCA Detritylation Reagent | | |
| DEPARTMENT OF TRANSPORTATION | HAZARD CLASSIFICATION | 8, corrosive liquid, poisonous, DOT12A, UN 2922 | |
| | SHIPPING NAME | Corrosive liquid, poisonous, n.o.s. | |

## II. Ingredients

| MATERIAL | % | TLV (Units) | PEL (Units) | CAS NUMBER |
|---|---|---|---|---|
| Trichloroacetic acid (TCA) | 2 | 1 ppm | 1 ppm | 76-03-9 |
| Dichloromethane (DCM) | 98 | 100 ppm | 500 ppm | 75-09-2 |
| | | | | |
| | | | | |
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| | | | | |
| | | | | |
| | | | | |

## III. Physical Data

| | | | |
|---|---|---|---|
| BOILING POINT, 760 mmHg | DCM only: 40°C (104°F) | FREEZING POINT | N/A |
| SPECIFY GRAVITY ($H_2O$ = 1) | N/A | VAPOR PRESSURE, mmHg | 349 @ 20°C (DCM) |
| VAPOR DENSITY (Air = 1) | 2.9 (DCM) | SOLUBILITY IN WATER, % BY WEIGHT | 3% |
| PERCENT VOLATILE BY VOLUME | 98% | EVAPORATION RATE (Butyl Acetate = 1) | N/A |
| APPEARANCE AND ODOR | Colorless liquid with a pleasant aromatic odor | | |

## IV. Fire and Explosion Hazard Data

| | | | |
|---|---|---|---|
| FLASH POINT (method used) | None | | |
| FLAMMABLE LIMITS | Lower: N/A | Upper: | N/A |
| FIRE EXTINGUISHING MEDIA | Dry chemical, foam, carbon dioxide, or Halon | | |
| SPECIAL FIRE FIGHTING PROCEDURES | Use self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. | | |
| UNUSUAL FIRE AND EXPLOSION HAZARDS | May form flammable vapor/air mixtures above 100°C. | | |

## V. Health Hazard Data

| | |
|---|---|
| EXPOSURE LIMITS | The current OSHA standard for DCM exposure is 500 ppm averaged over 8 hours. See Note 1. |

### EFFECTS OF ACUTE OVEREXPOSURE

| | |
|---|---|
| SWALLOWING | Harmful if swallowed. DCM is a mild narcotic. Effects of intoxication include headache, giddiness, stupor, irritability, numbness, and tingling in the limbs. |
| SKIN | Exposure to liquid may cause irritation. Continued contact with the skin may cause burns. |
| INHALATION | Severe exposure may cause halucinations, pulmonary edema, coma and death. |
| EYES | May cause severe irritation. |

### EFFECTS OF CHRONIC OVEREXPOSURE

Exposure to DCM may cause elevated carboxy-hemoglobin levels which may be significant in smokers or workers with anemia or heart disease.

### OTHER HEALTH HAZARDS

Points of attack include eyes, skin, respiratory system and central nervous system. Recent studies suggest that DCM is a potential carcinogen.

| CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: | NTP No | IARC No | OSHA No |
|---|---|---|---|

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY CHEMICAL OVEREXPOSURE

Anemias or cardiovascular disease.

### EMERGENCY AND FIRST AID PROCEDURES

| | |
|---|---|
| SWALLOWING | If conscious, give large volumes of water immediately and induce vomiting. Do not induce vomiting if unconscious. |
| SKIN | Remove contaminated clothing. Flush the contaminated area with large volumes of water for 15 minutes. Seek medical attention. |
| INHALATION | Provide fresh air and rest. If breathing is difficult, provide oxygen and get medical attention immediately. |
| EYES | Flush eyes immediately with large amounts of water for at least 15 minutes. Seek medical attention immediately. |

NOTES TO PHYSICIAN

None

## VI. Reactivity Data

| STABILITY: | CONDITIONS TO AVOID |
|---|---|
| STABLE [X] | Contact with strong oxidizers may cause fire or explosion. |
| UNSTABLE [ ] | |

INCOMPATIBILITY (materials to avoid) Strong oxidizers, strong bases, and chemically active metals such as aluminum or magnesium powders, sodium or potassium.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS Burning may release toxic vapors and gases, including phosgene, hydrogen chloride, and carbon monoxide.

| HAZARDOUS POLYMERIZATION: | CONDITIONS TO AVOID |
|---|---|
| MAY OCCUR [ ] | See Section IX. |
| WILL NOT OCCUR [X] | |

## VII. Spill or Leak Procedures

STEPS TO BE TAKEN Avoid inhalation and skin contact. Wear protective clothing. Ventilate area of spill or leak. Collect reagent with absorbent towels or pads and remove to a well ventilated area.

WASTE DISPOSAL METHOD This reagent should be disposed of as a regulated hazardous waste by a permitted hazardous waste management facility in accordance with federal, state, and local regulations.

## VIII. Special Protective Equipment

| | |
|---|---|
| RESPIRATORY PROTECTION (Specify Type) | An MSHA- or NIOSH-approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for high concentrations and emergencies. |
| VENTILATION | Handle within a well-ventilated area. Minimize open exposure to air. |
| PROTECTIVE GLOVES | Butyl rubber or similar type resistant to DCM and other chlorinated solvents. |
| EYE PROTECTION | Safety glasses with side shields, monogoggles or face shield. |
| OTHER PROTECTIVE EQUIPMENT | As necessary to prevent skin contact. |

## IX. Special Precautions

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE

Use with adequate ventilation. Do not breathe vapor. Avoid skin contact. Store in a secure, well ventilated area away from heat.

OTHER PRECAUTIONS Eye wash and safety shower should be in area of use. This reagent will attack aluminum surfaces and many forms of rubber, plastics, coating, and finishes.

## X. Additional Information

Note 1: For methylene chloride the STEL is 1000 ppm with a maximum peak exposure of 2000 ppm for 5 minutes in any 2 hour period. NIOSH has recommended a PEL of 75 ppm with a ceiling level of 500 ppm averaged over a 15 minute period. NIOSH recommends a further PEL reduction where carbon monoxide is present since a major metabolic product of methylene chloride is carbon monoxide. The ACGIH has proposed a TWA of 100 ppm and an STEL of 500 ppm. The IDLH level is 5000 ppm.

**CAUTION!**

THIS PRODUCT IS STRICTLY INTENDED FOR LABORATORY RESEARCH PURPOSES ONLY.
DISPOSE OF EMPTY BOTTLES PROPERLY .

Material Safety Data Sheet Number 920236

PREPARED BY: B.B. Moon

Date Issued: 11/18/85

The opinions expressed herein are those of qualified experts within Applied Biosystems, Inc., information from our suppliers, and data presented in various technical publications. We believe that the information contained herein is current as of the date of this Material Safety Data Sheet. It is the user's responsibility to determine the suitability of this information for the adoption of safety precautions as may be necessary. We reserve the right to revise Material Safety Data Sheets from time to time as new technical information becomes available.

# MATERIAL SAFETY DATA SHEET

850 LINCOLN CENTRE DRIVE
FOSTER CITY, CA 94404

EMERGENCY PHONE NUMBER
(USA) 415-570-6667, ext. 3333
(UK) 0925-825650

## I. Identification

| | | | |
|---|---|---|---|
| PRODUCT NAME | TETD/Acetonitrile, 180 mL | PART NUMBER | 401147 |
| CHEMICAL NAME | Tetraethylthiuram disulfide/Acetonitrile | MOL. WEIGHT | TETD - 296.54 acetonitrile - 41 |
| FORMULA | TETD - $C_{10}H_{20}N_2S_4$ Acetonitrile - $C_2H_3N$ (See Note 2) | STORAGE TEMP | 20-30°C |
| CHEMICAL FAMILY | TETD - thiuram Acetonitrile - nitrile | SHELF LIFE | 1 yr |
| SYNONYMS | sulfurizing reagent, Antabuse, Disulfuram, DNA synthesis, Methyl cyanide, Cyanomethane, Ethylnitrile | | |
| TRANSPORTATION INFORMATION: | Hazard Classification - 3 flammable liquid, poisonous | | |
| GROUND (DOT)<br>AIR (ICAO) | Acetonitrile, Flammable Liquid, NA 1648, Ltd Qty<br>Methyl Cyanide, 3, UN 1648, 6.1, Flammable Liquid | | |
| | TETD: NIOSH Registry Number: J01225000<br>DOT ID Number: NA9188<br>DOT Hazard Classification: ORM-E | | |
| TARIFF CODE | Not Applicable | | |

## II. Ingredients

| MATERIAL | % | TLV (Units) | PEL (Units) | CAS NUMBER |
|---|---|---|---|---|
| Acetonitrile | 85 | 40 ppm | 40 ppm | 75-05-8 |
| Tetraethylthiuram disulfide (TETD) | 15 | 2 mg/m$^3$ | 2 mg/m$^3$ | 97-77-8 |
| | | | | |
| | | | | |
| | | | | |

## III. Physical Data

| | | | |
|---|---|---|---|
| BOILING POINT | >82°C | FREEZING POINT | 0°C |
| SPECIFIC GRAVITY | .833 g/mL | VAPOR PRESSURE mmHg | Not Measured |
| VAPOR DENSITY | 1.3 (acetonitrile) | SOLUBILITY IN WATER, % BY WEIGHT | Miscible |
| PERCENT VOLATILE BY VOLUME | 85% | EVAPORATION RATE (Butyl Acetate=1) | 5.8 |
| APPEARANCE AND ODOR | Colorless liquid with an ether-like odor. | | |

## IV. Fire and Explosion Hazard Data

| | |
|---|---|
| FLASH POINT | Acetonitrile only: 5.6°C (42°F) (closed cup) |
| FLAMMABLE LIMITS | Lower: 4.4% Upper: 16.0% |
| FIRE EXTINGUISHING MEDIA | Dry chemical, alcohol foam, carbon dioxide or Halon™ |
| SPECIAL FIRE FIGHTING PROCEDURES | Use self-contained breathing apparatus and protective clothing to prevent contact with skin and eyes. |
| UNUSUAL FIRE AND EXPLOSION HAZARDS | Water spray may be ineffective except in cooling containers exposed to fire. Vapor is heavier than air and may travel considerable distance to ignition source. |

## V. Health Hazard Data

| | |
|---|---|
| EXPOSURE LIMITS | OSHA standard for acetonitrile is 40 ppm in air averaged over 8 hours, STEL is 60 ppm in air, IDLH is 4000 ppm. |

## EFFECTS OF ACUTE EXPOSURE

| | |
|---|---|
| SWALLOWING | In most cases of systemic acetonitrile poisoning, there is a latent period of several hours between exposure and onset of symptoms, perhaps due to the slow release of cyanide. (Also, See Note 1). |
| SKIN | Prolonged exposure to TETD may cause irritation of the skin or allergic dermatitis. |
| INHALATION | May cause flushing of the face, a feeling of chest tightness and irritation of the nose and throat. In high concentrations may cause nausea, vomiting, gastric distress, respiratory depression, elevated blood cyanide and possible death. |
| EYES | May cause irritation. |

## EFFECTS OF CHRONIC EXPOSURE

No chronic diseases have been reported for acetonitrile or TETD. May cause respiratory irritation.

## OTHER HEALTH HAZARDS

Teratogencity and embryotoxicity was reported in several animal studies for acetonitrile at very high doses.

| CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: | NTP NO | IARC NO | OSHA NO |
|---|---|---|---|

MEDICAL CONDITIONS GENERALLY AGGRAVATED BY CHEMICAL OVEREXPOSURE

TETD, also known as Antabuse, is useful in the treatment of alcoholism. No chronic affect has been reported. May cause respiratory irritation. Preclude from exposure those individuals with diseases of central nervous systems, heart and lungs.

## EMERGENCY AND FIRST AID PROCEDURES

| | |
|---|---|
| SWALLOWING | If conscious, give large volumes of water immediately. Get medical attention immediately. If not breathing, give artificial respiration. |
| SKIN | Remove contaminated clothing. Flush the contaminated area with large volumes of water for 15 minutes. Seek medical attention immediately. Wash skin with soap. |
| INHALATION | Provide fresh air and rest. If breathing is difficult, provide oxygen at once and get medical attention immediately. |
| EYES | Flush eyes immediately with large amounts of water for at least 15 minutes. Seek medical attention immediately. |

| | |
|---|---|
| NOTES TO PHYSICIAN | Do not rely on determination of blood cyanide or urinary thiocyanate for evidence of exposure. |

## VI. Reactivity Data

| STABILITY | CONDITIONS TO AVOID |
|---|---|
| STABLE X<br>UNSTABLE ___ | Avoid contact with strong oxidizers and reducing agents. Avoid contact with strong oxidizers, heat or flames. |

| | |
|---|---|
| INCOMPATIBILITY | Keep dry and tightly sealed. Contact with strong oxidizers (e.g., sulfuric acide, perchlorates, chlorosulfonic acid, etc.) may cause fire or explosions. |
| HAZARDOUS COMBUSTION OR DECOMPOSITION | Burning may release toxic vapors and gases, including carbon monoxide, carbon dioxide, nitrogen oxides, sulfer oxides and hydrogen cyanide. |

| HAZARDOUS POLYMERIZATION: | CONDITIONS TO AVOID |
|---|---|
| MAY OCCUR ___<br>WILL NOT OCCUR X | |

## VII. Spill or Leak Procedures

| STEPS TO BE TAKEN |
|---|
| Avoid inhalation and skin contact. Wear protective clothing. Remove all ignition sources. Small spills and leaks may be collected with absorbent towels or pads and removed to a well ventilated area, such as a fume hood. |
| **WASTE DISPOSAL METHOD** |
| This reagent should be disposed of as a regulated hazardous waste by a permitted hazardous waste management facility in accordance with federal, state, and local regulations. |

## VIII. Special Protective Equipment

| RESPIRATORY PROTECTION (Specify Type) |
|---|
| An MSHA- or NIOSH- approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for emergencies and high concentrations. |

| | |
|---|---|
| VENTILATION | Handle within a well-ventilated area. Minimize open exposure to air. |
| PROTECTIVE GLOVES | Butyl rubber, polyvinyl alcohol or similar type glove resistant to organic solvents. |
| EYE PROTECTION | Safety glasses with side shields, monogoggles or face shield. |
| OTHER PROTECTIVE EQUIPMENT | As necessary to prevent skin contact. |

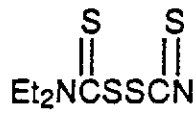
## IX. Special Precautions

| PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE |
|---|
| Handle as a flammable liquid. Use with adequate ventilation. Do not breathe vapor. Store in a secure, well-ventilated location suitable for flammable materials. Keep away from heat, sparks and flame. |
| **OTHER PRECAUTIONS** |
| Eye wash, safety shower and fire extinguisher should be in area of use. This reagent will attack many forms of rubber, plastics, coatings and finishes. |

## X. Additional Information

Note 1: Ingestion of alcoholic beverages after ingestion of TETD causes intense vasodilatation of face and neck, tachycardia and tachypnea followed by nausea, vomiting, pallor, hypotension. Occasionally convulsions, cardiac arrhythmias, myocardial infarction may occur. High doses of alcohol may cause dizziness, headache, dyspnea, unconsciousness and death.

Note 2: Chemical Structures:



tetraethylthiuram disulfide — TETD

$CH_3CN$ — acetonitrile

Note 3: Odor Threshold: Ether-like smell - 40 ppm

Note 4: Toxicity Data:

Acetonitrile

$TD_{LO}$ - Human, Oral - 570 mg/kg
$LD_{50}$ - Rats, Oral - 3800 mg/kg
$LD_{50}$ - Rat Intraperitoneal - 850 mg/kg
$LD_{50}$ - Rat Subcutaneous - 500 mg/kg
$LD_{50}$ - Intravenous - 1680 mg/kg
$LC_{LO}$ - Rats, Inhalation - 8000 ppm/4 hr.
$LD_{50}$ - Mice Intraperitoneal - 175 mg/kg
$LD_{LO}$ - Mice Subcutaneous - 700 mg/kg

TETD

$LD_{50}$ - Rats, Oral - 8600 g/k

Note 5: Nation Fire Protection Association Labeling (NFPA 704M)

Health - 2, Flammability - 3, Reactivity - 0

**CAUTION!**

**THIS PRODUCT IS STRICTLY INTENDED FOR LABORATORY RESEARCH PURPOSES ONLY. DISPOSE OF EMPTY BOTTLES PROPERLY.**

**PREPARED BY:** Alex Andrus **DATE ISSUED:** 12/14/90

**The opinions expressed herein are those of qualified experts within Applied Biosystems, Inc., information from our suppliers, and data presented in various technical publications. We believe that the information contained herein is current as of the date of this Material Safety Data Sheet. It is the user's responsibility to determine the suitability of this information for the adoption of safety precautions as may be necessary. We reserve the right to revise Material Safety Data Sheets from time to time as new technical information becomes available.**

ABI 392/394 INSTRUMENT WASTE PROFILE

Emergency Phone Numbers: (USA) 415-570-6667 Ext. 3333 (UK) 0925-825650

## I. IDENTIFICATION

All liquid waste from the 392/394 instruments is collected in a 10-Liter bottle located below the instrument. This waste is a complex mixture of reagents and solvents which may have properties of greater hazard than the individual waste components by themselves. HANDLE THIS MATERIAL WITH EXTREME CAUTION! DO NOT DISPOSE OF THIS WASTE IN SINKS OR DRAINS! THIS MATERIAL SHOULD BE DISPOSED OF AS A REGULATED HAZARDOUS WASTE!

## II. APPROXIMATE COMPOSITION (Standard Cycle)

| MATERIAL | % | TLV | PEL | CAS# |
|---|---|---|---|---|
| 1-Methylimidazole | ≤ 1 | N/A | N/A | 616-47-7 |
| Acetonitrile | 80% | 40 ppm | 40 ppm | 75-05-8 |
| Dichloromethane | 11 | 100 ppm | 500 ppm | 75-09-2 |
| Tetrahydrofuran | 5 | 200 ppm | 200 ppm | 109-99-9 |
| Phosphoramidites | ≤ 1 | N/A | N/A | N/A |
| Acetic Anhydride | ≤ 1 | N/A | N/A | N/A |
| Iodine | ≤ 1 | 0.1 ppm | 0.1 ppm | 7553-56-2 |
| 2,6-Lutidine | ≤ 1 | N/A | N/A | 108-48-5 |
| Trichloroacetic Acid | ≤ 1 | 1 ppm | 1 ppm | 76-03-9 |
| Pyridine | ≤ 1 | 5 ppm | 5 ppm | 110-86-1 |
| Tetraethylthiuram Disulfide | 1 | 2 mg/m$^3$ | 2 mg/m$^3$ | 97-77-3 |

## III. PHYSICAL DATA

| | | | |
|---|---|---|---|
| BOILING POINT, 760 mm: | N/A | FREEZING POINT: | N/A |
| SPECIFIC GRAVITY ($H_2O$ = 1) | 0.94 | pH RANGE: | 4-6 |
| VOLATILITY (vol %): | 96-98% | SOLUBILITY in $H_2O$: | Soluble |
| APPEARANCE AND ODOR: | Yellow to reddish-brown liquid with an acrid, unpleasant odor. | | |

## IV. FIRE AND EXPLOSION HAZARD DATA (Acetonitrile data only!)

FLASH POINT (Closed Cup): 5.6°C (42°F)

FLAMMABLE LIMITS: 4.4% LEL 16% UEL

FIRE EXTINGUISHING MEDIA: Dry chemical, alcohol foam, carbon dioxide or Halon.

SPECIAL FIRE FIGHTING PROCEDURES: Use self-contained breathing apparatus and protective clothing to prevent skin and eye contact.

## V. HEALTH HAZARD DATA

EXPOSURE LIMITS: See Section III. For acetonitrile, the STEL is 60 ppm and the IDLH level is 4,000. For Dichloromethane, the STEL is 1,000 ppm and the IDLH level is 5,000 ppm.

### EFFECTS OF ACUTE OVEREXPOSURE

SWALLOWING: Harmful is swallowed! Causes severe irritation of eyes, nose and throat. Higher concentrations may cause nausea, vomiting, convulsions, liver and kidney damage, unconsciousness and death.

SKIN: May cause severe irritation and burns. Allergic skin sensitization may also occur.

INHALATION: May cause severe irritation of eyes, nose, throat and lungs. Higher concentrations may cause pulmonary edema, unconsciousness and death.

EYES: May cause severe irritation or burns.

### EMERGENCY AND FIRST AID PROCEDURES

SWALLOWING: If conscious, give large quantities of water immediately and induce vomiting. Get medical attention immediately. Do not induce vomiting if unconscious.

SKIN: Remove contaminated clothing. Flush the contaminated area with water and wash with mild soap or detergent. Get medical attention.

INHALATION: Provide fresh air and rest. If breathing is difficult, provide oxygen and get medical attention immediately.

EYES: Flush eyes immediately with large amounts of water for at least 15 minutes. Get medical attention.

## VI. REACTIVITY DATA

STABILITY: Stable

INCOMPATIBILITY: Contact with strong oxidizing agents or concentrated acids or bases may cause fire or explosion.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS: Burning may release toxic vapors and gasses including phosgene, hydrogen cyanide, hydrogen chloride, carbon monoxide and oxides of nitrogen.

HAZARDOUS POLYMERIZATION: Will not occur.

## VII. SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN: Avoid inhalation and skin contact. Wear protective clothing. Ventilate area of spill or leak. Remove all ignition sources. Small quantities may be collected with absorbent towels or pads and removed to a well-ventilated area away from ignition sources. Larger amounts (1 liter or more) may be collected with an inert absorbent (kitty litter or similar material) or commercially available spill pillows designed for solvent collection. This waste material must not be allowed to enter confined spaces (such as a sewer!) because of the possibility of an explosion.

WASTE DISPOSAL: The instrument waste solution should be disposed of as a regulated hazardous waste by a properly permitted hazardous waste management facility in accordance with federal, state and local regulations. Recommended disposal methods include high temperature incineration or solidification for secure chemical landfill disposal.

## VIII. SPECIAL PROTECTIVE EQUIPMENT

RESPIRATORY PROTECTION: An MSHA- or HIOSH- approved respirator for organic vapors. A supplied-air or SCBA respirator is recommended for high vapor concentrations and emergency situations.

VENTILATION: Handle within a well-ventilated area. Minimize open exposure to air.

PROTECTIVE GLOVES: Butyl rubber to similar type resistant to strong solvents.

EYE PROTECTION: Safely glasses with side shields, monogoggles or face shield.

OTHER PROTECTIVE EQUIPMENT: As necessary to prevent skin contact.

## IX. SPECIAL PRECAUTIONS

PRECAUTIONS TO BE TAKEN: Handle as a flammable, poisonous liquid. Maintain adequate ventilation at all times. Do not breathe vapor. Do not get in eyes, on skin, or on clothing. Accidental contact should be washed away immediately. Keep away from heat, sparks and flame. Keep containers tightly closed. Eye bath, safety shower and spill collection materials should be in area of use.

OTHER: This waste solution has strong solvent properties and will attack many forms of rubber, plastics, coatings and finishes.

## X. ADDITIONAL INFORMATION

When not directly attached to the instrument, this waste material should be stored in a secure, well-ventilated location suitable for flammable materials. Store away from light, heat or potential ignition sources. Contact the appropriate state hazardous waste regulatory agency for proper disposal procedures and lists of registered service companies.

THIS WASTE MATERIAL IS HAZARDOUS AND SHOULD ONLY BE HANDLED BY PERSONS THOROUGHLY TRAINED IN HAZARDOUS MATERIALS HANDLING PROCEDURES!

### ACRONYMS USED

ACGIH: American Conference of Government Industrial Hygienists.

CAS#: Chemical Abstracts Service compound reference number.

IDLH: Immediately Dangerous to Life and Health.

LEL: Lower Explosion Limit.

MSHA: Mine Safety and Health Administration.

NIOSH: National Institute for Occupational Safety and Health.

OSHA: Occupational Safety and Health Administration.

PEL: Permissible Exposure Limit. The federal OSHA limit, usually expressed as a TWA for an 8 hour work shift.

PPM: Parts Per Million.

SCBA: Self-Contained Breathing Apparatus.

TLV: Threshold Limit Value. The ACGIH recommended TWA, usually for an 8 hour work shift.

TWA: Time Weighted Average.

UEL: Upper Explosive Limit.

# 1. INTRODUCTION

This manual will help you prepare for the installation of your Model 392 or 394 DNA/RNA Synthesizer. The first part describes (1) items that will be shipped with the instrument and (2) chemicals and equipment you should obtain before you receive the instrument. The second part of the manual describes site preparation. Careful attention to these requirements will simplify the installation procedure and ensure that the instrument works correctly. The site preparation section concludes with a Pre-Installation Checklist.

The Models 392 and 394 are available with four voltage options. **It is important that the internal voltage setting for your instrument agree with your site voltage.** Please see par. 2.3 for specific information.

## *If you purchased an Applied Biosystems installed instrument:*

Please do not unpack the crate until your local Applied Biosystems service representative arrives to make the installation. The Start-Up Chemical Kit will be shipped separately and should be unpacked upon arrival. This kit contains sufficient chemicals and reagents to demonstrate that the instrument is operating correctly. If additional DNA will be synthesized immediately following installation, additional chemicals should be ordered.

## *If you purchased a user installed instrument (U.S. only):*

The User Installation Manual will be sent to you. Please be aware that you will need to order reagents for the installation. We recommend that you order the Start-Up Chemical Kit (part No. 400971), which contains sufficient chemicals and reagents for demonstrating that the instrument operates correctly. The kit contains additional items for post-synthesis processing of the oligonucleotide. If additional DNA will be synthesized immediately following installation, additional chemicals should also be ordered.

# 2. CHEMICALS AND ACCESSORIES

## 2.1 Start-Up Chemical Kit

If an Applied Biosystems service engineer will install your Model 392 or 394, a Start-Up Chemical Kit will be sent to you for the installation. The kit contains chemicals and reagents (enough for at least 100 base additions), four synthesis columns, and a manual deprotection kit. The Start-Up Chemical Kit will be used during installation to verify instrument performance.

If you have purchased a user-installed instrument (U.S. only), you must order the Start-Up Chemical Kit, part No. 400971.

The contents of the Start-Up Chemical Kit are listed below:

| Part No. | Description | Quantity |
|---|---|---|
| 001753 | Seal, 16-oz bottle | 2 |
| 002267 | Seal, 8-oz bottle | 5 |
| 200140 | Septum, 13 mm, rubber | 2 |
| 400257 | Manual deprotection kit | 1 |
| 400330 | CE-phosphoramidite A, 0.5 g | 1 |
| 400331 | CE-phosphoramidite G, 0.5 g | 1 |
| 400332 | CE-phosphoramidite C, 0.5 g | 1 |
| 400333 | CE-phosphoramidite T, 0.5 g | 1 |
| 400443 | Acetonitrile, 4 L, Burdick & Jackson | 1 |
| 400445 | Trifluoroacetic acid, 10 mL | 1 |
| 400613 | Triethylamine acetate, 2 M, 200 mL | 1 |
| 400771 | Oligo purification cartridge | 1 |
| 400852 | Kit, B-CE large bottle reagents (includes the following items) | 1 |
| 400060 | Anhydrous acetonitrile, 30 mL | 2 |
| 400236 | Trichloroacetic acid, 450 mL | 2 |
| 400501 | 16-oz bottle seals, 10/pkg | 1 |
| 400606 | Tetrazole/acetonitrile, 180 mL | 1 |
| 400607 | Acetic anhydride, 180 mL | 1 |
| 400753 | Iodine/water/pyridine, 200 mL | 1 |
| 400785 | 1-methylimidazole, 180 mL | 1 |
| 400790 | 200-mL bottle seals, 10/pkg | 1 |
| 400953 | Small-scale A CPG column | 1 |
| 400954 | Small-scale C CPG column | 1 |
| 499955 | Small-scale G CPG column | 1 |
| 400956 | Small-scale T CPG column | 1 |

NOTE: The only additional reagent you will need for installation is concentrated analytical grade ammonium hydroxide. Because ammonium hydroxide is a common laboratory reagent, Applied Biosystems does not supply it in some countries, including the United States. If you do not have ammonium hydroxide in your laboratory, you can order it from Baker Laboratories (Concentrated reagent, 29.8%, Baker part No. 9721.1).

The Start-Up Chemical Kit includes a manual deprotection kit (part No. 400257) that contains the following:

| Part No. | Description | Quantity |
|---|---|---|
| 200243 | 1-mL disposable syringes with luer fitting | 10 |
| 200244 | Disposable needles with female luer | 10 |
| 200245 | No. 10 rubber stopper | 1 |
| 110127 | Male-to-male luer connector (Alltech 86506CTFE) | 5 |
| 400048 | DNA collection vial | 10 |
| 140048 | Teflon-lined vial cap | 10 |

The Models 392 and 394 deprotect automatically. You will need the supplies in the manual deprotection kit only if you choose to do manual deprotection.

## 2.2 Additional Chemicals and Columns

The supplies included in the Start-Up Chemical Kit will not last beyond the installation period. It is strongly recommended that an additional supply be ordered before installation. If you wish to order chemicals and columns for additional syntheses, please contact your local salesperson or call Applied Biosystems. All phosphoramidites, reagents, and synthesis columns should be ordered from Applied Biosystems with two exceptions:

- Ammonium hydroxide for the cleavage/deprotection step. Order concentrated analytical grade reagent. Purchase it in 500-mL bottles, keep it sealed tightly, and store it at 4°C. Do not use it for more than two weeks after you have opened a bottle.
- Acetonitrile (HPLC-grade) for base dilution as well as for washing the liquid delivery system during synthesis. The Models 392 and 394 are equipped to handle a 4-L bottle. Because this reagent is used to dissolve the phosphoramidites, the water content must be less than or equal to 100 ppm (0.001%). We have had success with Burdick & Jackson acetonitrile (B&J part No. 015-4).

If you are unsure of the water content of a local supplier's acetonitrile, or if you know that the water content is too high, you can control or avoid this problem by (1) checking the acetonitrile by Karl Fischer titration or (2) distilling HPLC-grade acetonitrile over $P_2O_5$. Redistill over $CaH_2$ to remove acid.

## 2.3 Shipping List

The instrument will be shipped in a crate with the following accessory items:

| Part No. | Description | Quantity |
|---|---|---|
| 003661 | Bracket, fraction collector | 1 |
| 100072 | Wrench, 1/4 in. x 5/16 in. | 1 |
| 100126 | Screwdriver, Pozidriv™, 6-10 | 1 |
| 100129 | Wrench, 1/2 in. x 9/16 in., open-end | 1 |
| 110005 | Connector, 1/4 MP-1/4 T, brass | 1 |
| 110096 | Nut, 1/4 in., brass tube | 1 |
| 110097 | Ferrule, 1/4 in., swage | 1 |
| 110207 | Fitting, 3/8-in. blind nut, Kynar | 1 |
| 140040 | Jerrican, 2-1/2 gal, polyethylene | 1 |
| 140041 | Safety carrier, 1-gal bottle | 1 |

Table, cont

| Part No. | Description | Quantity |
|---|---|---|
| 200270 | Solvent inlet filter, 40 µm | 1 |
| 212159 | Screw, 6-32 x 0.25 in., knurled, stainless steel | 1 |
| 225016 | Tube, 1/4-in. o.d., polypropylene | 10 ft (3 m) |
| 225075 | Tube, Tygon, 3/32-in. i.d. x 5/32-in. o.d. | 3 in. (76 mm) |
| 400501 | Bottle seals, 16 oz, 10/pkg | 1 |
| 400790 | Bottle seals, 200 mL, 10/box | 1 |
| 401082 | Kit, spare part, 392/4 | 1 |
| 600235 | Fraction collector cable | 4 |
| 602544 | Cap assembly, waste bottle | 1 |
| 602706 | Memory card, Version 1.01 (in user's manual) | 1 |
| 602182 | Assembly, trityl collection tube | 4 |
| 602278 | Assembly, flowtest tube | 1 |
| 602431 | Kit, setup, 392/4 | 1 |
| 602458 | Assembly, cap, 4 L, 1/8-in. tubing | 1 |
| 901237 | User's manual, 392/394 | 1 |

In addition a plug/voltage kit from the list below will be included with your instrument.

| Part No. | Description | Voltage and Frequency |
|---|---|---|
| 401081 | Plug/voltage kit for US/Canada | 120 V (60 Hz) |
| 401080 | Plug/voltage kit for Continental Europe | 220 V (50 Hz) |
| 401079 | Plug/voltage kit for United Kingdom | 240 V (50 Hz) |
| 401078 | Plug/voltage kit for Australia | 240 V (50Hz) |
| 401077 | Plug/voltage kit for Japan | 100 V (50/60Hz) |

Verify that the plug/voltage kit you received is the correct one for your installation site.

## 2.4 User-Supplied Equipment

All customer-supplied items (for trityl analysis and post-synthesis processing of your oligonucleotides) are listed below and should be obtained before the installation. The two most critical items are a **fraction collector** and a **spectrophotometer**. Although measuring trityl cation release is only an approximate indicator of synthesis success, it is a convenient assay, and so we use it rather than more meaningful HPLC or capillary electrophoresis analysis to confirm instrument performance.

The spectrophotometer must be capable of reading at a wavelength of 530 nm. We recommend a "boat" type fraction collector similar to the ISCO Retriever II.

The following items are supplied by the user:

- Fraction Collector

  To monitor synthesis efficiency, a fraction collector must be adjacent to the synthesizer to collect the trityl solution from the trityl collection port. Any commercially available fraction collector with an external advance switch will suffice. To collect tritylys from two to four columns, a "boat" type

fraction collector is useful because the trityls are delivered in parallel. We recommend that at least one hundred 10-15 mL volumetric tubes be available. The fraction collector cable and trityl delivery line are packed with the instrument. The fraction collector must be placed within 30 cm (12 in.) of the left side of the instrument.

> WARNING: The trityl effluent is composed of trichloroacetic acid and acetonitrile. Before you handle chemicals for the instrument, read the materials safety data sheets (MSDSs) and waste profile in the User's Safety Information section at the beginning of this manual. Always follow the safety precautions (eye protection, clothing, etc.) presented in the MSDSs. Dispose of waste in accord with all local, state, and federal health and environmental regulations and laws.

- Spectrophotometer

  To measure absorbance of the diluted trityl solutions you will need a visible spectrophotometer. Also required are 1-cm cuvettes and a 0.1-M toluene sulfonic acid/acetonitrile solution for dilution.

- Heating Bath or Block

  The laboratory must have access to a heating bath or block to complete the manual base deprotection of the synthetic oligonucleotides. The temperature of this bath or block is typically set at 55-60°C (131-140°F).

- Sample Concentrator

  The ammonium hydroxide deprotection solution is evaporated before the DNA is used or purified. Any device capable of removing an aqueous solution is acceptable.

- Toluenesulfonic Acid

  Purchase the 500-g bottle of p-toluenesulfonic acid monohydrate, 99% (part No. T-3751) from Sigma Chemical Co., St. Louis, MO 63178. This is used to dilute the trityl cation.

> WARNING: P-toluenesulphonic acid is hazardous and must be handled with great caution. Refer to the Sigma Chemical Co. MSDS to familiarize yourself with its hazards and the precautions that must be taken. All procedures must be conducted in a correctly operating fume hood. Use of eye protection, appropriate rubber gloves, and safety clothing is imperative.

- Top Loading Balance

  Flowrates are determined by measuring the weight of solvent delivered in a predetermined interval. A top loading balance should be available that can measure delivery weights to within ±0.01 g.

- Ammonium Hydroxide

  As mentioned in par. 2.1, you must purchase concentrated analytical grade ammonium hydroxide (preferably in 500-mL bottles) for deprotection. This reagent is not included in the Start-Up Chemical Kit.

> WARNING: Ammonium hydroxide is hazardous and must be handled with great caution. Refer to the supplier's MSDS to familiarize yourself with its hazards and the precautions that must be taken. All procedures must be conducted in a correctly operating fume hood. Use of eye protection, appropriate rubber gloves, and safety clothing is imperative.

# 3. SITE PREPARATION

## 3.1 Laboratory Space

Allocating sufficient laboratory space (Fig. 1) is critical for successful installation and trouble-free maintenance. A sturdy freestanding table with locking wheels is strongly preferred over a lab bench because it allows access to the rear of the instrument. Specifically, we recommend an open bench with plastic laminate top, which can be ordered from Bay Products, 8701 Torresdale Avenue, Philadelphia, PA 19136; (800) 523-3434 or (215) 338-7300. The Bay Products catalog No. is 6303B34.

Sufficient room must be provided for the user-supplied external fraction collector and the 4-L acetonitrile bottle container which will be positioned to the left of the synthesizer. A space within approximately 5 ft (1.5 m) of the instrument must also be provided for safely securing an argon gas cylinder (size 1A). The dimensions and weight of the Models 392 and 394 are as follows:

| | Width | Depth | Height | Weight |
|---|---|---|---|---|
| Model 392 | 66 cm (26.0 in.) | 46 cm (18.0 in.) | 50 cm (19.9 in.) | 41 kg (93 lb) |
| Model 394 | 66 cm (26.0 in.) | 46 cm (18.0 in.) | 50 cm (19.9 in.) | 43 kg (96 lb) |

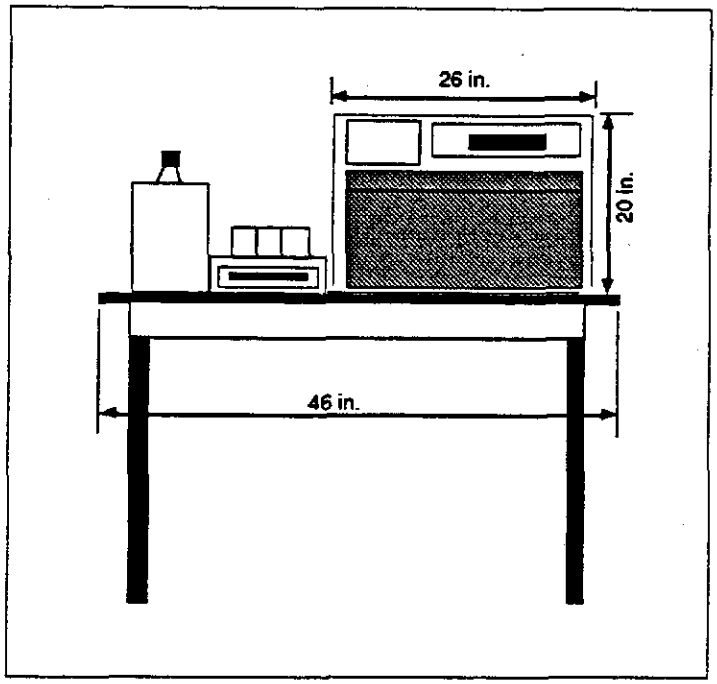



Fig. 1 Laboratory Space Requirement

## 3.2 Electrical Requirements

### 3.2.1 Power

The Models 392 and 394 can be configured for four different voltages. The instrument requires a dedicated electrical line with a circuit breaker (Fig. 2) and a power rating of at least 1.25 kVA. The outlet must be located close to the instrument. The supplied power cord is 8-ft (2.5-m) long.

The voltage options for the Models 392 and 394 are listed in par. 2.3. Check to make sure that the part number of the kit you received is appropriate for the supplied power.

### 3.2.2 Grounding

Certain types of electrical noise are exaggerated by poor or improper electrical ground connections. To prevent these problems, the installation site should have a dedicated 1.25-kVA power line and isolated ground between the instrument and building main electrical service, as depicted below.

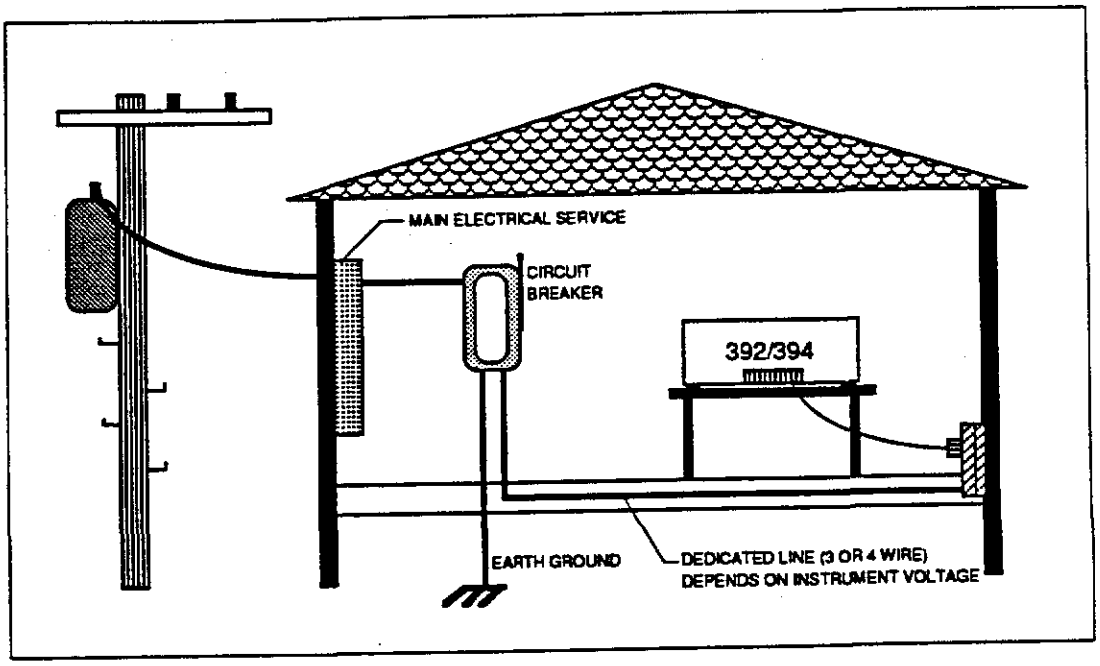



Fig. 2 Dedicated Power Line

### 3.2.3 Power "Quality"

#### Line Voltage Fluctuation

The line voltage must be within 5% of the nominal figures. High or low voltage conditions will have an adverse effect on electronic components of the instrument. In areas where the supplied power is subject to fluctuations exceeding the above limits, a power line regulator is strongly suggested. Please consult your local Applied Biosystems service representative if you suspect a problem.

### Voltage Spikes

Short-duration, high-voltage spikes can be caused by other devices (such as large motors) using the same power source or by outside influences such as lightning. If your laboratory environment contains devices that are electronically "noisy," or if you are in an area with frequent electrical storms, a line conditioner may greatly enhance reliability of your system. The line conditioner must have a rating of at least 0.5 kVA. Several sources of line conditioners are as follows:

- Best Power, P. O. Box 280, Necedah, WI 54646 - Tel: (608) 565-7200
- RTE Deltec, 2727 Kurtz St, San Diego, CA 92120 - Tel: (619) 291-4211
- Elgar, 9250 Brown Deer Rd, San Diego, CA 92121 - Tel: (619) 450-0085

## 3.3 Cooling Requirements

The Models 392 and 394 generate a maximum of 600 Btu/h. While there are no special cooling requirements, a constant laboratory temperature between 16-22°C (60-72°F) is recommended. Avoid placing the instrument close to heaters or cooling ducts. Temperatures below 16°C (60°F) must be avoided because they will cause the tetrazole to precipitate from solution.

## 3.4 Argon

A regulated cylinder (size 1A) of prepurified (99.998%) argon must be connected to the rear panel of the synthesizer. The instrument has a gas input that accepts 1/4-in.-o.d. Parflex™ tubing with appropriate Swagelok® tube fittings (1/4-in. nut and ferrule).

A 10-ft length of Parflex™ tubing (1/4-in. o.d.) and the necessary Swagelok® fittings to connect this tubing to the instrument are provided.

The argon tank requires a two-gauge regulator with a CGA (Compressed Gas Association) 580 argon cylinder adapter on the inlet side and a Swagelok® fitting that accepts a 1/4-in.-o.d. tube. The primary gauge (0-3000 psi; 0-25,000 kPa recommended) measures tank pressure, and the secondary gauge (0-200 psi; 0-2000 kPa recommended) measures regulated pressure. The regulator must be obtained by the user prior to installation.

Typical argon consumption is approximately 3.5-4.5 $m^3$ every four weeks. Size 1A cylinders have a capacity of 8.2 $m^3$ and should last approximately two months. The tank should be regulated to approximately 65 psi (450 kPa).

> WARNING: Compressed gas cylinders must be safely attached to the wall or bench by means of approved restraints. Failure to do so could cause the cylinders to fall over and explode, which could result in physical harm. Turn off or cap the cylinders when they are not in use.

## 3.5 Ventilation

Gaseous waste is vented from the waste bottle. Your facility must be equipped with a ventilated laboratory chemical fume hood or the equivalent to remove harmful vapors. The waste vent should be routed to the chemical exhaust system by one of the methods shown in the User's Safety Information section at the beginning of this manual.

## 3.6 Liquid Waste Disposal

WARNING: Please review the waste profile for information on contents of the Model 391 and 391 EP liquid waste so that it can be disposed of in accordance with all local, state, and federal health and environmental regulations and laws. Please also read the MSDSs to determine the protective equipment required to work with and dispose of these chemicals. The waste profile and MSDSs are in the User's Safety Information section at the beginning of this manual.

## 3.7 Operator Training at Installation (Applied Biosystems Service Installed Instruments Only)

An important part of in-laboratory installation is operator training. Those persons who will be operating the Models 392 and 394 should set aside two uninterrupted days to work with the Applied Biosystems service representative. If this is not possible, the installation should be rescheduled.

## 3.8 Proof of Performance

Unless previous arrangements have been made, an oligonucleotide of less than 25 bases will be synthesized to demonstrate instrument performance. It is necessary to have the fraction collector, spectrophotometer, and p-toluenesulfonic acid solution prepared so that the trityl assay can be performed. The passing on-site specification is 98.0% (±0.5%) average coupling yield.

## 3.9 Printer

A printer option consisting of a Hewlett-Packard Think-Jet™ printer and interface cable is available from Applied Biosystems. The appropriate part number depends on the site voltage as follows: part No. 401084, 120 V; part No. 401083, 220 V; part No. 401085, 240 V; and part No. 401086, 100 V. The interface cable (part No. 100390) may be ordered separately. Although the printer uses any standard 8-1/2 x 11-in. (European size A4) single or fanfold paper, best print quality is ensured by using H-P specified Ink Jet paper. Additional paper stock is available from Hewlett-Packard:

Part No. 92261M: Ink Jet paper, 500 sheets, single sheets

Part No. 92261N: Ink Jet paper, 2500 sheets, fanfold

# 4. PRE-INSTALLATION CHECKLIST

The following checklist is designed to ensure that all necessary preparations have been made prior to installation of the Model 392/394. If there are any questions concerning the installation procedure or preparations, please contact your local Applied Biosystems service representative.

| | Check (✓) if ready |
|---|---|
| 1. Pre-installation manual read and understood | |
| **Chemicals and Equipment:** | |
| 2. Correct plug/voltage kit received | |
| 3. Start-Up Chemical kit received | |
| 4. Additional reagents, including concentrated ammonium hydroxide and acetonitrile (4 L) ordered | |
| 5. Fraction collector(s) | |
| 6. Spectrophotometer | |
| 7. Heating block | |
| 8. Sample concentrator | |
| 9. Toluenesulfonic acid | |
| 10. Top loading balance | |
| 11. All material safety data sheets (MSDSs) and waste profile material read and understood | |
| **Site Preparation:** | |
| 12. Correct laboratory space | |
| 13. Constant laboratory temperature | |
| 14. Argon cylinder, size 1A (regulator and adapter) | |
| 15. Correct ventilation | |
| 16. Waste disposal method established | |

For installation, service, or support of your ABI instrument, contact your Field Service representative at the nearest location.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# *Appendix VI*

# Manual Phosphoramidite Preparation
# How to Store Dissolved Phosphoramidites
# Manual Cleavage and Deprotection
# Dimethoxytrityl (DMT) Cation Assay
# OPC (Oligonucleotide Purification Cartridge)

The information and procedures provided in this appendix are required only for special manual preparation of phosphoramidites, when one of the standard auto-dilution procedures is not used, or for manual cleavage and deprotection, when the standard instrument deprotection and cleavage procedures are not used.


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# Manual Phosphoramidite Preparation

Since the phosphoramidites are extremely sensitive to acid, oxygen, and water, you must take special care when dissolving them. The following instructions will help avoid contamination, prevent degradation, and ensure high coupling yields.

## Important Factors to Consider When Dissolving Phosphoramidites

1. Use anhydrous acetonitrile with less than 100 ppm water to dissolve the phosphoramidites.

   Use of Applied Biosystems anhydrous acetonitrile, Part Number 400060, is strongly recommended. *Do not* use HPLC-grade acetonitrile. Its higher water content will decrease coupling efficiency. After opening the acetonitrile, keep it blanketed with argon to avoid contamination with air.

2. When transferring the acetonitrile to a phosphoramidite bottle, use a clean, dry, glass syringe with a needle. Follow these precautions:
   - Store the syringe in a 100-120°C oven to prevent atmospheric moisture contamination.
   - Keep a syringe dedicated to acetonitrile transfer.
   - Use acetonitrile to rinse the syringe. *Do not* use water.
   - *Do not* contaminate the acetonitrile bottle with traces of phosphoramidites (i.e., do not allow the syringe needle to contact the phosphoramidites).

3. When preparing phosphoramidites, add the correct amount of acetonitrile to each phosphoramidite as shown in Table A6-1. Both standard and FOD phosphoramidites are diluted with the same volume of acetonitrile.

## Table A6-1
## Adding Acetonitrile to Phosphoramidites

| β-Cyanoethyl Phosphoramidite | Volume of Acetonitrile(ml) | Weight of Phosphoramidite (grams) | Molarity (M) |
|---|---|---|---|
| A | 2.8 | 0.25 | 0.10 |
| | 5.6 | 0.50 | 0.10 |
| | 11.2 | 1.00 | 0.10 |
| | 11.2 | 0.50 | 0.05* |
| G | 2.9 | 0.25 | 0.10 |
| | 5.8 | 0.50 | 0.10 |
| | 11.6 | 1.00 | 0.10 |
| | 11.6 | 0.50 | 0.05* |
| C | 2.9 | 0.25 | 0.10 |
| | 5.9 | 0.50 | 0.10 |
| | 11.8 | 1.00 | 0.10 |
| | 11.8 | 0.50 | 0.05* |
| T | 3.3 | 0.25 | 0.10 |
| | 6.6 | 0.50 | 0.10 |
| | 13.2 | 1.00 | 0.10 |
| | 13.2 | 0.50 | 0.05* |
| Deoxyinosine | 3.4 | 0.25 | 0.10 |

* 0.05 molar phosphoramidites should be used with the Primer cycle only.

## How to Dissolve Phosphoramidites

1. Prepare the phosphoramidite bottle as follows:
   a. Pull back the aluminum tab in the direction of the arrow. *Do not* yet remove it, simply expose the septum.
   b. Place a needle (any gauge) without a syringe into the rubber septum. This vents the pressure in the bottle when the anhydrous acetonitrile is added. Venting also prevents accidental splashing when the phosphoramidite bottle is opened and placed on the instrument.
2. Unscrew the cap from the anhydrous acetonitrile bottle and quickly replace it with a clean rubber septum.

   The acetonitrile is bottled under argon. Since argon is heavier than air, argon should still blanket the acetonitrile after the septum transfer.
3. Remove the syringe/needle from the oven and allow it to cool to room temperature.

4. Pierce the septum of the acetonitrile bottle with the needle and remove the correct amount of acetonitrile.

5. Pierce the septum of the phosphoramidite bottle a few millimeters with the needle/syringe and slowly add the acetonitrile.

   Make sure the needle does not touch the phosphoramidite powder or solution.

6. When finished adding the acetonitrile, remove both the venting needle and the needle/syringe and gently swirl the bottle to dissolve the phosphoramidite.

7. Once it is dissolved, place the phosphoramidite on the instrument.

   Use the Bottle Change menu and follow the prompts (see Section 3 for details). Also, refer to "Installing Reagent Bottles," in Section 2, for instructions on attaching the bottles to the instrument.

8. Blanket the anhydrous acetonitrile bottle with argon as follows:
   a. Place a needle on the upper male luer fitting on the front of the synthesizer (remove the column if necessary).
   b. Select *Manual Control*, on page 2 of the Main Menu, and activate Function 2, a reverse flush. Argon will then flow out of the needle.
   c. Next, push the acetonitrile bottle onto the needle so that the needle pierces the bottle's septum. Hold it for 5 seconds and then remove the bottle and the needle simultaneously. The pressure will immediately be released through the needle, but the bottle will remain blanketed with argon.
   d Remove the needle from the bottle and continue dissolving additional phosphoramidites as necessary.

   If you cannot use the phosphoramidites within about two weeks, you can remove them from the instrument, store them, and reuse them later. *Do not* use the Bottle Change Procedure for this. Instead, follow the procedure "How to Remove and Store Dissolved Phosphoramidites," in the next subsection.

# How to Remove and Store Dissolved Phosphoramidites

Once in solution, phosphoramidites are stable for at least two weeks. After this time, coupling efficiencies will begin to decrease. If phosphoramidites cannot be used within this time, it is possible to freeze, store, thaw, and reuse them. The stored phosphoramidites will show some loss of activity, depending on storage technique and age.

1. Using *Manual Control* (on page 2 of the Main Menu), activate valve #45 for 10 seconds to deliver argon to the phosphoramidite bottles. At the end of each set period, the valves will automatically shut off.

2. Remove the bottles (without using the Bottle Change procedure) and quickly cap with previously unused rubber septa (Aldrich catalog number Z10,074-9) to minimize exposure to water vapor.

   Remove a bottle by firmly pulling it straight down while pressing the black button above its receptacle. If the bottle seems to stick, carefully move it side to side while pulling it off.

3. Seal the bottles with paraffin film, place them in a desiccator containing Drierite, and put the desiccator in a freezer at –20°C.

4. Rinse the delivery lines as follows:

   Activate functions F70 to F77 for 10 seconds each. This rinses the delivery lines with acetonitrile. Then flush the lines with argon by activating functions F6 to F13 for an additional 10 seconds each.

| Acetonitrile to Reservoirs | Argon to Reservoirs |
|---|---|
| F 70 18 to A | F 6 Flush to A |
| F 71 18 to G | F 7 Flush to G |
| F 72 18 to C | F 8 Flush to C |
| F 73 18 to T | F 9 Flush to T |
| F 74 18 to 5 | F 10 Flush to 5 |
| F 75 18 to 6 | F 11 Flush to 6 |
| F 76 18 to 7 | F 12 Flush to 7 |
| F 77 18 to 8 | F 13 Flush to 8 |

5. When ready to reuse the phosphoramidites, prepare them as follows:

   Thaw the phosphoramidites, remove them from the desiccator. and place them on the instrument using the Bottle Change menu. Be sure to enter an appropriate alarm setting.

# Manual Cleavage and Deprotection

The information and procedures provided in this appendix are required only for special manual cleavage and deprotection of oligonucleotides when one of the standard ending procedures is not used.

## Reagents, Solvents, and Apparatus

Reagent-grade, concentrated ammonium hydroxide is provided in the installation reagent kit. Additional quantities should be purchased from a local supplier. Small DNA collection vials with a rubber-lined screw cap are available from Applied Biosystems (Part No. 400048) or can be purchased from a local source. However, use Teflon-lined caps with the vials because the rubber-lined caps can leach contaminants into the DNA-ammonium hydroxide solution. They can be ordered from Wheaton: Part Number 240408, size 13-425.

## The Double Syringe Method (for manual ammonia cleavage)

1. Attach an empty luer tip syringe, with plunger fully inserted, into one end of the synthesis column.

***IMPORTANT*** *The concentration of ammonia is critical. Use fresh, concentrated ammonium hydroxide that has been opened less than one month.*

2. Load 2 ml of conconcentrated ammonia in another luer tip syringe and attach to the other end of the column

3. Holding a syringe in each hand, carefully inject the reagent through the column to the empty syringe and return the reagent through the column several times.

4. Allow it to stand for at least one hour at room temperature.

5. Drain all of the reagent into one syringe, detach and eject contents carefully into an appropriate vial for heating to achieve complete deprotection.

***IMPORTANT*** *Use a tightly sealed DNA collection vial that can withstand positive pressure. The vial must also have a Teflon-lined cap. Rubber-lined caps have contaminants that leach out of the cap liner during base deprotection.*

*Remember that the product DNA is now in solution and no longer bound to the support. Save the column until the cleavage is confirmed.*

6. If you are using standard phosphoramidites, the exocyclic amine base-protecting groups (benzoyl and isobutyryl) are removed by treating the vial of DNA at 55° C for 8 to 15 hours.

   If you are using FOD phosphoramidites, remove the exocyclic amine base-protecting groups (dimethylformamidine and isobutyryl) by treating the vial of DNA at 55° C for 1 hour or by allowing it to remain at room temperature for 8 hours.

   In either case, longer treatment is advisable if the ammonium concentration is questionable.

7. After completing deprotection, cool the ammonium hydroxide-DNA solution and perform the appropriate analysis or purification step:
   a. When purifying by OPC, dilute the DMT-on oligonucleotide with water and then load it directly onto the cartridge. No other preparation is needed. For details, refer to "Oligonucleotide Purification Cartridge (OPC)," in Section 6.
   b. If the DMT group was removed previously as a part of the synthesis cycle, the DNA is ready for analysis and/or purification by PAGE or ion-exchange HPLC. Analysis and purification procedures are discussed in User Bulletin 13 (Revised).
   c. When purifying by trityl-specific, reverse-phase HPLC, remove the ammonia by vacuum. To prevent accidental detritylation, keep the solution basic by adding one drop of distilled triethylamine every 10 minutes. Also, avoid heating the sample.

8. After collection and concentration of the product from reverse-phase HPLC, perform the following procedures:
   a. Detritylate the dried sample by dissolving it in 200-500 µl of 80% acetic acid. Since the acetic acid is an aqueous solution, the trityl cation will react with water and form tritanol and will not give an orange color.
   b. After 20 minutes, add an equal volume of 95% ethanol and lyophilize the sample. The dried sample is lyophilized from ethanol until no acetic acid remains. The hydrolyzed DMT group and remaining salts can be removed from the vial by methods such as the OPC desalting procedure, discussed in User Bulletin 59.

# Dimethoxytrityl (DMT) Cation Assay

## Introduction

Determining the stepwise yield of coupling reactions during synthesis is a convenient indicator of the performance of an automated DNA synthesizer. The standard assay for determining stepwise yield is to spectrophotometrically measure the amount of DMT cation liberated at each 5'-deprotection step in the synthesis process. (The DMT group is the 5'-hydroxyl protecting group.) All Applied Biosystems DNA synthesizers can automatically collect, with the aid of a fraction collector, the solution containing the DMT cation during the detritylation step of the synthesis cycle. Because the group is completely ionized in the acidic solution in which it was cleaved, the yield calculation can be very precise (± 0.5%) by measuring the DMT cation at 498 nm.

This assay is valid only when used in conjunction with a synthesis cycle utilizing a capping procedure that quantitatively blocks unreacted oligonucleotide chains. If these chains are not capped, the assay will give higher, incorrect yield data due to the coupling and subsequent detritylation of the deletion sequences.

If the DMT cation measures greater than 98%, the instrument is probably performing properly. Measurement of the DMT cation will detect improper flow rates or "wet" acetonitrile. Each can decrease the stepwise yield by as much as 5%—a figure that is difficult to detect visually. Also, when a problem in the synthesis process has occurred, whether instrument-related or chemical in nature, it is usually much easier and quicker to detect if the DMT cation results have been quantified and documented.

## The Assay Procedure

It is difficult to have a standard procedure for the DMT cation assay because of the different fraction collectors, spectrophotometers, and other equipment used. We find the ISCO Retriever II, with a four-column adapter, suitable for two- and four-column instruments. Both can accommodate various test tube sizes, such as a graduated 15 ml tube (VWR No. 21054-008). Each sample should be diluted with proper mixing to 10 ml with 0.1 M toluene sulfonic acid monohydrate in acetonitrile. The samples are then mixed and the absorbance read at 498 nm.

Quantitatively, the stepwise yield is determined by dividing the last absorbance value, or the lowest, by the second detritylation, or the highest. This value represents the overall yield. When it is multiplied exponentially by the reciprocal of the number of couplings, the stepwise yield is obtained. The first base detritylation is not relevant because no coupling has occurred yet. The DMT absorbance of each subsequent base should show a steady decline. Rising or fluctuating absorbance values may indicate synthesis problems or faulty assay technique.

Table A6-2 shows theoretical data from an assay of the synthesis of a 15-mer in which the yield at each step was exactly 98.0%. In this simplified example, the average stepwise yield is equivalent to the result calculated from the overall yield. In actual cases, these numbers will differ slightly. Since the DMT assay depends upon small differences between large numbers, there are significant statistical fluctuations.

Expected stepwise yields from this assay should be in the range 98% ± 0.5%. Yields below 97% indicate possible synthesis difficulties that may affect final product quality. However, stepwise yields in the expected range *do not* ensure product quality. There are factors that occasionally may result in inadequate quality despite the fact that stepwise yields are high. Hence, there can be false positives when using this assay alone.

The only way to confirm product quality is to evaluate the crude oligonucleotide either by PAGE or one of the several HPLC procedures. The DMT assay used in conjunction with either PAGE or HPLC will ensure product quality while minimizing the time required for analysis and purification.

When time does not permit reading every sample, perform a visual inspection to determine any gross changes.

## Table A6-2
## Theoretical Results from the DMT Cation Assay

### DMT Yield Sample Calculation

| No. | Base | Absorbance |
|---|---|---|
| 1 | Support T | |
| 2 | A | 0.289 |
| 3 | G | 0.274 |
| 4 | C | 0.269 |
| 5 | T | 0.262 |
| 6 | G | 0.258 |
| 7 | A | 0.251 |
| 8 | A | 0.247 |
| 9 | C | 0.243 |
| 10 | T | 0.243 |
| 11 | G | 0.242 |
| 12 | C | 0.233 |
| 13 | T | 0.226 |
| 14 | A | 0.221 |
| 15 | G | 0.217 |
| 16 | C | 0.210 |
| 17 | A | 0.204 |
| 18 | G | 0.198 |

Overall yield = last or lowest/second or highest
$0.198/0.274 = 0.723$ (72%)

Stepwise yield = overall yield$^{1/couplings}$
$0.723^{1/16} = 0.98$ (98.0%)

# OPC (Oligonucleotide Purification Cartridge)

## Introduction

OPC (Part No. 400771) is a rapid purification cartridge used specifically for synthetic DNA. It provides the level of purity required for the common applications of synthetic DNA. Using OPC eliminates the need to purify by time-consuming and labor-intensive polyacrylamide gel electrophoresis (PAGE) or HPLC. If you currently only desalt your synthetic oligonucleotides, you can now use OPC to desalt *and* purify in less time than it takes to desalt alone.

OPC is fast, easy to use and delivers consistent results. The oligonucleotide is synthesized with the 5' DMT left on (DMT On). Complete purification, from the deprotected, crude oligonucleotide to its use in an experiment, requires only 20 minutes.

After completing deprotection and cleavage, an aliquot of the crude oligonucleotide in ammonia is diluted with water and then loaded directly onto the OPC cartridge. This eliminates the need to evaporate the sample prior to purifying. The remaining portion of the crude synthesis can then be stored in ammonia either refrigerated or at -20°C without harming the DNA.

Detritylation is also done on the OPC, eliminating the need for post-purification work-up. The purified oligonucleotide is eluted as a trityl-off species in a volatile media (20% acetonitrile/water) and quantified spectrophotometrically. Only the volume containing the amount required for the experiment need be evaporated. The reminder can be stored at -20°C for later use. Once dry, the pure oligonucleotide can be redissolved in any appropriate media and used in your experiment. The product obtained from OPC is high-purity DNA that can be used with complete confidence.

The OPC purification protocol appears at the end of this section. Note that for sequences greater than 40 bases long, you should perform an additional washing step, number 9a. This adds several washes with dilute ammonia to remove shorter sequences that had trityl-protecting groups attached and thus were co-purified with the desired product.

Dilution of the ammonia sample with water prior to loading affects the capacity of the OPC cartridge to bind DNA. While ammonia confers a denaturing effect, minimizing secondary structures that hinder purification, it also decreases capacity of the oligonucleotide to bind to the OPC medium. For maximum capacity, use an equal volume of water to dilute the ammonia sample. This will increase the OPC capacity up to 10 odu of purified oligonucleotide.

The OPC purification process can be facilitated with the use of any one of a number of commercially available vacuum manifold devices. These are designed to process many solid-

phase cartridges concurrently. The operator simply delivers the reagents in reservoirs (syringe barrels) mounted on the cartridge and directs the device to collect the cartridges' effluents or deliver them to waste.

For multiplexed OPC purification, we have found the Analytichem "Vac Elut" sample processing stations to be especially effective and appropriate. They are capable of conducting up to 10 or 24 OPC purifications in approximately 20 minutes, using the same reagent deliveries as the manual procedure. These devices require less manipulation, give more consistent results, and are far faster than the manual OPC procedure.

For concerns about depurination and OPC purification, please refer to the lysine treatment detailed in User Bulletin No. 52.

# OPC Purification Protocol

## Solutions Needed

- HPLC grade acetonitrile, 5 ml
- 2.0 M triethylamine acetate (Part No. 400613), 5 ml
- Deionized water, 20 ml
- Dilute ammonium hydroxide, 15 ml (1:10 dilution of conc. ammonium hydroxide in deionized water)
- 2% Trifluoracetic acid in deionized water, 5 ml (Neat TFA Part No. 400137)
- 20% v/v acetonitrile/deionized water, 1 ml

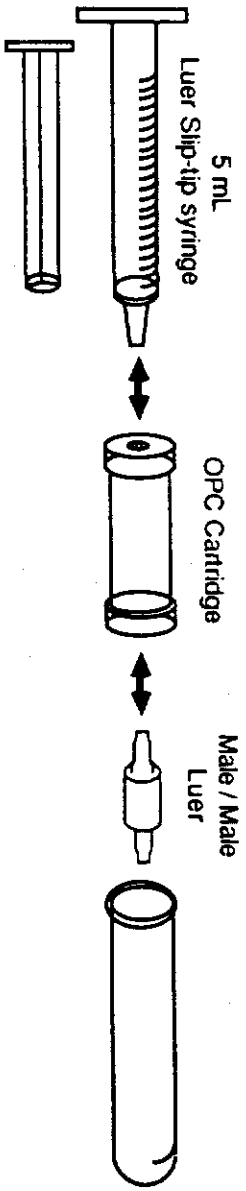


Figure VI-1
OPC Apparatus

## Protocol Procedure

1. After completion of a trityl-on synthesis, cleave the oligonucleotide from the support and deprotect.

2. Connect an all-polypropylene syringe (Aldrich Zl 1686-6) to one end of the OPC column and a male-to-male Luer tip to the other end. See figure to the left. Make sure all fittings are snug.

3. Flush the cartridge with 5 ml HPLC-grade acetonitrile, followed by 5 ml 2.0 M triethylamine acetate.

*NOTE* *Keep the flow rate at 1 to 2 drops per second for all reagent additions.*

4. Dilute the ammonium hydroxide solution containing the cleaved, deprotected, "trityl-on" crude oligonucleotide with one-third volume of deionized water.

5. Load 20-40 odu of the above solution into the syringe and then gently push it through the cartridge, saving the eluted fraction. Reload this fraction, and again push it through the cartridge.

   This will load 1 to 5 odu of the trityl oligonucleotide (depending on length, sequence, and synthesis quality) onto the cartridge. This final eluted fraction in ammonia can be stored at -20° C and put through another cartridge until exhausted of trityl oligonucleotide, if desired.

6. Flush cartridge with 3 × 5 ml dilute ammonium hydroxide.

7. Flush cartridge with 2 × 5 ml deionized water.

8. Detritylate the OPC-bound oligonucleotide with 5 ml of the 2% trifluoroacetic acid solution. Gently push ~1ml through the cartridge, incubate for 5 minutes, and then flush the remaining TFA solution through the cartridge.

9. Flush cartridge with 2 × 5 ml deionized water.

9a. For sequences ≥40 bases, add this step. Flush cartridge with 1 × 5 ml dilute ammonium hydroxide, then 2 × 5 ml deionized water.

10. Elute the purified, detritylated oligonucleotide by flushing the cartridge with 1 ml of the 20% acetonitrile solution.

11. Evaporate to dryness an aliquot of the eluate from Step 10 and dissolve in water to determine the odu's at 260 nm.

12. Store any unused OPC-purified oligonucleotide as a dry solid at -20° C.

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| UB 17 - | Annotated Bibliography of Publications Involving Oligonucleotides made with Applied Biosystems DNA Synthesizers and/or Chemicals |
| UB 49 - | Aminolink 2™ |
| UB 50 - | HPLC Purification and Processing of 10-Micromole Synthetic Oligonucleotides |
| UB 51 - | OPC™ Purification of Long Oligonucleotides |
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| UB 58 - | Sulfurization With TETD: Phosphorothioate Oligonucleotide Synthesis Via Phosphoramidite Chemistry |
| UB 59 - | New Applications for the Oligonucleotide Purification Cartridge |
| UB 61 - | 40-nanomole Polystyrene: New Highly Efficient DNA Synthesis Columns |

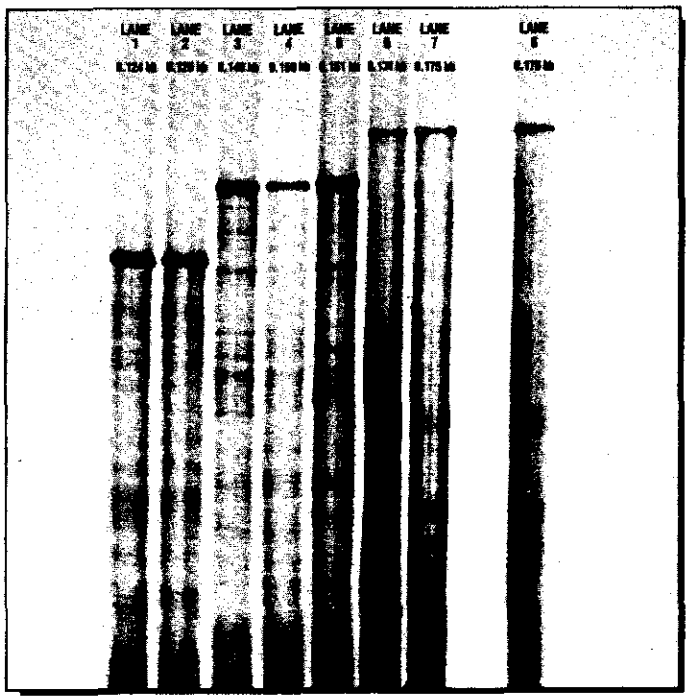
*As manuals are revised, the material provided in User Bulletins is gradually incorporated into a manual. For this reason, the User Bulletins provided with a manual are not in any particular number order and provide information not in the manual.

Applied Biosystems

# USER BULLETIN

DNA SYNTHESIZER
MODEL 380 / 381

Issue No. 13-Revised
April 1, 1987



# THE EVALUATION AND PURIFICATION OF SYNTHETIC OLIGONUCLEOTIDES

$15.00

**Inside front cover:**

The *Evaluation and Purification of Synthetic Oligonucleotides* is one of a continuing series of User Bulletins produced by Applied Biosystems, Inc. for its DNA synthesizer users. All rights are reserved. Permission to photocopy for personal use is hereby granted. This publication or any portion of it may not be reproduced for sale without the express permission of Applied Biosystems, Inc.

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**A note about the use of the word *oligonucleotide.***

In the interest of clarity and simplicity, the several authors and editors of this Bulletin have chosen to use the word *oligonucleotide* as having no specific reference to the *length* of the synthesized product. It is becoming preferred in the literature to use *oligonucleotide* when referring to a product *under twenty bases* and *polynucleotide* for a product *over twenty bases*. To have followed the preferred usage in this Bulletin would have frequently created awkward and confusing sentences.

**About the cover:**

An autoradiogram of kinased crude reaction mixtures of long oligonucleotides. From left to right: 124-, 125-, 149-, 150-, 151-, 174-, and 175-*mers* synthesized on an Applied Biosystems' Model 381A DNA synthesizer.

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# FORWARD

When we conceived *'User Bulletin 13'* in mid-1984, we were responding to the fact that users of our DNA synthesizer were spending most of their time in post-synthesis work. We designed this User Bulletin to begin the process of educating ourselves and our users how best to evaluate a synthesis and then to purify the desired product. What we did not know was the demand for such a document; our first printing was consumed in six short weeks. Through two additional printings, the demand has continued very strong.

Realizing this, we continued our work into the analysis and purification of DNA; this publication is the result. What we have learned since 1984 has not been from the study of synthesis alone. It has been the synergism of our development of instruments to automate DNA extraction (our Model 340A) and DNA sequencing (our Model 370A), as well as the continuing effort in DNA synthesis. These developments have all relied on improvements in and a better understanding of DNA analysis and purification. As an example, we needed to develop an HPLC method for the analysis of digested DNA, obtained from the Model 340A Extractor, to ascertain the degree of base modification, if any. This research led to the chemical authenticity assay for synthetic DNA found in this revised Bulletin.

What we have learned has also come from the more than one thousand users of our DNA synthesizers world-wide. And, the learning process for us is continuing. We are in the initial stages of investigating the biological activity of synthetic DNA; we expect interesting preliminary results will soon be published. You can be assured that, at Applied Biosystems, we will continue to provide the means to make your molecular biology research simpler and easier.

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# INTRODUCTION

## PURPOSE OF THE BULLETIN

DNA synthesis has become a reliable, economical and relatively simple technology due to the advances in synthesis chemistry and subsequent automation. Oligonucleotide synthesis that once took weeks, can now be accomplished in a matter of hours. Intermediate purification is no longer necessary due to solid phase synthesis. Consequently, there is heavy reliance on the synthesis chemistry to consistently couple nucleotides at each cycle and on purification methods to isolate the pure oligonucleotide from the crude reaction mixture.

At Applied Biosystems, we are committed to bringing expert technical support to our users worldwide. This support includes the full spectrum of DNA chemistry, from basic organic chemistry to the investigation of the biological activity of the synthetic product. This publication contains a collection of methods used to analyze and purify synthetic oligonucleotides. We believe them to be the best currently available. They also represent our continuing effort to make DNA synthesis an even more efficient and reliable technology.

Since many of the purification methods are more difficult and time consuming than the actual synthesis, it is advisable to become familiar with the options that are available in developing a post-synthesis strategy. By accurately predicting what is necessary to obtain oligonucleotides of sufficient quantity and quality, one can avoid costly errors. Additionally, there are often alternative methods for achieving analysis or purification. By evaluating the various methods described in this Bulletin, one can tailor a strategy which is optimal for specific applications.

For all syntheses, a thorough analysis of the oligonucleotide is advisable. Factors to consider when devising strategies for analysis and purification are: the oligonucleotide sequence, the amount necessary for a particular application, the equipment necessary for the chosen method, and time constraints. All methods discussed in this Bulletin (except as noted) have been successfully used by Applied Biosystems DNA research chemists as well as by most of our users.

## SELECTION OF A SYNTHESIS METHOD

The synthesis method used has a dramatic effect on the amount of purification required to obtain high quality product. Before the advent of solid phase methods, syntheses were performed *in solution* where the product of each reaction required purification (there are four reactions per synthesis cycle in the phosphoramidite chemistry). Solid phase synthesis eliminates the necessity for intermediate purification because the unreacted reagents are removed by simple filtration. In solid phase synthesis, the 3′-nucleoside is anchored to an insoluble support. Propagation is achieved by delivering synthesis reagents to the support in a stepwise fashion, determined by the desired sequence and the chemistry. The solid phase methodology has facilitated development of automated DNA synthesizers as well as a simple manual synthesis technique[1].

## The Advantages of Automation

Automation offers many advantages over manual solid phase synthesis. The most important are associated with throughput and yield. Instruments can work 24 hours a day. In addition, with the true simultaneous synthesis available from Applied Biosystems, the number of oligonucleotides synthesized on a daily basis can easily support the needs of most laboratories. The phosphoramidite chemistry, the most widely used method for oligonucleotide synthesis, requires very precise synthesis conditions which are more easily achieved in automated instruments. Therefore, the high yields possible with this chemistry are more consistently realized.

## Phosphoramidite Chemistry

The phosphoramidite method[2] has become the synthesis chemistry of choice. In comparison with other approaches, the inherently high coupling yields (routinely 98% ± 0.5%) result in a product which is easily purified. However, the phosphoramidite chemistry alone does not guarantee good results. The key to consistent, high quality product from an automated instrument is the synthesis cycle. Every step in a cycle is important to produce good results. At Applied Biosystems, we have the most experience in cycle development and optimization.

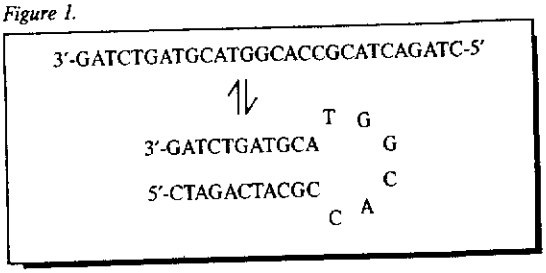
One important factor that ensures ease of purification is the capping step. The crude reaction mixture collected at the conclusion of each synthesis contains different lengths and sequences of oligonucleotide. The major species is the desired product. There are also small quantities of deletion sequences that range in size from one base to lengths one base shorter (the *n-1 mer*) than the desired product.

These deletion sequences, which failed to undergo coupling, must be chemically capped during each base addition cycle. Without capping, they would have coupled during subsequent steps to form *n-1 mers* which create two significant problems. First, in subsequent coupling reactions, these uncapped deletion sequences can compete with the full length oligonucleotide for phosphoramidites. Secondly, the separation of *n-1 mer* oligonucleotides from the desired product is more difficult than the separation of truncated oligonucleotides that are much shorter in length. Clearly, efficient capping during synthesis simplifies subsequent purification.

# OLIGONUCLEOTIDE SEQUENCE

It is important to consider the effect that the sequence of an oligonucleotide has on its chromatographic, electrophoretic and biochemical behavior. If possible, avoid sequences that might result in secondary structures. Even though such sequences are synthesized correctly, the secondary structures they are likely to produce may inhibit purification or affect biological activity in subsequent experiments. If there is a choice, sequences which have a total purine concentration significantly greater than 50% should be avoided. There is a high probability of side reactions which will be discussed later. Additionally, self-complimentary or palindromic sequences also should be avoided. These enhance the possibility of intrastrand base pairing or secondary structures which could affect both purification and biochemical activity. An example of secondary structure is shown below.

*Figure 1.*




*If there are complimentary regions in its sequence a single stranded oligonucleotide has the ability to fold back on itself to form the structure shown.*

# SYNTHESIS YIELD

It is possible to estimate how much product one can expect from a synthesis. The yield is determined by the amount of the initial support-bound nucleoside and the subsequent length of the oligonucleotide.

The flexibility of all Applied Biosystems DNA synthesizers allows the choice of three different synthesis scales without any hardware changes: small scale (approximately 0.2 micromole of starting nucleoside), 1 micromole and 10 micromole. The amount of final product desired determines the synthesis scale. The economical small scale is widely used for a variety of applications in molecular biology.

When relatively large quantities of oligonucleotides are needed, the 1-micromole scale can produce a good supply of oligonucleotide. The 10-micromole scale is used primarily for physical studies (such as drug-DNA interactions) or for commercially produced probes and primers. Persons needing to do 10-micromole syntheses of sequences greater than 60 bases should contact an Applied Biosystems Product Specialist as the synthesis of these compounds is very difficult. Table 1 gives the approximate crude product yield at each scale when all synthesis parameters are optimized.

*Table 1*

| SCALE | YIELD OF A 20-MER | YIELD OF AN 80-MER |
|---|---|---|
| Small Scale | 0.6 - 0.8 mg | 2.4 - 3.5 mg |
| 1 μmol | 3.8 - 4.5 mg | 13.0 - 16.0 mg |
| 10 μmol | 33.0 - 36.0 mg | Contact Applied Biosystems |

*Approximate crude product yields of typical oligonucleotides for each scale of synthesis.*

# EVALUATION OF THE CRUDE PRODUCT

## INTRODUCTION

This section presents information on crude product evaluation before analysis by polyacrylamide gel electrophoresis (PAGE) or high performance liquid chromatography (HPLC). Post-synthesis processing, completed before analysis and purification, includes complete deprotection of the oligonucleotides, quantification of yield and possible de-salting.

## DEPROTECTION

Complete deprotection of an oligonucleotide includes elimination of the protecting groups on the phosphate groups, the exocyclic amino groups on the bases and the 5′-hydroxyl moiety. Incomplete deprotection of synthetic oligonucleotides will adversely affect analysis and purification as well as biological activity.

The method for phosphate deprotection depends on whether the synthesis was performed with β-cyanoethyl or methyl protected phosphoramidites. Methyl phosphoramidite oligonucleotides require treatment with thiophenol for deprotection. This step is performed prior to cleavage of the product from the support with concentrated ammonium hydroxide.

Although the methyl groups can be cleaved from the internucleotide phosphates by ammonium hydroxide, serious problems may result. Our studies have shown that demethylation with ammonia leads to the methylation of thymidine residues at the N-3 position[3]. See page 42 for details. β-cyanoethyl phosphoramidite oligonucleotides require only treatment with concentrated ammonium hydroxide to simultaneously cleave the product from the support and deprotect the phosphate groups.

Deprotection of the exocyclic amines of deoxyadenosine, deoxycytidine, and deoxyguanosine is achieved by incubating the sample in concentrated ammonium hydroxide at 55°C for a minimum of eight hours. It is important that only fresh ammonium hydroxide be used because a very strong base is required to remove the exocyclic amino protecting group of deoxyguanosine (isobutyryl). We recommend purchase of ammonium hydroxide in small bottles (250 mL) and storage at 4°C. Each bottle should be dated when opened, sealed with paraffin film after use, and re-stored at 4°C. Bottles that are open more than one month or that have warmed to room temperature should not be used for deprotection. They may be used for other applications such as buffer preparation, however.

Phosphoramidite synthesis employs a capping scheme which involves acetylation of the 5′-hydroxyl molecules which have failed to undergo coupling. These truncated sequences remain capped during synthesis. The acetyl groups are later removed during base deprotection in ammonia resulting in free 5′-hydroxyl groups.

The product-length molecules, which are not acetylated, have 5′-hydroxyls which are protected by dimethoxytrityl (DMT) groups. The DMT group can be automatically cleaved using the instrument or left uncleaved if trityl-on reverse phase HPLC analysis or purification methods are used.

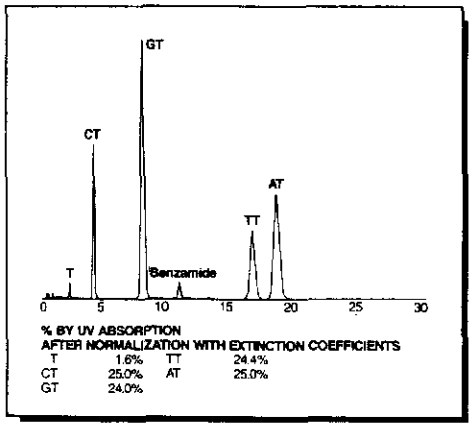
Manual removal of the DMT group is achieved easily by treatment of a dried oligonucleotide with 80% acetic acid (see Appendix 2.1 for the exact protocol). The existence of the DMT group does not interfere with quantification by UV absorbance. However, it blocks the phosphorylation of the 5′-hydroxyl during analysis by autoradiography. It should be noted that a free 3′-hydroxyl is created when the synthesized oligonucleotide is cleaved from the support; no further deprotection is required.

# COMPOSITION OF THE CRUDE REACTION MIXTURE

The crude mixture, dissolved in ammonium hydroxide from the final deprotection, contains a variety of oligonucleotides along with some extraneous ammonia salts from the removed protecting groups. The oligonucleotides in the crude mixture include the desired full length product, the truncated oligonucleotides resulting from incomplete coupling and subsequent capping, and strand scission product oligonucleotides that were cleaved during ammoniolysis. Depurination, from a number of sources, results in strand scission during ammoniolysis at apurinic sites.

Syntheses that have mixed-base sites produce a more complex crude reaction mixture. Instead of having one product in the mixture, there are many. This presents problems in analysis and purification. One important aspect in the synthesis of mixed-base oligonucleotides is competitive coupling which occurs at each mixed-base site. Competitive coupling results in equivalent amounts of the desired bases at each degenerate site. Without competitive coupling, the relative proportions of each oligonucleotide in the product mixture will be incorrect, which will affect purification and subsequent use. At Applied Biosystems, we test every DNA synthesizer for competitive coupling at mixed-base sites. Figure 2 is a chromatogram of the 4-base degenerate dimer d[(AGCT)T] that shows the relative peak areas after normalization by extinction coefficients.

*Figure 2*




*A chromatogram showing the relative proportions of each dimer in the synthesis of the degenerate dimer when the bases are added simultaneously.*

## De-Salting the Sample

The organic salts include benzamide, isobutrylamide and ammonium acetate. Although de-salting the sample may be unnecessary for some applications, one may wish to consider de-salting the crude sample before analysis and purification. There are several methods of de-salting oligonucleotides including ethanol precipitation, size exclusion chromatography and reverse phase chromatography. The choice of method depends on the quantity of oligonucleotide to be de-salted, the materials available and the time available. Some methods of de-salting have the added value of being pre-purification methods as well.

Perhaps the quickest and easiest de-salting method is to use a commercially available C-18 reverse phase cartridge[4] (like the Sep-Pak or NENsorb cartridges). These cartridges are disposable and have Luer fittings which easily attach to syringes. In Appendix 2.2, there is a protocol for using Sep-Pak cartridges for general de-salting. Appendix 2.3 is a Sep-Pak protocol that utilizes an acetonitrile gradient scheme which has been optimized for the separation of the tritylated product from the 5'-hydroxyl oligonucleotides.

The latter trityl-on protocol results in purification that may be satisfactory for some applications. This pre-purified material can also be loaded on a gel for final purification.

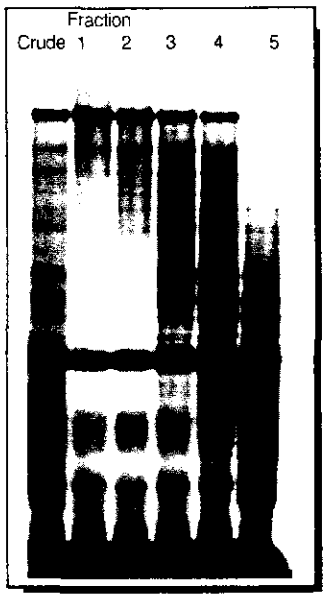
It is important to note that the cartridges above have a loading capacity that is generally less than the synthetic yield of an average small-scale synthesis. Therefore, several cartridges must be used to de-salt the entire sample.

Size exclusion chromatography is an excellent de-salting method that also results in partial size fractionation of the crude mixture as demonstrated in Figure 3. The crude reaction mixture is loaded onto a size exclusion column (Sephadex G-50 or G-75, 10 mL), collected as 1-mL fractions and analyzed. The advantage of using such a method is that an entire synthesis can usually be loaded onto one column.

Ethanol precipitation is a quick and efficient method for de-salting with the inherent ability to purify large quantities of oligonucleotide. Therefore only one precipitation is needed to de-salt a typical synthesis, and no carrier RNA is required. The drawback to this method is that recovery and de-salting are somewhat less reproducible than other methods. An advantage is that some of the lower molecular weight species of truncated molecules may not precipitate, so partial size fractionation can be achieved. A protocol can be found in Appendix 2.4.

Sequences greater than 50 bases have a higher concentration of salt due to the increased amount of removed protecting groups. For almost any manipulation, de-salting should be done. Fragments less than 50 bases may not require de-salting prior to use.

*Figure 3*




*An autoradiogram comparing a crude reaction mixture and the collected fractions of the mixture separated by gel exclusion chromatography. Note the enrichment of the* n-mer *oligonucleotide in fractions 1 and 2.*

# DIMETHOXYTRITYL (DMT) CATION ASSAY

## Introduction

Determining the stepwise yield of coupling reactions during synthesis is a reliable indication of final product quality. Yield data are also excellent for measuring the performance of an automated DNA synthesizer.

The standard assay for determining stepwise yield is to spectrophotometrically measure the amount of DMT cation (the DMT group is the 5′-hydroxyl protecting group) liberated at each 5′-deprotection step in the synthesis process. All Applied Biosystems DNA synthesizers can automatically collect, with the aid of a fraction collector, the solution containing the DMT cation during the detritylation step of the synthesis cycle. Because the group is completely ionized in the acidic solution in which it was cleaved, the yield calculation can be very precise (± 0.5%) by measuring the DMT cation at 498 nm.

This assay is valid only when used in conjunction with a synthesis cycle that utilizes a capping procedure that quantitatively blocks unreacted oligonucleotide chains during the synthesis cycle. If capping is not used, the assay will give higher, incorrect yield data due to the coupling and subsequent detritylation of the deletion sequences.

Although the DMT cation assay is time consuming, it is an excellent means to determine the performance of a DNA synthesizer. It is an important assay for determining synthesis efficiency, solely from a coupling point of view. For instance, if the DMT cation measurement is greater than 98% when using controlled pore glass as the solid phase support, the instrument is probably performing properly. Measurement of the DMT cation will detect improper flow rates or "wet" acetonitrile. Each can decrease the stepwise yield by as much as 2 to 5% — a figure that is virtually impossible to detect visually. Also, when a problem in the synthesis process has occurred, whether instrument related or chemical in nature, it is usually much easier and quicker to detect if the DMT cation results have been quantified and documented.

## The Assay Procedure

It is difficult to have a standard procedure for the DMT cation assay because of the different fraction collectors, spectrophotometers, and other equipment used. We find the ISCO Cygnet fraction collector convenient for one-column instruments, and the ISCO Retriever II, with a four-column adaptor, suitable for three-column instruments. Both can accommodate various test tube sizes. We prefer a graduated 15-mL tube (VWR No. 21054-008). Graduated tubes, though more expensive, give more reliable results since dilutions are done on the fractions obtained from the instrument.

Dilutions are required because the absorbance of the DMT cation directly from the instrument is too concentrated for accurate results. Deviations from Beer's Law at these concentrations cause significant errors which result in an inaccurate assay.

Each sample should be diluted with proper mixing to 10 mL with 0.1 M toluene sulfonic acid monohydrate in acetonitrile. An aliquot is taken from each sample and diluted again to give a final dilution of approximately 60-fold (assuming 2.5 mL of original solution). The samples are then mixed and the absorbance read at 498 nm.

The number of micromoles of DMT cation is determined for each step using the following calculation:

$$\text{Micromoles of DMT} = \frac{(A)\quad(\text{Dilution})\quad(\text{Volume})}{e}$$

Where:

A = absorbance at 498 nm

Dilution = second dilution

Volume = final volume after the first dilution (in mL)

e = extinction coefficient at 498nm

= 70 mL/micromole

As an example, if the absorbance is 0.48, the equation would be as follows:

$$\text{Micromole} = \frac{(0.48)\quad(15.0)\quad(10.0)}{70}$$

= 1.03 micromole of DMT cation

= 1.03 micromole of nucleoside

Table 2 details two procedures for measuring the stepwise yields by the DMT cation assay. The first requires a standard spectrophotometer that measures absorbance at 498 nm. The second requires a spectrophotometer with a 2-mm absorbance cell that is dipped into the diluted sample. Use of this small cell eliminates the need of the second dilution, but individual calibration of each cell is necessary. The second procedure will save valuable time in laboratories that measure many DMT samples.

*Table 2*

***Procedure 1***

1. Collect trityl fractions in 15-mL graduated tubes.
2. For a one-micromole scale synthesis, dilute each trityl collected to 10 mL with 0.1 M toluene sulfonic acid (monohydrate) in acetonitrile.
3. Mix well with a vortex mixer or by bubbling air through the solution with a Pasteur pipette.
4. Dilute further by adding 150 uL of this solution to 2.75 mL of 0.1 M toluene sulfonic acid in acetonitrile in a 1-cm path length cuvette.
5. Mix well.
6. Read the absorbance of each sample at 498 nm.
7. Record data and perform calculations as shown above.

***Procedure 2***

1. Collect trityl fractions in 15-mL graduated tubes.
2. For a one-micromole scale synthesis, dilute each trityl collected to 10 mL with 0.1 M toluene sulfonic acid (monohydrate) in acetonitrile.
3. Mix well with a vortex mixer or by bubbling air through the solution with a Pasteur pipette.
4. Read the absorbance on a colorimeter equipped with a 2-mm path probe and a 490-nm filter. (Brinkman PC 800).
5. Convert to micromoles based on the calibration curve obtained for your colorimeter. This calibration curve will probably not be linear but can be fit to the equation:

$$u\mathit{mol} = ax^4 + bx^3 + cx^2 + dx$$

where a, b, c and d are constants and x is the absorbance. A programmable calculator can be used to solve this equation for any absorbance.

*Procedures for measuring the concentration of DMT cation fractions.*

Table 3 shows theoretical data from an assay of the synthesis of a 15-mer where the yield at each step was exactly 99.0%. In this simplified example, the average stepwise yield is equivalent to the result calculated from the overall yield. In actual cases, these numbers will differ slightly. Since the DMT assay depends upon small differences between large numbers, there are significant statistical fluctuations.

Expected stepwise yields from this assay should be in the range of 98% ± 0.5%. Yields below 98% indicate possible synthesis difficulties that may affect final product quality. However, stepwise yields in the expected range do not ensure product quality. There are factors that occasionally may result in inadequate quality despite the fact that stepwise yields are high. Hence, there can be false positives when using this assay alone.

The only way to confirm product quality is to evaluate the crude oligonucleotide either by PAGE or one of several HPLC procedures. The DMT assay used in conjunction with either PAGE or HPLC will ensure product quality while minimizing the time required for analysis and purification.

When time does not permit reading every sample, measurement of every fifth sample is sufficient to determine general trends in the synthesis. If the exact yield at each step is not important, the overall yield can be obtained by pooling all the samples. Perform a visual inspection to determine any gross changes. Then measure the total number of micromoles by the DMT cation assay. This number can be divided by the theoretical number of micromoles (the scale of synthesis multiplied by the number of DMT cation samples in the synthesis) to obtain the overall yield and the average stepwise yield. For example, if a value of 33.7 micromoles of DMT cation was calculated from the total amount of solution collected, from the synthesis of a 50-*mer* configured *trityl-off*, the overall yield would be $(\frac{33.7}{50})$ or 67.5% which would equate to a 99.2% stepwise yield ($0.675^{1/49}$).

## Synthesis Problems Detected by the DMT Assay

Understanding how to diagnose synthesis problems detected by the DMT cation assay can be very useful. Valuable instrument time can be saved because the user can often make repairs or more accurately describe conditions to an Applied Biosystems service engineer. Understanding the following situations will help in the diagnosis of instrument problems.

*Table 3*

***Record of Trityl Cation Data and Calculations***

| Base Added | | Absorbance | Micromoles* | (%) Step Yield |
|---|---|---|---|---|
| Support | T | .380 | 1.00 | — |
| 2 | A | .376 | .990 | 99.0 |
| 3 | T | .372 | .979 | 99.0 |
| 4 | G | .369 | .971 | 99.0 |
| 5 | C | .365 | .961 | 99.0 |
| 6 | C | .361 | .950 | 99.0 |
| 7 | A | .358 | .942 | 99.0 |
| 8 | G | .354 | .932 | 99.0 |
| 9 | C | .351 | .924 | 99.0 |
| 10 | T | .347 | .913 | 99.0 |
| 11 | G | .344 | .905 | 99.0 |
| 12 | C | .340 | .895 | 99.0 |
| 13 | C | .337 | .887 | 99.0 |
| 14 | T | .333 | .876 | 99.0 |
| 15 | T, Trityl-on | — | — | Av. = 99.0 |

*If Procedure 1 is used: Micromoles =

$$\text{Overall yield} = \frac{\text{last absorbance}}{\text{first absorbance}} = \frac{.333}{.380} = 87.6\%$$

$$\text{Stepwise yield} = (\text{overall yield})^{\frac{1}{N-1}} = (.876)^{\frac{1}{13}} = 99.0\%$$

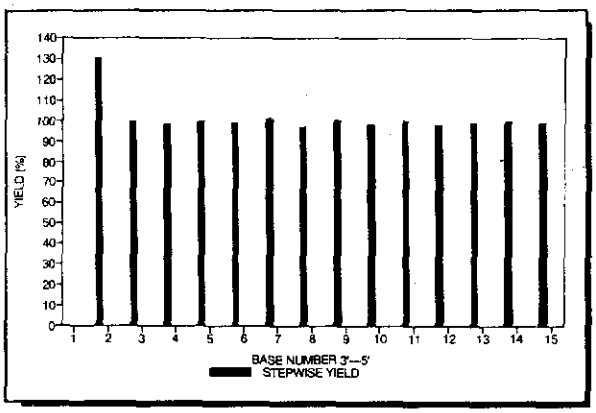
Where N = number of trityl fractions collected.

*Theoretical results from the DMT cation assay.*

Table 4 illustrates a situation often encountered when controlled pore glass is used as the solid phase support. The data reveal a first coupling yield of 130%. Actually, the DMT cation collected from the detritylation of the support-bound nucleoside is low, due to spontaneous detritylation that occurred during storage of the support. The DMT cation, cleaved in this manner, is lost in the initial washes of the synthesis cycle and is not collected in the DMT cation fraction. While this has no effect on the synthesis, it does affect the assay. When this problem occurs, it is best not to use that data in calculating the overall yield. Depending upon the scale of synthesis used, one can insert that value into the yield data.

Additionally, there are variations in the calculations that show yields greater than 100% intermittently in the synthesis. These variations are somewhat common and do not signify problems with syntheses. They are caused by incomplete mixing of the sample prior to measurement, inaccurate dilutions, and/or stray light in the spectrophotometer.

*Table 4*



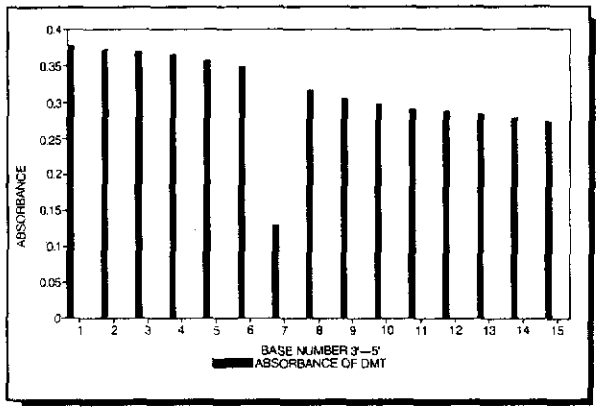

*Typical DMT cation result showing spontaneous detritylation of a CPG support.*

Table 5 depicts a synthesis that proceeds normally until the DMT cation assay reveals a low yield, immediately followed by yields consistent with the synthesis prior to the low yield. This may or may not affect product quality. There are three possible sources.

The first source could be that only a portion of the DMT cation solution was delivered to the collection tube in the low yield step. This may be caused by misalignment of the collection tube or a mechanical problem causing the solution to be delivered to the waste bottle instead of the collection tube. Neither would result in poor product quality.

The second source of the problem could be incomplete detritylation. This may be caused by a failure to deliver proper quantities of the detritylating reagent, a faulty reagent, or incomplete rinsing of the support prior to detritylation. This would affect product quality and would be detected by evaluation of the crude product.

*Table 5*



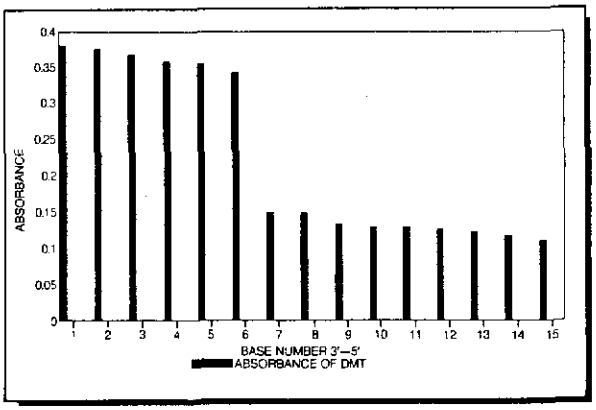

*DMT cation results depicting a low value followed by an inconsistent high value.*

The third source could be a true coupling failure combined with inefficient or faulty capping — an unlikely combination of events at only one cycle in the synthesis. This would affect product quality and could be determined with further analysis.

The data in Table 6 represent a true coupling failure with efficient capping. The amount of DMT cation liberated will remain low in subsequent synthesis cycles. The oligonucleotides, bound to the support, that failed to couple in the low-yield coupling reaction were completely capped. Thus, these oligonucleotides could not react in the subsequent coupling reactions. A failure of this type can be confirmed because a major side-product appears in an HPLC or PAGE analysis.

Table 7 shows another possible problem, a low yield at the first coupling step (the second DMT cation fraction). This indicates a problem with the phosphoramidite added during this cycle or with inconsistent delivery of either tetrazole or trichloroacetic acid. In automated synthesis, this is often the result of not purging phosphoramidite and tetrazole lines prior to synthesis. These lines are more permeable to air or moisture than the bottles. On humid days, it is necessary to purge these lines prior to synthesis if the instrument has been idle for more than six hours. Regardless of the humidity, the instrument will need to be purged if idle for more than twelve hours. All Applied Biosystems DNA synthesizers have an automated purge procedure which can be used at the start of the synthesis.

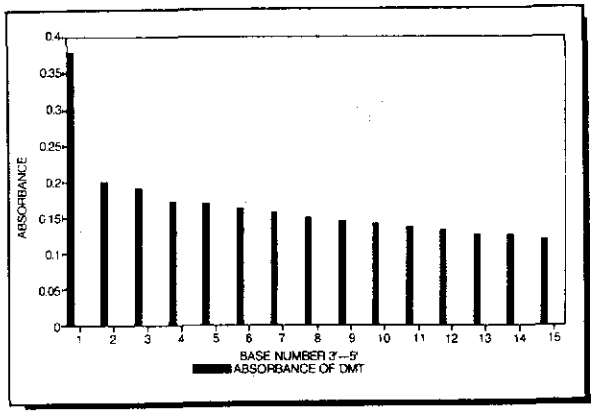
*Table 6*




*DMT cation data showing a true coupling failure with efficient capping.*

Phosphoramidites have a life span on the instrument of two weeks. Syntheses with compounds older than two weeks will have DMT cation results similar to those in Table 7. If the TCA reagent is not delivered to the column during detritylation or is of inferior quality, detritylation will not be quantitative at the level required. Subsequent DMT cation results, because the scale of synthesis is lower, will appear normal in relation to the low value.

*Table 7*




*DMT cation data showing low yields at the first coupling step.*

## Synthesis Problems Not Detected by the DMT Cation Assay

Unfortunately, the DMT cation assay will not detect several conditions that may be detrimental to the final product quality. In these instances, the DMT assay gives positive results. However, the product will be of inferior quality, or there may be no product at all.

The first problem is inefficient oxidation. During synthesis, the trivalent phosphorus internucleotide triester must be quantitatively oxidized to its pentavalent form at each cycle. If oxidation is not complete, the residual phosphite triester will be degraded by exposure to the acidic detritylating reagent which is the next step in the synthesis cycle. Figure 4 depicts what can happen. The phosphite function, because of its instability in acid, can be cleaved at any of its triester moieties to give an *n-1* oligonucleotide

bearing a 5′-hydroxyl or a 5′-phosphorus function. In either case, the DMT cation is liberated normally, giving normal results in the assay. The decrease in product quality is purely a matter of statistics. Depending upon the amount of phosphite triester remaining after each oxidation step and the size of the oligonucleotide being synthesized, the crude mixture will be very complex, and may or may not have any desired product at all.

Figure 5 illustrates the second problem, depurination, which is the cleavage of the purine bases from their deoxyribose sugars at the glycosidic bond. It is known that the glycosidic bond of deoxyribopurine compounds, especially when derivatized with chemical protecting groups, is quite unstable to acids[5]. Great care has been taken in developing detritylation conditions where the degree of depurination is minimal. Similar to the oxidation problem, it is a matter of statistics.

In oligonucleotides that have many purine bases, a low level of depurination is unavoidable. And, like the oxidation problem, there will be normal liberation of the DMT cation that will depict adequate yields at each synthesis step. Since the depurination is random, the crude oligonucleotide mixture will be much more heterogeneous than the DMT cation yield data would indicate.

Another cause of depurination is side reactions during the synthesis cycle which cause base modification that renders the glycosidic bond even more prone to cleavage[6]. Data show that there is no easy way to detect where possible depurination sites exist. The problem, like many others in DNA synthesis and purification, seems to be sequence dependent.

## Conclusions of the DMT Cation Assay

Given the above, the question of whether to spend the time to obtain good DMT cation yield data is a pertinent one. It is a useful procedure for most syntheses, though there are problems with its reliability. We believe that the DMT cation solution should be collected during each synthesis and measured to give yield data. However, if time is limited, an analysis of the oligonucleotide mixture itself is more important.

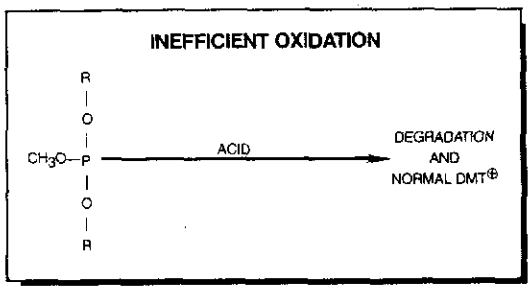
We know from our own experience and that of our synthesizer users that calculation and documentation of DMT cation yield data is very useful in the early diagnosis of instrument-related problems. It should only be used as a prediction of the quality of the oligonucleotide synthesized. Low DMT cation data will inform the user of inferior product quality much quicker than analysis of the oligonucleotide. However, yield data within the instrument's specification should only be regarded as presumptive with regard to product quality; analysis of the oligonucleotide mixture is the only way to positively confirm product quality.

# QUANTIFICATION OF THE CRUDE PRODUCT BY UV ABSORPTION

## Introduction

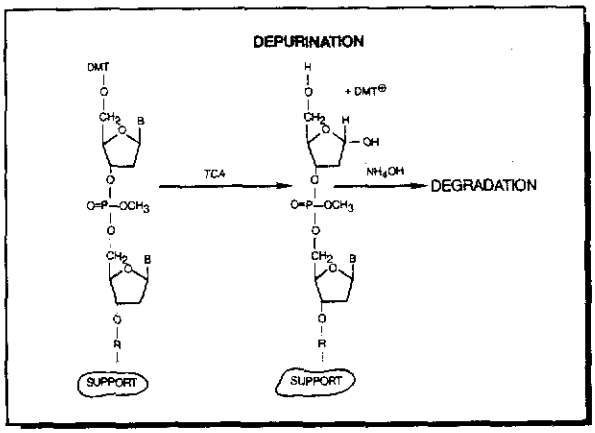
Quantification by UV absorbance is a method which is used to determine synthesis yield. The comparison between the estimated theoretical yield and the measured yield is a good prediction of synthesis quality. A low overall yield may indicate poor coupling reactions during synthesis, incomplete cleavage from the support, or inaccurate calculation from the absorbance reading.

*Figure 4*




*The problem with inefficient oxidation. The phosphate backbone undergoes degradation but there is still liberation of DMT cation.*

*Figure 5*




*The problem with depurination. The purine base is cleaved from the oligonucleotide but, again, there is still liberation of DMT cation.*

## Quantification Methods

The average absorbance maximum of the four bases is approximately 260 nm, which is why quantification is done at this wavelength. The absorbance spectra of each of the four bases are shown in Figure 6. Deoxyguanosine has a characteristic doublet in its spectrum.

Since it is laborious to calculate the expected absorbance from the base composition, an average extinction coefficient of 10,000 is used. This is a satisfactory approximation when the pH of the solution ranges from 7.0 to 7.4. The procedure for measuring the absorbance of a sample is described in Appendix 2.5. A typical spectrum of a crude 18-mer is shown in Figure 7. Oligonucleotides that are very rich in either purines or pyrimidines could have absorbance maxima above or below 260 nm, depending upon the base predominance.

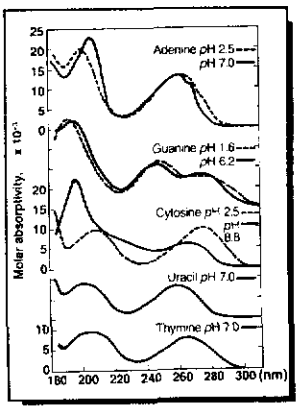
It should be noted that for a 1-micromole synthesis, the expected recovery of the crude reaction mixture in optical density units (OD) is approximately five times the length of the sequence. For example, a 20-mer synthesis should yield approximately 100 OD. The factor for small-scale synthesis columns is about 1.5. Hence, the same 20-mer would yield approximately
30 OD.

One should verify the yield of a synthesis before discarding the synthesis column; one reason for low yield could be partial cleavage from the solid phase support. The most common causes of incomplete cleavage are poor delivery of ammonia to the column or the use of ammonia which is not fresh. A manual method for treatment of a synthesis column with ammonia is described in Appendix 2.6.

# PURIFICATION AND ANALYSIS: HPLC AND PAGE

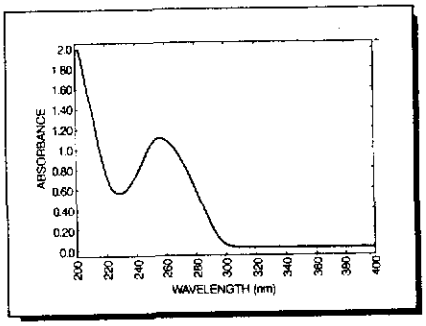
The following sections contain techniques for analysis and purification of oligonucleotides, and discussion of the interpretation of results. Presented are optimized methodologies for both HPLC and PAGE analysis and purification of oligonucleotides of up to approximately 50 bases. For longer syntheses, PAGE is generally used. However, there is ongoing research at Applied Biosystems to develop optimized separations of longer oligonucleotides by HPLC as well. Table 8 is a chart which outlines some of the advantages and disadvantages of both methods.

*Figure 6*




*The individual UV spectra of the four deoxynucleosides, and uracil.*

*Figure 7*




*The UV spectrum of a typical oligonucleotide, an 18-mer.*

*Table 8*

| | PAGE | HPLC |
|---|---|---|
| **Advantages** | • Inexpensive equipment.<br>• Can be used for any length.<br>• Multiple sequences of same size are not resolved (important for mixed-base probes).<br>• Approximate size standard can be run with sample. | • Good yields.<br>• Volatile buffer—lyophilization is fast.<br>• Quantitation is possible through integration.<br>• Data collection is fast. |
| **Disadvantages** | • Recoveries are variable.<br>• Separation takes 2-5 hours.<br>• Quantitation not reliable. | • Equipment can be expensive.<br>• Size confirmation is not good.<br>• Sequence-dependent problems with resolution of sequences larger than 40-60 bases. |

*A comparison of PAGE and HPLC analysis and purification methods.*

# EVALUATION AND PURIFICATION BY POLYACRYLAMIDE GEL ELECTROPHORESIS

## INTRODUCTION

Polyacrylamide gel electrophoresis (PAGE) is a widely used methodology for the analysis and purification of oligonucleotides. The three major steps involved in PAGE methodology are: sample preparation, electrophoresis and visualization of the gel. Since the methods for analysis vary somewhat from those used for purification, these topics are discussed separately.

## ANALYSIS

Post-synthesis analysis by PAGE is one procedure to verify synthesis quality. Because the DMT cation assay alone is not an accurate indicator, analysis of the oligonucleotide is critical to assure product quality. We recommend analysis by autoradiography of radiolabeled crude mixtures. UV shadowing can be utilized, but often it does not reveal how the synthesis proceeded. In developing an analysis strategy, it is important to realize that methods of analysis and purification differ with increasing oligonucleotide length.

### Long Versus Short Oligonucleotides: Two Sets of Analysis Conditions

PAGE can be used for oligonucleotides of any length, although there are modifications to the methodology depending on the length. The major modification is in the gel matrix. A gel of 12% polyacrylamide has the larger pores required for molecules (i.e., for oligonucleotides greater than 50 base residues) to enter the gel and migrate with adequate resolution. However, since resolution is inversely proportional to the pore size of a gel, it decreases in 12% gels as compared to 20% gels used for shorter oligonucleotides. This decrease in resolution can be overcome by the use of a thinner gel, nominally 0.4 mm.

The second modification is associated with sample size. The surface area of the sample well on a thin gel is smaller than on a thicker gel. Thus, normal loading of an oligonucleotide sample will clog the gel and remain in the well. By decreasing the amount of oligonucleotide loaded, the sample will enter the gel easily and give excellent resolution. Normally, for oligonucleotides greater than 50 bases, a 10-picomole, radiolabeled sample is loaded on a 0.4 mm thick, 12% polyacrylamide gel. For oligonucleotides less than 50 bases, 100 picomoles of radiolabeled sample are loaded on a 0.8 mm thick, 20% polyacrylamide gel (See Appendix 3.10 for a procedure for preparing a stock acrylamide-bis solution).

### Sample Preparation

Before the oligonucleotide undergoes electrophoresis, it must be quantified by optical density, sampled, de-salted if necessary, and evaporated to dryness. If autoradiography is to be used for analysis, the sample is then radiolabeled either at the 5′- or 3′-end.

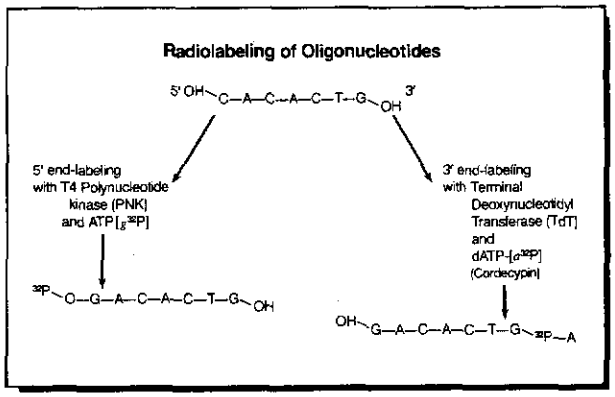
5′-end labeling is the most common method of radiolabeling. $T_4$ polynucleotide kinase is used to catalyze the phosphorylation of the 5′-hydroxyl. As shown in Figure 8, [gamma-$^{32}$P]-ATP serves as the phosphate donor. This method can be used at different scales depending on the quantity and length of the oligonucleotide to be labeled. Variations are also used for phosphorylating oligonucleotides for ligations as well as for radiolabeled probes. There are two scales needed for analysis: the 100-picomole scale for sequences that are less than 50 bases long, and the 10-picomole scale for longer sequences (See Appendix 2.7 for details).

For analyzing crude compounds, there must be an excess of ATP in the reaction for competitive labeling of each oligonucleotide species in a sample. At limited ATP concentrations, the kinase enzyme will phosphorylate oligonucleotides of approximately 10 to 20 bases before it will phosphorylate longer sequences. The addition of radiolabeled ATP, which is low in molarity, does not meet the concentration needed for efficient phosphorylation. The molar excess is achieved with the addition of unlabeled ATP.

We have developed a 3′-end labeling procedure for use when the 5′-hydroxyl has been derivatized (see Figure 8). *Oligonucleotides with a 5′-linker such as Aminolink 1,* (the Applied Biosystems adaptor molecule for the attachment of ligands to oligonucleotides[7]) can be 3′-end labeled for analysis. Terminal deoxynucleotidyl transferase adds single nucleotides to the 3′-end of the oligonucleotide. To prevent chain propagation, a radiolabeled 2′,3′-dideoxyadenosine triphosphate analogue, [alpha-$^{32}$P]-cordecypintriphosphate, is used. The 3′-end labeled sample will be one base longer than the 5′-end labeled sample as shown in Figure 9 (See Appendix 2.8 for a protocol).

There is occasionally a radioactive contaminant which is a by-product of the 3′-labeling procedure. This contaminant is frequently a type of enzyme-cordecypin complex which co-migrates with the sample and may appear on the autoradiogram as a diffuse band in the region of a 16-mer. It is recommended that a blank sample containing the constituents of the reaction without the oligonucleotide be loaded on the gel to be compared with the oligonucleotide sample.

*Figure 8*




*A comparison of 5′- and 3′- end labeling.*

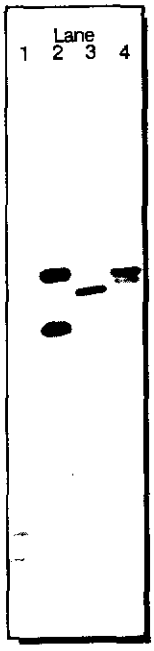
## Electrophoresis

To determine the product quality of a crude reaction mixture by analytical electrophoresis, denaturing conditions should be maintained to minimize the formation of secondary structures. Both the high concentration of urea (7M) in the gel and sample loading in formamide serve as the denaturants. It should be noted, however, that there can be secondary structure effects on the gel, even in 7M urea.

Colored tracking dyes, such as bromophenol blue and xylene cylanol blue, can be loaded on the gel with the oligonucleotide. This will help to determine the migration of the oligonucleotide not visible during electrophoresis.

After the gel has been allowed to polymerize usually for two hours or more, it is placed on the electrophoresis apparatus and the buffer (1X TBE) is added (see Appendix 3.1). Before the sample is applied, the wells must be cleaned and flushed with buffer to remove debris and urea which can affect resolution. The gel is then pre-run with a blank sample of dye-formamide for about 30 minutes at approximately 25 to 30 milliamp. Pre-running increases the temperature of the gel which helps to prevent secondary structures and serves as visible confirmation that the gel is functioning properly.

*Figure 9*




*An autoradiogram showing the differences in mobility between the 3′- and 5′-end labeled oligonucleotides of the same sequence. Lane 1 is an oligonucleotide with the 5′-end nearly blocked quantitatively by Aminolink 1. Lane 2 is 5′-Aminolink 1 oligonucleotide 3′-end labeled; notice the lower band which is a contaminant of the reaction. Lane 3 is a 5′-end labeled oligonucleotide. Lane 4 is a 3′-end labeled oligonucleotide.*

If an oligonucleotide is thought to form secondary structures, the sample should be heated to 90°C for several minutes just prior to loading. The warming of the gel and the sample serves to aid in the denaturation process. We prefer to run our gels at 60 watts constant voltage. By attaching a metal plate to the gel apparatus, the heat generated at this wattage will be efficiently dispersed. This will avoid cracking the gel plates and melting the polyacrylamide.

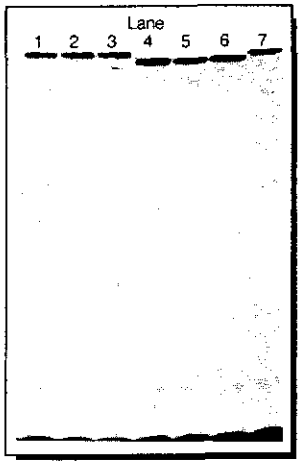
After pre-running and just prior to loading, the sample wells again are flushed to remove urea. The samples are carefully loaded on to the surface of the well. To avoid smearing or uneven loading, the sample should be a concentrated solution usually not greater than 10 uL per 1.5-cm well in analytical-size gels. It is recommended that a Hamilton-type syringe or a pulled glass capillary tube be used to load the sample. Once the samples are loaded, electrophoresis should be started immediately to avoid diffusion, again at 60 watts constant power.

Both the length and sequence of an oligonucleotide affects the mobility of DNA through the gel. The order of migration of mononucleotides is C>A>T>G, with C being the fastest[8]. Oligonucleotides of the same length but with different sequences will not migrate at the same rate. Therefore, it is not recommended to compare directly an "oligonucleotide size marker" with another oligonucleotide to verify the correct length. Figure 10 depicts the migration of different sequences of the same length in a single gel. Figure 11 shows an autoradiogram of oligonucleotides that have the same 10 bases on the 5'-end and only differ in the 3'-base. The results clearly show the effect of base composition.

## Visualization of the Gel

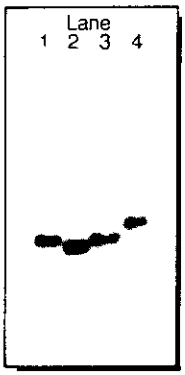
Autoradiography is used to visualize the electrophoresis of radiolabeled oligonucleotides. The radioactive oligonucleotide molecules emit beta particles which expose a piece of X-ray film placed on top of the gel. To speed up the exposure process, an intensifying screen may be used. The quality of results obtained from analyzing crude products by autoradiography is depicted in Figure 12.

*Figure 10*



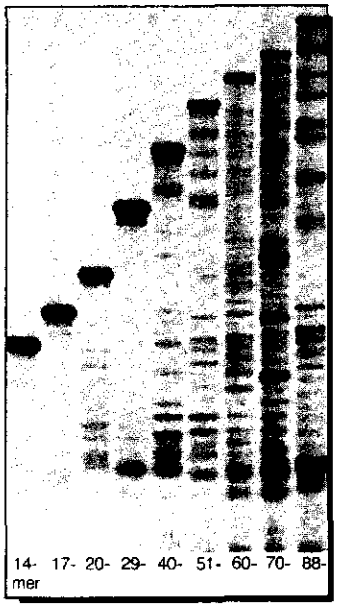

*The migration of three sequences (Lanes 1 to 3, lanes 4 to 6, and lane 7) of the same length (18-mer) through a gel. Note the differences in migration.*

*Figure 11*




*An autoradiogram showing the effect of base composition on migration of oligonucleotides through a gel. The bands correspond to oligonucleotides with the same 5'-10-mer root with different bases on the 3'-end. Lane 1 ends in dA; lane 2 is dC; lane 3 is dT; lane 4 is dG.*

*Figure 12*




*An autoradiogram of typical crude reaction mixtures.*

Film exposure time is critical. Figure 13 shows the effect of exposure on the interpretation of results. In the upper autoradiogram (Figure 13a), the exposure is too short to accurately analyze the performance of each synthesis. In the lower autoradiogram (13b) the exposure is longer, so that the amount of shorter sequences can be visualized. However, exposure is not linear. In longer exposures, the comparison of *n-mer* product to shorter sequences is not correct. See the first three lanes in Figure 22, on page 24, that show a long exposure (Lane 1), a correct exposure (Lane 2), and a short exposure (Lane 3).

UV shadowing, the visualization technique most commonly used for purification, can also be used for analysis of crude mixtures. However, a relatively large oligonucleotide concentration is required for visualization by UV shadowing, it may be wasteful to use this technique for routine analysis. Also, it is often difficult to see minor species.

Gel staining[9] can be done with a DNA-specific stain such as methylene blue. Staining can be particularly useful if the gel is to be preserved by drying. As an analytical tool, staining has limitations because some sequences in smaller concentrations may not be visible. Nevertheless, staining is much more sensitive than UV shadowing and is considered the more useful analytical technique. Refer to Appendix 2.9 for the protocol.

*Figure 13a*

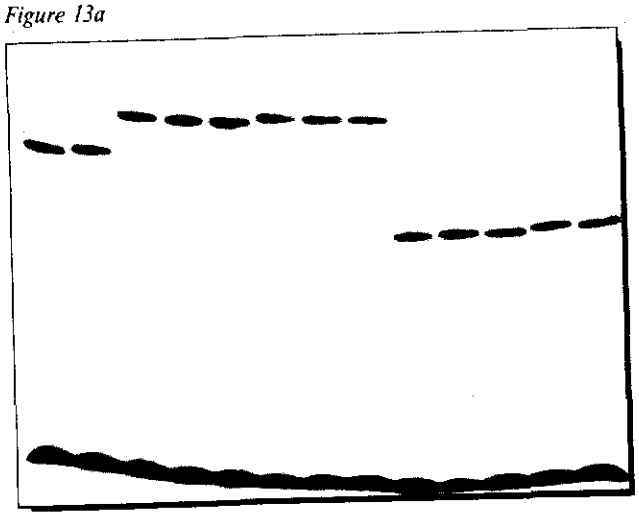
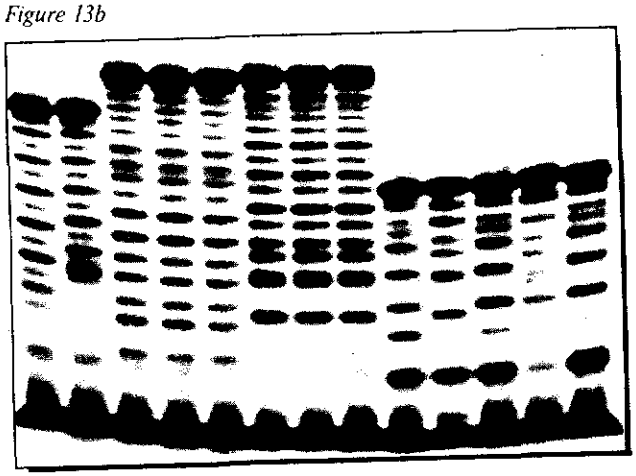


*Figure 13b*



*The effect of exposure on the interpretation of synthesis results.*

## Interpretation of Results

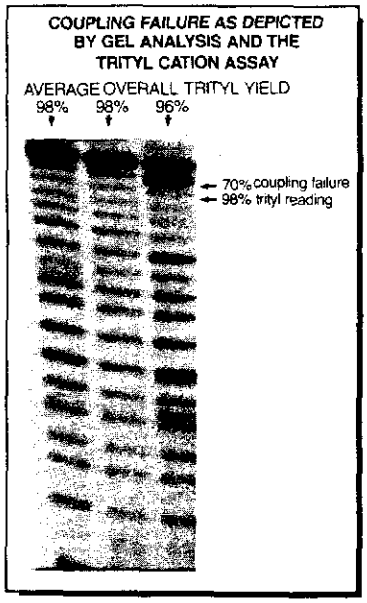
Gel analysis can usually confirm that the DMT cation data is an accurate prediction of product quality. Below are some examples of these comparisons.

Figure 14 shows how well the DMT cation data can predict product quality. The six syntheses on the left have lighter truncated sequences compared to the six syntheses on the right. This correlates with the average coupling yields determined by the DMT cation assay — syntheses on the left have DMT cation yields above 99%; syntheses on the right have values of 97%.

Figure 15 shows an autoradiogram of a gel that depicts a coupling failure (a dense band below the product band) which was detected by the DMT cation assay.

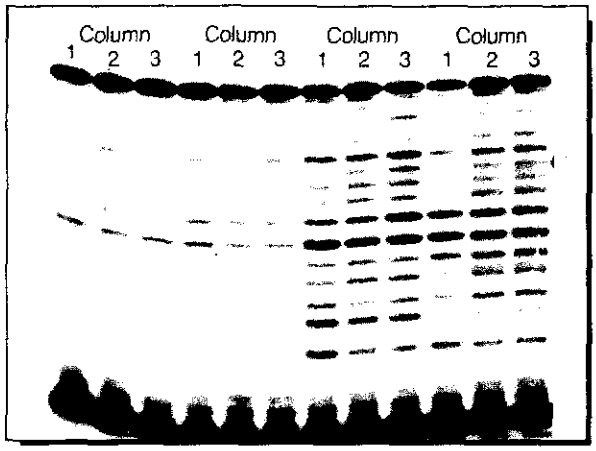
Figure 16 shows true synthesis failures early in the syntheses on the first and third column of a 3-column instrument. Efficient capping of the failed sequences halted the syntheses.

*Figure 15*



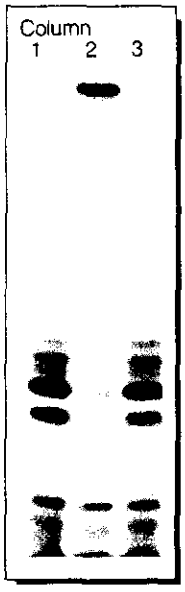

*A coupling failure at the penultimate base addition (DMT cation data showed a 70% yield).*

*Figure 14*




*An autoradiogram showing the correlation of DMT cation data and what can be observed by autoradiography. The differences in DMT cation results are 99% stepwise yield in the six lanes on the left, and 97% stepwise yield in the six lanes on the right.*

*Figure 16*




*Gross coupling failures in lanes 1 and 3 where efficient capping eventually stopped synthesis. Lane 2 shows an efficient synthesis of the same oligonucleotide as in lanes 1 and 3.*

## Problem Areas

There are three general categories of problems associated with these analysis techniques that can lead to the misinterpretation of results: the oligonucleotide sequence, sample preparation, and electrophoresis.

The oligonucleotide sequence can have a marked affect on the resolution of the gel. The most common problem associated with the sequence is secondary structure formation. The ability of the oligonucleotide to aggregate into secondary structures causes changes in its migration through the gel. Because secondary structures are in equilibrium with denatured molecules or other secondary structures, banding on the gel can be very complex. This makes interpretation of the gel very difficult. Hence, the synthesis could be characterized as inferior even though it proceeded normally.

Figure 17 shows an autoradiogram of a 20-mer that has secondary structure-related problems. In this figure, the 20-mer (Lane 2) has a major band migrating at the same rate as a 17-mer (Lane 1) and faint bands migrating above the major band. The major band is probably the product with secondary structures. The faint bands are probably truncated sequences and a population of product molecules with no secondary structure.

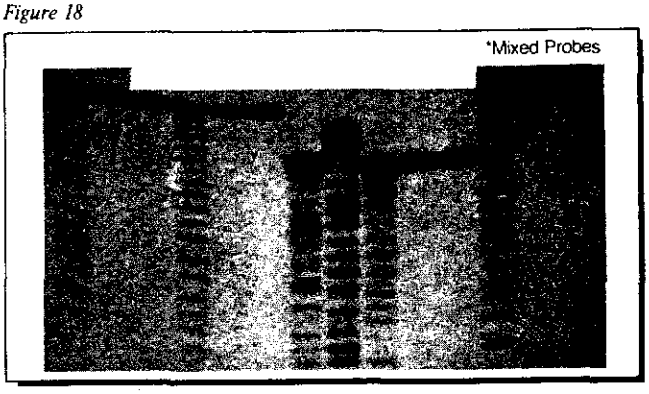
*Figure 17*

*An autoradiogram depicting the effects of secondary structure on the migration of oligonucleotides through a gel.*

In the synthesis of mixed probes of high degeneracy, the inherent complexity of the crude product will give decreased resolution. The product band, because of base composition differences between the oligonucleotides in the mixture, will appear much thicker than normal and might migrate with some *n-1* oligonucleotides (see Figure 18).

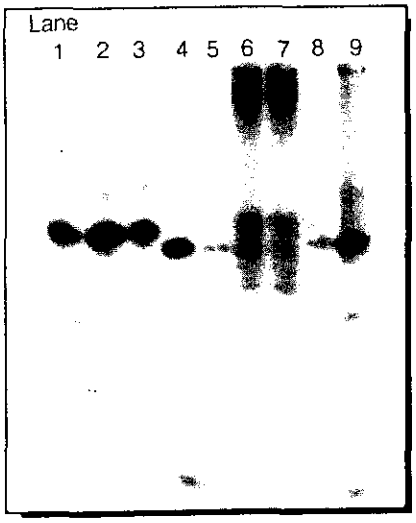
Deoxyguanosine-rich oligonucleotides can migrate quite differently through a gel because of the high degree of secondary structures these sequences can form[10]. It is often very hard to discern the product quality of these sequences. Figure 19 shows an autoradiogram that compares a homodecamer of deoxyguanosine to homodecamers of the other three bases.

*Figure 18*




*An autoradiogram of degenerate oligonucleotides. Note the width of the bands in comparison to the other syntheses on the gel.*

*Figure 19*




*The problem with oligonucleotides with high amounts of deoxyguanosine. An autoradiogram of crude reaction mixtures of homodecamers: lanes 1-3, dT; lanes 4-5, dC; lanes 6-7, dG; lanes 8-9, dA. Note the diffuse radioactivity high in the gel in the dG lanes as well as the diffuse banding pattern in the area around the* 10-mer *region.*

Sample preparation can have an effect on gel results as well. Samples with the DMT group on the 5'-end will not end-label with kinase. Hence, product quality will appear to be minimal at best. Figure 20 shows an autoradiogram of a tritylated oligonucleotide.

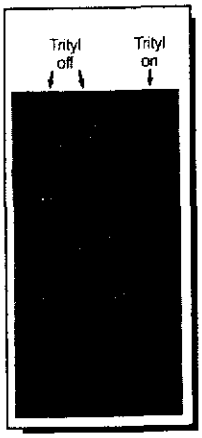
Samples with base-protecting groups still attached will appear diffuse and of a larger molecular weight than deprotected samples because the base-protecting groups hinder migration. Additionally, the base-protecting groups hamper the ability of $T_4$ kinase to phosphorylate the product molecules. We have determined that to achieve quantitative base deprotection, samples should be incubated for a minimum of eight hours. We recommend this even though samples that have been incubated less *appear* deprotected by PAGE analysis (see Figure 21).

Excessive salt in the sample may affect migration and may interfere with labeling reactions. Some commercially available radiolabeled ATP may contain contaminants, which co-migrate with the oligonucleotide and then may appear falsely as truncated sequences (See Figure 3).

Other sample-related problems are due to inaccurate quantification of the sample, inactive enzyme, and inaccurate ATP concentrations. There is a marked affect on the results obtained when enzymatically phosphorylating with kinase under limiting ATP concentrations. If radioactive ATP is the sole source of ATP, there will not be sufficient reagent for use as the phosphorylating agent. The enzyme, under these conditions, will preferentially label a certain set of oligonucleotides based on their length. Figure 22 shows the effect of ATP concentration on the analysis results. By the adding unlabeled ATP to the reaction mixture, efficient phosphorylation can be accomplished independent of the length of the molecules.

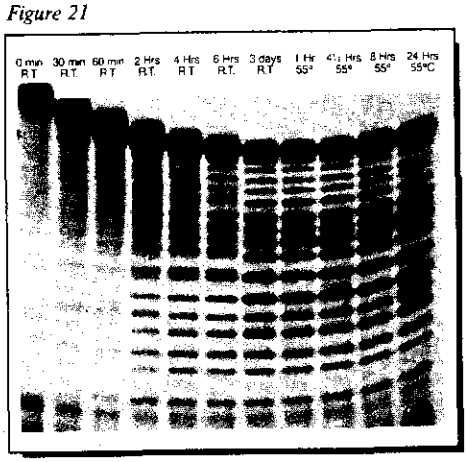
Problems with electrophoresis, aside from gel composition and sample loading, usually are the result of incorrect polymerization. This can occur if too much TEMED is added or if the gel has uneven spacers. Gels should polymerize slowly away from cool or varying air currents. The gel plates also should not be stressed inside of the spacers.

*Figure 20*



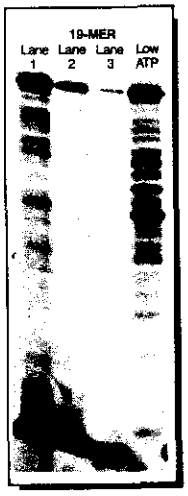

*An autoradiogram of kinased crude reaction mixtures of the same oligonucleotide where: lanes 1-2, the oligonucleotide had a free 5'-hydroxyl; Lane 3, the oligonucleotide has a 5'-DMT group still attached.*

*Figure 21*




*An autoradiogram of a time study of deprotection. Notice the migration of the diffuse product band at time 0 and the absence of truncated sequences due to acetylation of their 5'-ends.*

*Figure 22*




*The effect of ATP concentration on the kination of oligonucleotides. An autoradiogram showing the differences obtained when there is limiting ATP concentration in the kinase reaction mixture.*

# PURIFICATION

## Introduction

The goal of electrophoretic gel purification is to separate the product from the other oligonucleotides in the crude mixture. The key steps in this process are: separation, UV shadowing, product excision from the gel, and recovery of the product from the gel matrix. Figure 23 depicts an analytical gel of crude mixtures and their subsequently purified products.

The methods used in gel purification are generally identical to those used in analysis. There are two major differences. First, the gels used for purification are usually thicker to accommodate the loading of more oligonucleotide. Second, the oligonucleotide has to be loaded on the gel in a sufficient concentration to be visible by UV shadowing.

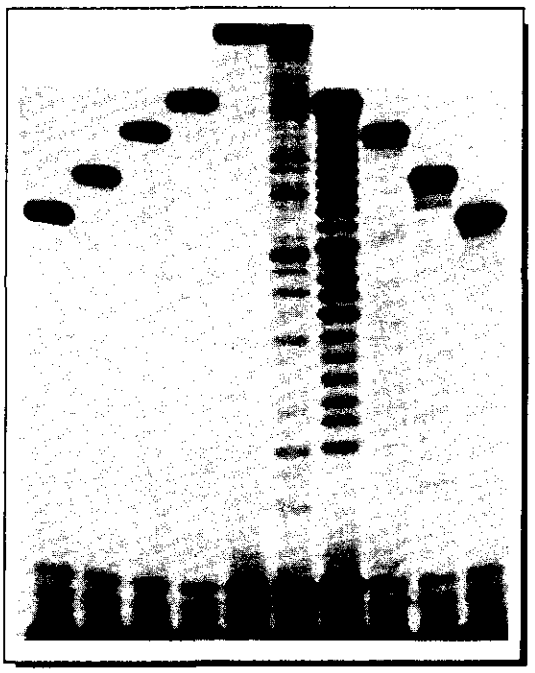
## Sample Preparation

The samples are quantified and prepared in a manner similar to that done during analysis. In some cases, especially for long oligonucleotides, de-salting is recommended before electrophoresis. It is convenient to dissolve the pellets in deionized formamide to a concentration of 1 to 2 OD per microliter of formamide. Samples which don't readily dissolve in the formamide should be heated to approximately 60°C and mixed. It is recommended that marker dyes not be loaded with the sample to avoid possible contamination and masking of the UV shadow. A dye-formamide sample can be loaded in the outermost wells of the gel as a gauge to monitor the migration of the oligonucleotide.

## Electrophoresis

The gel conditions are essentially the same for purification as they are for analysis. Since gels are thicker (1-1.5mm), the separation by electrophoresis takes longer. Often the gels are run overnight at lower power (10 to 20 watts) to accommodate this. Additionally, it is recommended that the sample migrate as far down the gel as possible to achieve optimum product separation. Because the purpose is to *separate* the oligonucleotide from the crude mixture, it is acceptable to run the truncated sequences off the bottom of the gel.

*Figure 23*



*A comparison of gel-purified and crude oligonucleotides showing a variety of lengths (from left to right) 10-, 12-, 16-, 20-, and 30-mers.*

## UV Shadowing

UV Shadowing is done by placing a gel, which has been removed from its glass plates and placed on a clear plastic film, on top of a fluorescent TLC plate. The UV-absorbing oligonucleotide masks the fluorescent emission of the fluorescein in the plate and appears as a shadow. A short wave UV lamp (approximately 240 to 300 nm) is used. Care should be taken to minimize the exposure of the oligonucleotide to UV light which can cause damage such as thymidine dimerization.

Figure 24 is a photograph of a preparative gel being illuminated by a hand-held UV light source. The important idea to keep in mind, when cutting the oligonucleotide out of the gel, is that the product is visualized as a shadow. Like any shadow, it can move in relation to the light source producing it. Therefore, the UV lamp must be held *directly* overhead when trying to detect the product band on the gel.This prevents movement of the shadow from its correct position on the product band to another position which could cause excision of contaminants.

## Product Excision

The product is excised with a clean razor blade which has been rinsed with 95% ethanol to remove any nucleases. If the oligonucleotide is not degenerate, the cuts should be slightly to the interior of the product band to eliminate *n-1* mer contamination. To avoid missing some of the possible sequences in a degenerate product mixture, the cuts should be be at or slightly outside of the diffuse product band.

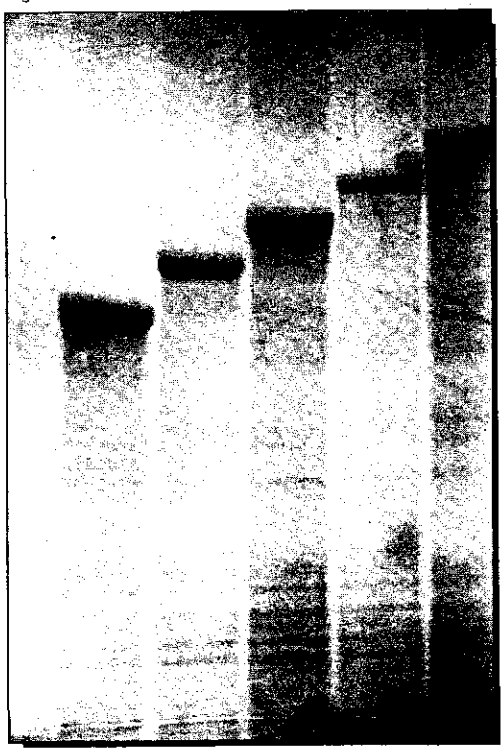
The excised band is then placed in a tube or a fritted column, with forceps that have also been ethanol rinsed. If more than one sequence is run on the same gel, care should be taken in handling to prevent cross-contamination.

## Oligonucleotide Recovery

Once the product band has been cut out of the gel, it is necessary to recover the oligonucleotide from the acrylamide. The two most common ways are soaking or electroelution. Both methods are effective, but there advantages and disadvantages to both. Soaking is inexpensive, easy, effective and can be accomplished without monitoring.

Electroelution is quicker, gives consistent yields but requires expensive apparatus and must be monitored. We prefer the use of a Sep-Pak cartridge[11] to recover oligonucleotides which have been "soaked out" of a gel. A protocol for this type of recovery is found in Appendix 2.10. For electroelution, the IBI electroeluter, Model EUA is recommended.

*Figure 24*



***A photograph of oligonucleotides, 90 to 130 bases in length, being illuminated as a UV shadow.***

## Differences in the Purification of Long and Short Oligonucleotides

Table 9 is a simple chart to aid in planning oligonucleotide purification. Sequences greater than 50 bases are considered long oligonucleotides. Crude samples of long oligonucleotides contain substantially more truncated sequences due to the increased number of coupling cycles. Hence, the product is in a lower concentration in relation to the crude reaction mixture than is found in shorter sequences.

Therefore, it is necessary to load more of a long oligonucleotide on a preparative gel so that the *n-mer* oligonucleotide can be visualized. Since there is a finite amount of DNA that can be loaded onto a gel, there may be problems associated with the electrophoresis of long oligonucleotides. When these problems exist, enrichment of the crude reaction mixture by size exclusion chromatography prior to gel purification will result in an increased concentration of the *n-mer* oligonucleotide sufficient for visualization. Table 9 shows the differences in procedures for the two types of oligonucleotides.

*Figure 25*

*An autoradiogram depicting the effect of loading on the determination of oligonucleotide purity.*

*Table 9.*

| Procedure | Long Oligonucleotides | Short Oligonucleotides |
|---|---|---|
| Sample Concentration | 2 OD/ μL | 1 OD/ μL |
| %Acrylamide in gel | 12% | 20% |
| Amount of crude DNA loaded per sq mm | 0.05 - 0.5 OD | 0.4 - 0.8 OD |
| De-salting | yes | no |

*The differences in the purification of large and small oligonucleotides.*

## Analysis of Purity

In order to verify that pure product has been excised from the preparative gel, a second analysis is performed. We prefer a high resolution technique such as thin gel autoradiography. Figure 25 shows the effect of different amounts of the same labeled, purified sample in relation to the interpretation of their purity. It is not until the lighter exposure band (corresponding to less material loaded) is visible that one could be confident that the product was pure. Overloaded samples appear indistinct or somewhat "smeary" and are difficult to interpret.

# EVALUATION AND PURIFICATION BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

## INTRODUCTION

High performance liquid chromatography (HPLC) is an efficient method for the analysis and purification of synthetic oligonucleotides. The advantages of HPLC are ease of automation, quantification of the separated products, speed, and ease of pure product recovery. There are many HPLC methods for evaluation and purification of oligonucleotides, but the two most prominent are reverse phase chromatography and ion exchange chromatography.

Reverse phase chromatography separates oligonucleotides based on hydrophobic differences; ion exchange chromatography separates oligonucleotides based on charge. Because of resolution limitations, HPLC is a useful system for routinely evaluating and purifying oligonucleotides less than 50 bases in length. For most separations, the only difference between the analysis and purification procedures is the amount of material injected.

These chromatographic modes require an HPLC with an injector, a binary pumping system, a UV detection system, a chart recorder (one that integrates peaks is preferred), and a gradient former. In configuring the system, it is imperative that the post-column volume be minimized. Most HPLC's are configured only for analysis, therefore the dead volume and mixing points are often minimized only up to the detector. For preparative work, both the dead volume must be minimized and mixing points eliminated between the detector and point of collection.

A finite period of time is required for a compound to pass from the detector to the collection port. This time interval can be determined by injecting any UV-absorbing compound, and collecting the fractions at specific time points, and measuring the absorbance of the fractions individually. The time, corresponding to the fraction containing the strongest UV absorption minus the time of the peak maximum on the chart recorder, will be the time lag before samples should be collected. This time interval should be measured a number of times initially to ensure an accurate result and repeated periodically because pump flow rates may vary over a period of time.

It is also very helpful to have a digital readout of the absorbance on a real-time basis. This can be extremely important in the purification of complex mixtures where the collection process plays a key role in obtaining pure material.

The HPLC purification process is quite different from analytical HPLC. The resolution requirements for purification are not as strict as in analyses. Because the amount of product needed for most applications is quite small, only a portion of the peak need be collected. Hence, good collection techniques and post-purification analysis can routinely produce pure product even when the analytical chromatogram shows complex mixtures.

## REVERSE PHASE CHROMATOGRAPHY

### Introduction

There are two types of reverse phase chromatography used in the evaluation and purification of oligonucleotides. One utilizes the separation of tritylated from non-tritylated oligonucleotides in the crude oligonucleotide mixture[12]. The other utilizes the hydrophobic differences between oligonucleotides of varying lengths and sequence. Each method will be discussed separately.

## TRITYL-ON CHROMATOGRAPHY

### The Chemistry

Theoretically, when all the reactions involved in an oligonucleotide synthesis have worked properly, the only compound bearing the terminal 5′-protecting group, the DMT moiety, is the product oligonucleotide. All the other species in the crude reaction mixture will have been acetylated at the 5′- position during capping. These truncated chains, upon deprotection in ammonia, will have free 5′-hydroxyl functions.

Trityl-on HPLC utilizes the difference in hydrophobicity between the trityl- and non-trityl-bearing species. Within certain solvent conditions, a reverse phase column will easily separate the two types of oligonucleotides. The tritylated product, being more hydrophobic, will be retained longer on the column than the truncated sequences and other components of the crude reaction mixture.

A typical chromatogram is shown in Figure 26. The initial peak at the void volume includes all truncated sequences. The second peak at 4.45 minutes contains the benzamide and isobutyryl amide from the deprotection of the exocyclic amines by ammonia. The major peak at 15.10 minutes is the tritylated product.

In Figure 26, the number of peaks in the region where trityl-bearing compounds elute shows that there is not a homogeneous trityl species. The heterogeneity is a result of strand scission of the tritylated full length product during the deprotection in ammonia, though it also could be due to incomplete detritylation. The strand scission in ammonia is thought to be the result of cleavage at apurinic sites on some molecules of the product[13].

The apurinic sites are believed to be caused by chemical modification of purine bases at a low level during the synthesis process. This modification destabilizes the glycosidic bond (the bond between the base and the deoxyribose sugar) to acids. Separation of the trityl-bearing species in the reaction mixture can be accomplished by trityl-on HPLC. Separation is based on length. Longer trityl-bearing species have less overall hydrophobicity than shorter species due to increased number of charges on the larger compound.

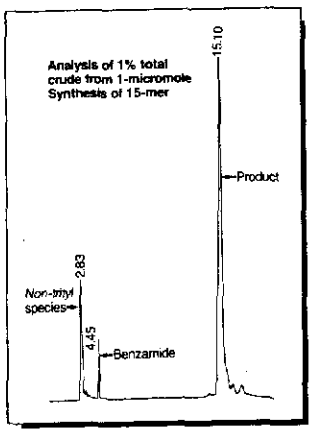
### Sample Preparation

When reverse phase trityl-on chromatography is used to evaluate and purify synthetic oligonucleotides, it is necessary to leave the final 5′-DMT group attached to the oligonucleotide. With an Applied Biosystems DNA synthesizer, this can be done by choosing the trityl-on end function. After treatment with ammonia at 55°C, the oligonucleotide will be completely deprotected except for the DMT group.

To further prepare the sample, the ammonium hydroxide used for cleavage and deprotection is removed by evaporation (a Savant vacuum centrifuge is preferred). It is important to note that the DMT group is unstable to heat and acid. Therefore, the evaporation should be done at room temperature and, as a precaution, a drop of triethylamine should be added periodically throughout the evaporation process. After the sample is dried, it should be redissolved immediately in 995 $\mu$L of 0.01M triethylammonium bicarbonate (TEAB), pH 7.0 and 5 microliters of triethylamine. Samples can be stored at -20°C up to 2 years with minimal detritylation.

It is always a good technique to filter HPLC samples prior to injection especially if any cloudiness appears. Keep in mind that cellulose or glass filters will irreversibly bind some of your sample. Many suitable filters and microfiltration devices are available for filtering HPLC samples.

*Figure 26*




*A chromatogram of a typical trityl-on crude reaction mixture.*

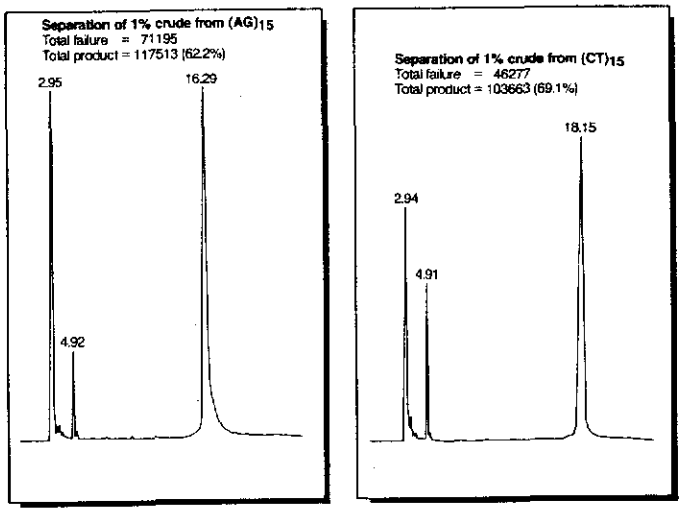
## Results

In order to demonstrate that HPLC sufficiently resolves different DMT-bearing species, we synthesized a number of similar compounds. Table 10 shows the size, sequence, and retention times of five DMT-bearing synthetic oligonucleotides. Compound 1 has the same sequence as compounds 2 to 5 except that it is lacking the 5'-nucleotide. Therefore, Compound 1 would be a likely contaminant of the other compounds, and the hardest to separate. The differences in retention time are also shown.

To show that trityl heterogeneity is a result of depurination, repeating $d(AG)_{15}$ and $d(CT)_{15}$ 30-mers were synthesized and analyzed using this methodology. Figure 27 shows the chromatograms of both crude oligonucleotide mixtures. The trityl peak of the pyrimidine compound is virtually homogeneous while there is some heterogeneity in the purine compound.

By comparing the UV absorbance in the product peak to the total UV absorbance as a measure of synthesis yield, the pyrimidine compound was superior (69% versus 62%). If stepwise yields were calculated from these ratios, one would generate a value representative of yield data taking into account factors that the DMT cation assay can not detect. When compared to the DMT cation assay result, this value could reflect the extent to which side reactions contribute to decreased product quality.

*Figure 27*



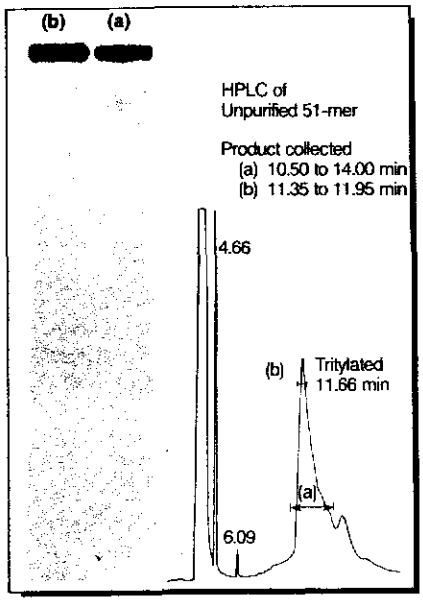

*The chromatograms of homopurine and homopyrimidine oligonucleotides.*

*Table 10*

| No. | Compound | Length | $R_t$(Min) | $R_t$1-$R_t$2-5 (Min) |
|---|---|---|---|---|
| 1 | DMTr- TCA CAG TCT GGT CTC ACC | 18 | 8.72 | — |
| 2 | DMTr-A TCA CAG TCT GGT CTC ACC | 19 | 7.94 | 0.78 |
| 3 | DMTr-C TCA CAG TCT GGT CTC ACC | 19 | 7.61 | 1.11 |
| 4 | DMTr-G TCA CAG TCT GGT CTC ACC | 19 | 6.70 | 2.02 |
| 5 | DMTr-T TCA CAG TCT GGT CTC ACC | 19 | 9.81 | -1.09 |

*The sequences of five similar trityl-bearing oligonucleotides.*

*Figure 28*




*Chromatogram of a crude reaction mixture of the synthesis of a 51-mer. In separate preparative runs, two different cuts were collected. In the autoradiogram of the detritylated, kinased-collected oligonucleotides, Lane A was collected from 10.50 and 14.00 minutes, and Lane B was collected between 11.35 and 11.95 minutes.*

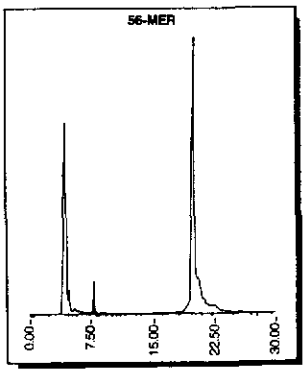
The efficiency of HPLC purification of complex synthesis mixtures is shown in Figure 28. The chromatogram portrays the synthesis of a 51-mer which did not proceed very well at all, though pure product was isolated. There were two problems affecting the purification: the degree of trityl heterogeneity and the size of the oligonucleotide. Because differences in hydrophobicity are very small between trityl-bearing species in this size range (greater than 40 bases), separation is minimal.

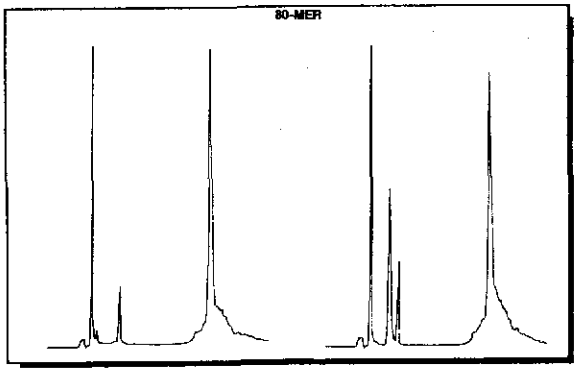
The key to obtaining pure product is in the collection process. The differences in purity, as a function of points of collection, are shown in the autoradiogram of the electrophoresis of the radiolabeled pure fractions.

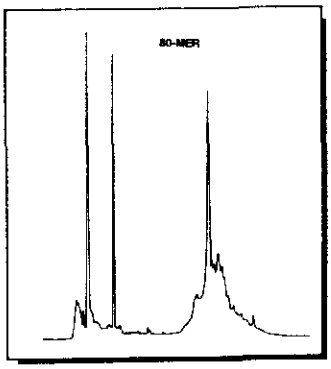
Purification of long oligonucleotides is best accomplished with a strongly hydrophobic reverse phase column. Because the hydrophobicity of these long trityl-bearing species is greatly decreased, the difference in hydrophobicity in relation to the hydroxylated species is quite small. Hence, separation of these types of compounds can be aided by a more hydrophobic column.

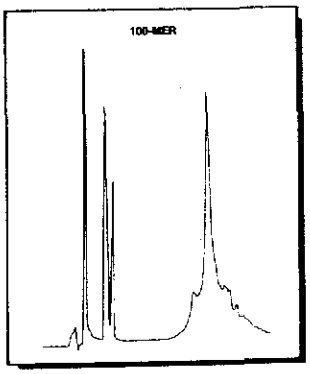
The efficiency of a strong hydrophobic column in purifying long oligonucleotides is depicted in Figure 29. Separation of long tritylated oligonucleotides from 56 to 100 bases in length and their respective hydroxylated species can be achieved. The column also appears to perform relatively well in separating the trityl species in the mixture.

*Figure 29*




*Crude reaction mixtures of some large oligonucleotides using a highly hydrophobic column.*

## The Chromatography System

The HPLC that we recommend is the Applied Biosystems Model 152A Separation System. The instrumentation, columns and reagents have been carefully selected to perform the isolation, purification, and analyses required. The use of this high performance separation system expedites many of the most demanding separations required in nucleic acid research.

Various commercial supports have been utilized for the separation of tritylated versus non-tritylated species. Supports with small pores (nominally 80 Angstroms) have been used but seldom give adequate separation of similar trityl species. Analyses of oligonucleotides purified from these supports show contamination with species of different length or base composition. Repeated attempts with the same chromatographic system often do not result in adequate purity for most applications.

We recommend a C-8, 10-micron particle size, 300-Angstrom pore size column from Applied Biosystems (RP300). This column is especially useful for separating the trityl species in a crude mixture. A standard size column (4.6 mm x 23 cm) is suitable for analysis and most purifications. A larger column (10 mm x 23 cm) gives better separation with a preparative level loading. It can also be used for large scale purification (normally used for 10-micromole syntheses).

The mobile phase is a solvent buffer system, acetonitrile and 0.1M triethylammonium acetate (TEA-Ac), pH 7.0, first reported by Khorana, et al.[14].

Most crude reaction mixtures in the range of 15 to 40 nucleotides can be evaluated and purified using the linear gradient system in Table 11.

*Table 11.*

| Time | Flow (mL/min) | % Acetonitrile |
|---|---|---|
| 0.0 | 1.0 | 16 |
| 10.0 | 1.0 | 22 |
| 40.0 | 1.0 | 22 |

*The standard gradient for trityl-on chromatography.*

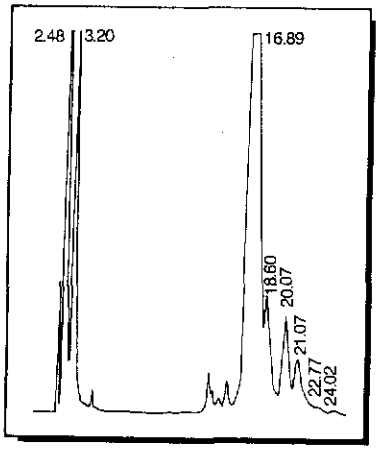
For longer sequences or oligonucleotides where one base predominates, the conditions may need to be altered. Base composition and sequence length will affect mobility. The elution order of the nucleotides is G>>C>A>T and the retention time will decrease as chain length increases.

## Evaluation of the Analytical Chromatogram and Purification

The initial analytical separation of a crude reaction mixture is performed under the conditions described in the previous section. About 1% of the total sample from a 1-micromole synthesis (10 microliters) is sufficient when the range of the detection system is set at 0.1 OD AUFS. For most syntheses, the peaks will be full scale.

When proper conditions have been determined, examine the chromatogram for the best possible points of collection. Since the unwanted trityl peaks will usually elute later in the chromatogram, appearing as separate peaks or as shoulders of the main peak (see Figure 30), it is important to know when to start and stop collecting. Generally speaking, it is safe to stop collecting when the absorbance is 20% below the peak maximum on the down side of the peak. Figure 31 depicts a chromatogram of an oligonucleotide purified by this collection process.

*Figure 30*



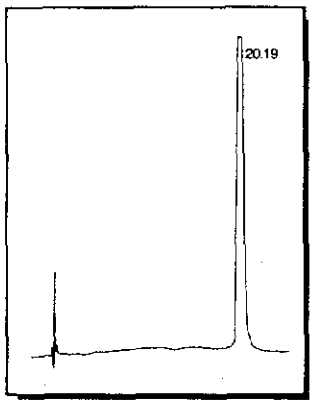

*Trityl-on chromatogram of a preparative injection of an 18-mer.*

Because only part of the product peak is collected, the trityl-on methodology is not preferred for the purification of mixed probes. This methodology also does not work well with oligonucleotides rich in deoxyguanosine. The chromatographic conditions described are not denaturing to any extent. Hence, oligonucleotides bearing many deoxyguanosine residues, especially those with four contiguous deoxyguanosines, will behave chromatographically like oligonucleotides with self-complimentarity. If the number of deoxyguanosines is sufficiently large, the chromatogram will often have no major peaks at all. Gel electrophoresis is a better way to analyze and purify these oligonucleotides.

## Workup of the Final Product

After collection of the purified product from trityl-on chromatography, the oligonucleotide needs to be detritylated to render a biologically active compound. The first step is to evaporate the solution under vacuum. The evaporated sample, which could be an oil, is treated with approximately 300 microliters of 80% acetic acid at room temperature for twenty minutes. This acidic treatment does not cause depurination because the bases are much more stable to acid after deprotection. An equal volume of ethanol is added, and the solution is evaporated (again the resulting sample could be an oil). Several co-evaporations with distilled deionized water may be necessary to remove the remaining volatile salts, apparent from the presence of white precipitate in the sample. The hydrolyzed trityl group can be removed by organic/aqueous extraction, ethanol precipitation, or de-salting on a gel exclusion matrix or Sep-Pak chromatography.

*Figure 31*




*A chromatogram of the purified oligonucleotide from Figure 30.*

# TRITYL-OFF CHROMATOGRAPHY

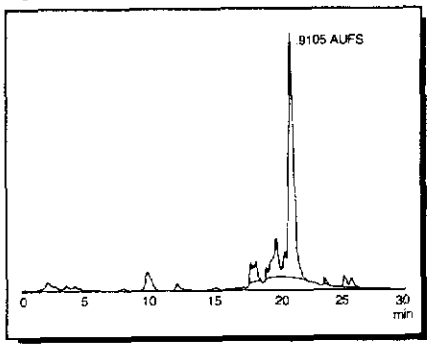
## The Chemistry

In this method the DMT group on the 5'-end of the oligonucleotide is removed prior to analysis and purification. Separation of the crude reaction mixture is achieved by utilizing the differences in hydrophobicity among the various oligonucleotides.

The differences between oligonucleotides of similar length and sequence are very subtle. However, resolution is sufficient to make this a viable approach for routinely evaluating and purifying oligonucleotides under 35 base residues in length. The advantages of this method are ease of sample preparation and recovery of the purified compound in a ready-to-use form.

A typical chromatogram is shown in Figure 32. The major peak at 21.43 minutes is the product, an 18-mer. The peak is surrounded by minor peaks which constitute the various truncated and strand-scission products of the crude reaction mixture. It is very difficult to identify these peaks based on relative retention times because of the effect of base composition on retention time. Compounds of shorter length than the product can elute faster or slower than the product. Retention time is based solely on their surface hydrophobicity.

*Figure 32*




*A chromatogram of a typical trityl-off crude reaction mixture.*

## Sample Preparation

With trityl-off reverse phase HPLC, sample preparation is much simpler than with trityl-on HPLC. Because the product does not have a trityl group, it is much more stable during preparation for analysis and purification. The ammonia solution containing the crude mixture can be easily and quickly evaporated to dryness with mild heat (35 to 40°C) and without addition of triethylamine.

The compound after evaporation can be reconstituted in distilled, deionized water and stored at -20°C indefinitely. Upon reconstitution, there may be a small precipitate in the sample. This can easily be removed by simple centrifugation and removal of the liquid.

## Results

The resolution of trityl-off chromatography for the separation of oligonucleotide mixtures of similar length and sequence is depicted in Figure 33. The chromatogram shows the separation of two similar 18-mers. The only difference between the sequences is three bases on the 3'-end. The resolution of the 18-mers is greater than five minutes.

Figure 34 depicts HPLC resolution of four oligonucleotides, 15 to 18 base residues in length, with identical sequence roots. Thus, the 15-, 16-, and 17-mers are the contaminants found in the synthesis of the 18-mer. The chromatogram shows resolution of the four compounds that were loaded in approximately equal proportions. Therefore, even if the synthesis of the 18-mer proceeded with very poor yields in the final three coupling steps, the final product could still be purified from the mixture using conservative collection methods.

## The Chromatography System

The solid support and the mobile phase used for trityl-off HPLC are identical to that used for trityl-on. Separating the non-trityl bearing species requires a decrease in the acetonitrile concentration. The gradient used is shown in Table 12.

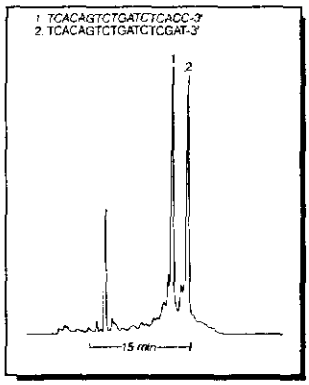
*Table 12.*

| Time | Flow (mL/min) | % Acetonitrile |
|---|---|---|
| 0.0 | 1.0 | 07 |
| 50.0 | 1.0 | 17 |
| 60.0 | 1.0 | 17 |

*The standard gradient for trityl-off chromatography.*

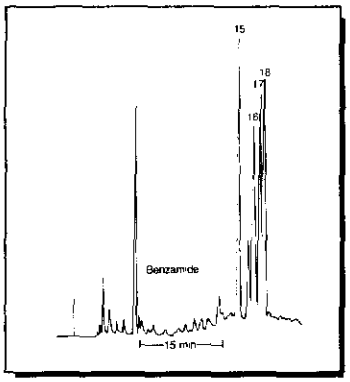
For longer sequences, the conditions may need to be altered by increasing the concentration of acetonitrile. Base composition affects mobility under the general rule: the elution order is G>>C>A>T.

*Figure 33*




*The chromatogram of the separation of two similar 18-mers.*

*Figure 34*




*A chromatogram of four oligonucleotides ranging from 15 to 18 in length.*

## Evaluation of the Analytical Chromatogram and Purification

Using the conditions described in the mobile phase section, about 1% of the crude mixture from a one-micromole synthesis (10 microliters) can be injected for analysis. Detection at 0.1 OD AUFS is sufficient for routine examination of the chromatogram. The oligonucleotide, under most circumstances, will elute between 10 and 15% acetonitrile, depending upon its length and sequence.

Like the trityl-on method, the collection process is very important. Because the impurities can elute both before and after the product peak, great care must be taken in deciding where to start and stop collecting. In a chromatogram, the collection can be done beginning at 50% of the peak maximum on the upside of the peak to 50% of the peak maximum on the downside.

If the analysis reveals a more complex mixture, the collection is not as simple. It is important to remember that for most molecular biology applications, only small quantities of pure product are needed. Hence, it is better to be more conservative in the collection process and get less pure product than to try to get a larger amount that would require further purification.

Problems that arise in using trityl-off chromatography to purify oligonucleotides with mixed sites, oligonucleotides with regions of self-complimentarity, and deoxyguanosine-rich sequences are similar to those for trityl-on HPLC which were covered earlier in this Bulletin.

## Workup of the Final Product

In trityl-off chromatography, because the purified product does not have a 5'-DMT group, sample workup is very simple. The solution containing the pure product can be evaporated under vacuum with mild heat (35 to 40°C) and used directly in any number of applications. If the evaporated sample leaves a white film on the bottom on the vial, it is probably some residual TEA-Ac still in the sample. Addition of 100 microliters of distilled, deionized water and reevaporation will remove these salts.

## The Limitations of Reverse Phase HPLC

The major limitations in both types of reverse phase HPLC are related to the size of the synthesized oligonucleotide and possible secondary structures formed by intrastrand base pairing. The problems related to size are two-fold. The first is yield. As the synthesis proceeds, the overall yield of the final product decreases making purification more difficult.

The second problem relates to the ability of the system to achieve complete resolution. Longer oligonucleotides, because of steric problems, cannot enter the 300-Angstrom pores of the solid phase matrix where the majority of the bonded phase exists. Hence, resolution is decreased because the oligonucleotide has a limited number of sites with which to interact on the support.

Secondary structure problems can occur with oligonucleotides of any length. This sequence-dependent phenomenon impacts resolution because the secondary structure can mask differences in hydrophobicity of chemically similar compounds to the extent that separation of these is virtually impossible. Also, the individual compounds elute as broader peaks which further complicate the separation.

The degree of secondary structure can be so severe, as with palindromic sequences, that the product will elute as a series of peaks. This series represents a population of oligonucleotides of identical length and sequence that have different degrees of secondary structure. Hence, they will elute at different retention times. The concept of secondary structure problems is shown in Figure 35 on the following page.

Figure 35a illustrates all the different types of oligonucleotides that can appear in a crude reaction mixture: the desired product (*n*-mer), the acetylated failure sequences (including *n-1* mer), the strand scission fragments that result in short DMT-bearing sequences and short 5'-OH sequences, desired product-length oligonucleotides (*n*-mers) that have base modifications, and an array of DMT-bearing *n-1 mers* resulting from incomplete detritylation.

Figure 35b presents all the possible iterations of secondary structures. The desired product can form loops and hairpins with itself, the equilibrium of which is sequence dependent. The desired product can also form duplexes with other products in the crude. Clearly, there is a variety of secondary structures that can form.

Reverse phase HPLC is still a very powerful technique for analysis and purification, even with the problems presented here. Oligonucleotides purified by these techniques have been used in a variety of applications, from gene synthesis to physical studies by NMR and X-ray crystallography.

*Figure 35a*

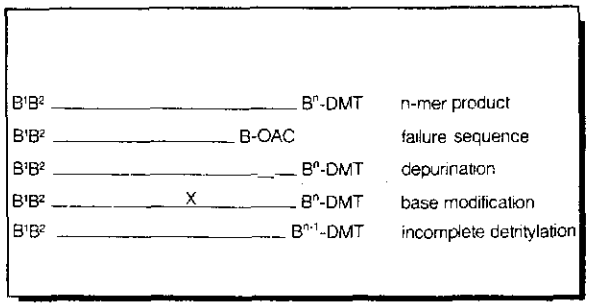
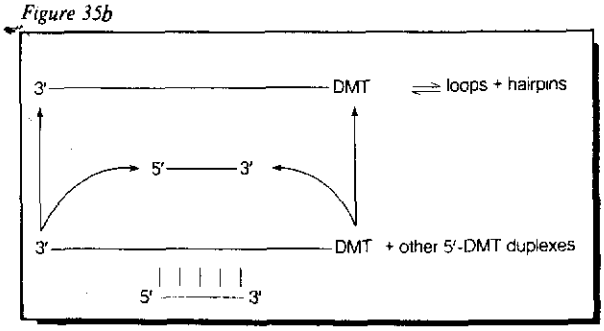



*Figure 35b*




# ION EXCHANGE HPLC

## The Chemistry

Ion exchange HPLC is another efficient technique for analysis and purification. Separation is largely based on charge. Each oligonucleotide in the crude mixture has a different net charge based on the number of phosphate groups in the molecule (base length) and on the respective charges on the bases (base composition). It would be advantageous to neutralize the positive charges on the bases by raising the pH of the mobile phase, however, most silica-based solid phase supports dissolve above pH 8.0. Hence, base composition does play a role in the separation process, but not a significant one.

Separation of the crude mixture is accomplished by slowly increasing the ionic strength of the mobile phase. By doing so, the longer, more charged oligonucleotides will elute later than shorter ones. An important aspect of this method is minimizing secondary structures. Phosphate groups masked by secondary structures will cause serious problems when separating compounds of similar length. This can be achieved by adding the strong denaturant, formamide, to the mobile phase[15].

## Sample Preparation

With ion exchange chromatography, sample preparation is much simpler than methods used for trityl-on HPLC. Because the product does not have a trityl group, it is much more stable during preparation. The ammonia solution containing the crude mixture can be easily and quickly evaporated to dryness with mild heat (35 to 40°C) without addition of triethylamine.

The compound, after evaporation, can be reconstituted in distilled, deionized water and stored at -20°C indefinitely. After reconstitution, if a small precipitate is present, it can easily be removed by simple centrifugation.

*All the possible oligonucleotides present in a crude trityl-on reaction mixture and the possible secondary structures that can result from intra- and inter-strand base pairing are shown.*

## Results

Figure 36 shows typical chromatograms. Oligonucleotides elute separately by size with the product peak eluting last. Separation decreases as larger oligonucleotides are eluted because the relative difference in charge between longer oligonucleotides is less than in shorter compounds. Also, there is relative band widening as a function of increasing oligonucleotide length.

With ion exchange HPLC, using identical mobile phase conditions, the retention time of *n*-mer oligonucleotides will be quite predictable. Therefore, sequence length can be confirmed with reliability.

Chromatograms of crude mixtures of various lengths are shown in Figure 37. The major peaks represent products that have been resolved from the truncated chains.

## The HPLC System

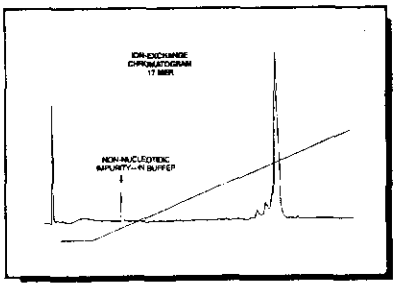
This method utilizes a standard 4.6 mm x 25 cm strong anion exchange column. The mobile phase is a potassium dihydrogen phosphate buffer system, pH 6.3, with 60% formamide added. A standard gradient is 1 to 300 mM of the phosphate buffer over 60 minutes.

## Evaluation of the Analytical Chromatogram and Purification

The initial analytical separation of a crude reaction mixture is performed under the conditions described in the previous section. Injection of about 1% of the sample from 1-micromole syntheses (10 microliters) is sufficient when the AUFS is set at 0.1 OD. This absorbance scale will present major peaks at full scale to allow easy interpretation of the data.

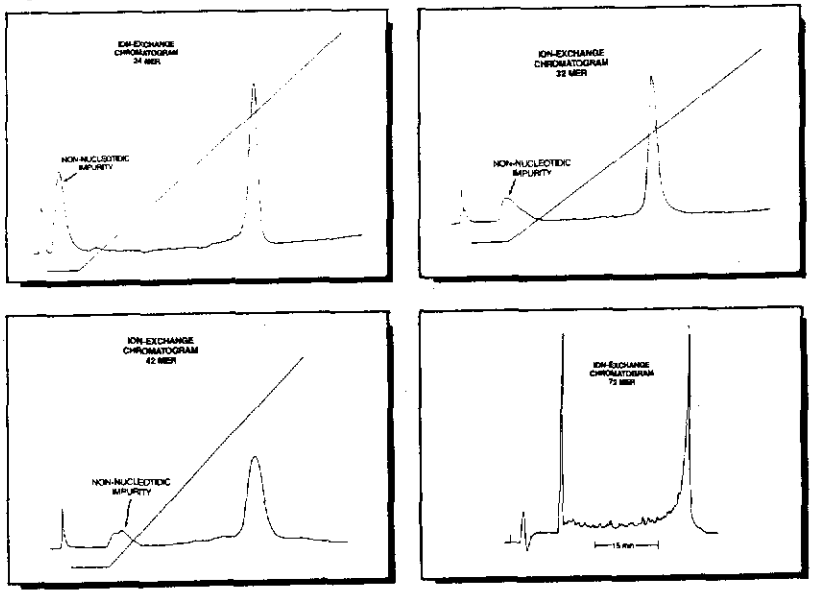
Depending upon the length of the synthesized oligonucleotide, the gradient can be decreased to give better resolution. Crude mixtures under 25 bases in length will probably need a shallower gradient, up to 200 mM phosphate buffer.

*Figure 36*




*A typical chromatogram of a crude oligonucleotide mixture using ion exchange methods.*

*Figure 37*




*Ion exchange chromatograms of oligonucleotides ranging from 24-72 bases in length.*

Since reaction contaminants will elute before the product peak, it is best to collect most of the sample from the down side of the peak. A conservative collection process is recommended by starting the collection when the absorbance is about 70% of its maximum on the up side of the peak and continuing until the absorbance has decreased to 50% on the down side. Complex mixtures will require an even more conservative approach.

### Workup of the Purified Product

While there is no 5'-DMT group in ion exchange HPLC, the workup is compounded because there are non-volatile salts in the chromatography buffers. The pure product must be de-salted prior to use. De-salting can be achieved by various procedures. We prefer gel exclusion chromatography or Sep-Pak chromatography.

## ADDITIONAL ANALYSIS AND PURIFICATION

The purified sample should always be analyzed for contaminants. Labeling by $^{32}$P-kination, followed by gel electrophoresis and autoradiography is the recommended analysis method. Re-injection of a small aliquot of the pure oligonucleotide under the conditions of the original analysis can also be done.

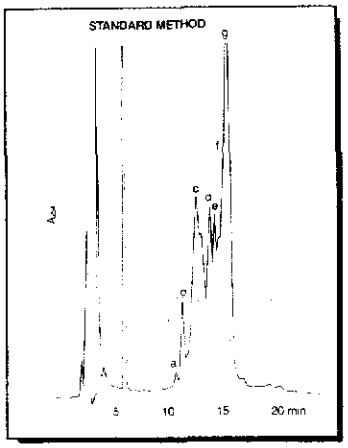
If further purification is necessary, an alternative method that will separate the mixture using a different set of parameters is highly recommended. This should help ensure that a pure compound is isolated. If only one type of chromatography is available, changing the gradient slightly and taking more conservative collections will give better results. Using different purification techniques is the best approach to acquire consistently pure oligonucleotides, regardless of the length and sequence.

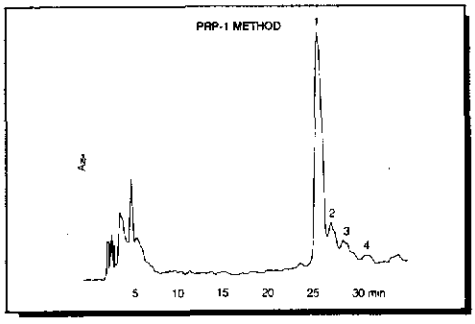
## CONCLUSIONS

The preceding data show that the analysis and purification of oligonucleotides can be accomplished reliably and efficiently with several HPLC procedures. Clearly, there is potential for increasing chromatographic separation, especially regarding long oligonucleotides. There have been some recent references[16-18] that explore that potential.

Applied Biosystems is responding to this potential in a very positive way. Our acquisitions of Brownlee Labs and the chromatography division of Kratos Analytical demonstrate that we believe there are large, unrealized opportunities in the chromatography of biological macromolecules. We are beginning to apply the chromatography expertise developed in our protein and peptide group to the separation of oligonucleotides. Our Model 152A DNA Separation System is the first step toward implementing what we believe to be major advances in this field.

*Figure 38*



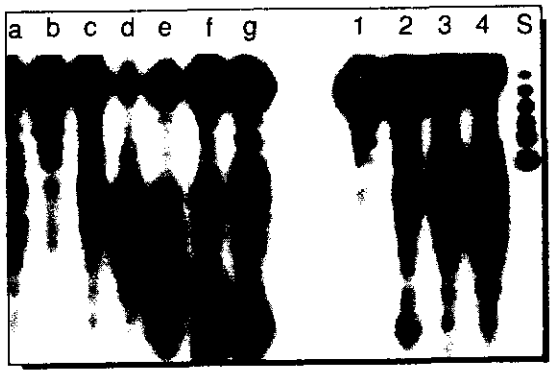

*Chromatograms of the same crude oligonucleotide reaction mixture using standard trityl-on reverse phase methods and the new PRP-1 trityl-on reverse phase method using formamide and increased temperature as denaturants.*

One recent methodology worthy of note has been developed by Gerry Zon, formerly at the FDA at NIH, who has joined our staff. Dr. Zon's approach utilizes a PRP-1 resin in a trityl-on reverse phase method that contains a 50% solution of formamide in acetonitrile and is accomplished at 60°C. The high pH of the solution and increased temperature, not a problem for PRP resins, act as denaturants in the reverse phase process. These denaturants eliminate the problems associated with inter- and intra-strand base pairing resulting in the improved separation shown in Figure 38.

The first chromatogram shows the profile of a crude mixture with a high degree of secondary structure. There is a large amount of heterogeneity within the DMT-bound region. Clearly, the identification and isolation of the desired product would be extremely difficult.

The second chromatogram shows the same oligonucleotide mixture when applied to a PRP-1 resin with formamide and run at 60°C. This method seems to decrease secondary structure more effectively. Figure 39 is an intentionally overexposed autoradiogram of the detritylated, kinased fractions noted in each chromatogram. The oligonucleotide in Lane 1 appears approximately 95% pure.

*Figure 39*




*An autoradiogram of the collected fractions from each chromatogram in Figure 38.*

# CHARACTERIZATION OF THE OLIGONUCLEOTIDE

## VERIFICATION OF SEQUENCE

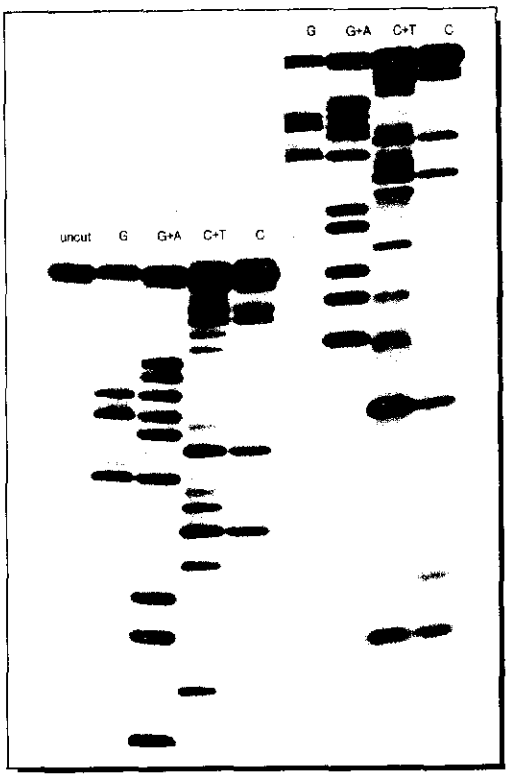
To verify the sequence of purified oligonucleotides, a modified Maxam-Gilbert method[19] is recommended. Base-specific chemical reactions are performed on a radiolabeled oligonucleotide causing cleavage at the glycosidic bond of the specific base. The conditions of these reactions have been modified to permit only one modification per molecule. By treating with a base, strand scission occurs at these modified sites. Each of these four reactions then undergo electrophoresis in contiguous lanes on a gel followed by autoradiography. The sequence is determined by analyzing the banding of each lane. The appearance of a band in a given base-reaction lane denotes the presence of that base in the molecule. The position of a band within a lane denotes the sequence order of that base. Figure 40 shows an autoradiogram of a sequencing procedure. Details of the procedure can be found in Appendix 2.11.

## CHEMICAL AUTHENTICITY

Another important aspect, in addition to the verification of sequence, is the chemical authenticity of a purified oligonucleotide. This concept relates to the fidelity of the chemical structure after the rigorous chemistry of the synthesis process. Though the growing oligonucleotide is chemically protected at various points during synthesis, there are sites at which the very reactive synthesis chemicals can attack. Because modification can have a marked affect on biological activity, assessing chemical authenticity is important when characterizing a purified synthetic oligonucleotide.

At Applied Biosystems, we have developed a reliable and efficient assay to quantify chemical authenticity[20]. After purification, we enzymatically degrade the oligonucleotide to its constituent nucleosides with snake venom phosphodiesterase and phosphatase. This nucleoside mixture is then injected onto a reverse phase HPLC system that efficiently separates the desired nucleosides and any modified nucleosides. The sensitivity of the chromatography is sufficient enough to detect modifications at the 0.05% level (in relation to total UV absorbance). See Appendix 2.12 for this assay procedure.

*Figure 40*




*An autoradiogram of a Maxam-Gilbert sequencing gel.*

Typical results of this assay are shown in Figures 41 and 42. Figure 41a shows chromatograms of the enzymatic digestion of a 72-mer, purified by gel electrophoresis, synthesized from methyl and β-cyanoethyl phosphoramidites. The results show little, if any, detectable modification in syntheses prepared from these phosphoramidites. The deoxyinosine (dI) present is the result of a contaminant in one of the enzyme preparations that converts deoxyadenosine to dI. The difference in modification between syntheses of this length prepared from the two phosphoramidites is minimal. Figure 41b shows an expanded region of the area around N-3 methyl thymidine to show that, even at a higher sensitivity, there are no detectable amounts produced in either chemistry.

*Figure 41a*

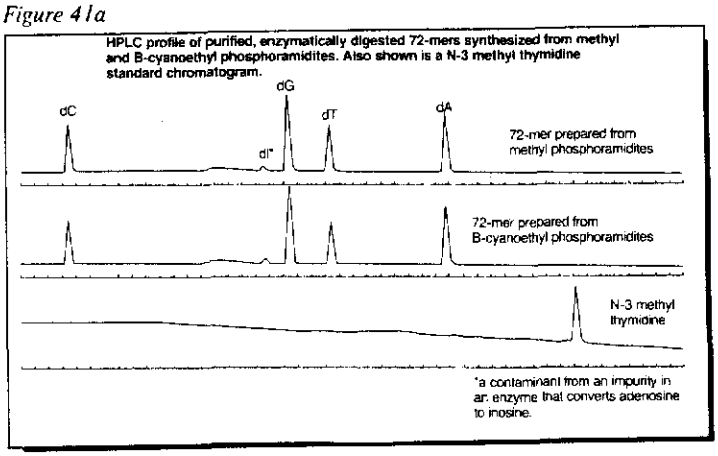
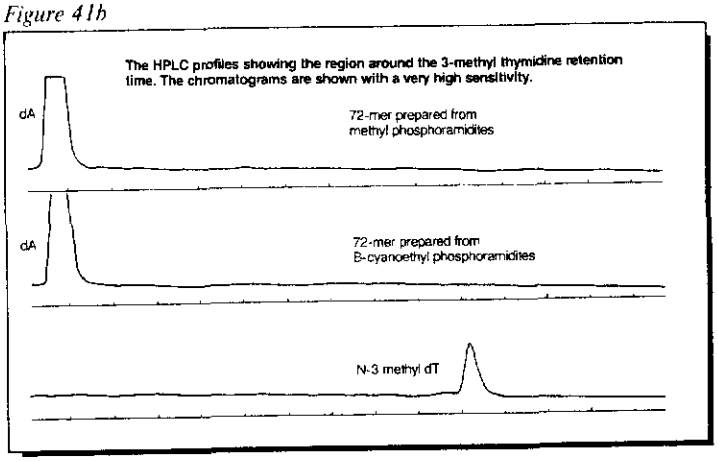



*Figure 41b*



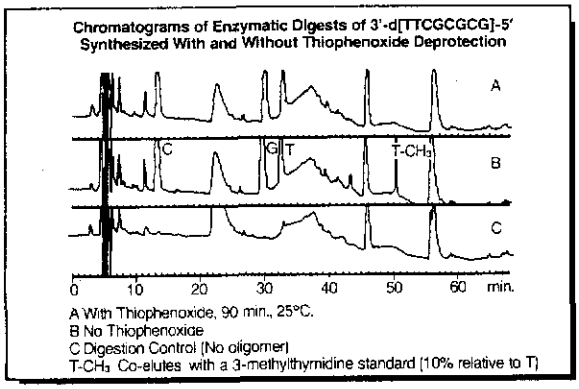

*Chromatograms of the digestion of identical 72-mers synthesized from methyl and β-cyanoethyl phosphoramidites. Also shown is a 3-methyl thymidine standard.*

Figure 42 shows chromatograms of the enzymatic digestion of a purified 8-mer synthesized from methyl phosphoramidites. In one synthesis deprotection was accomplished with thiophenol to remove the methyl phosphate groups prior to ammoniolysis. In the other, deprotection was accomplished with ammonia alone. As the chromatograms show, syntheses prepared from methyl phosphoramidites must be deprotected initially with thiophenol to prevent the methylation of thymidine at the N-3 position.

For base composition to be a verification of an oligonucleotide sequence, the fidelity of the automated synthesizer's performance must be a given. The HPLC method used in the chemical authenticity assay produces quantitative data. These data allow determination of the base composition of a purified oligonucleotide with a high degree of accuracy. Errors from this assay, in syntheses up to the 50-mer range, are less than one base. Hence, this procedure is a fast, efficient means for verifying sequences in this range. Figure 43 demonstrates the accuracy of the assay.

Figure 43a reveals data of several 18-mers that were assayed by this method. The results correlate very well with the theoretical data. Figure 43b shows data from the synthesis of a nested set of oligonucleotides in the 50-mer range. Again, the results correlate well with the theoretical data. The data distinguishes sequences ± one base from the 50-mer *and* a single G to A base substitution.

*Figure 42*




*Digestions of the same 8-mer synthesized from methyl phosphoramidites where deprotection was done with and without thiophenol to demethylate the internucleotide bonds.*

*Figure 43*

**BASE COMPOSITION STUDIES OF SIMILAR 18-MERS**

| | Exp | Theory | Exp | Theory | Exp | Theory | Exp | Theory | Exp | Theory |
|---|---|---|---|---|---|---|---|---|---|---|
| dC | 3.9 | 4 | 7.0 | 7 | 2.0 | 2 | 5.0 | 5 | 3.9 | 4 |
| dG | 4.0 | 4 | 4.0 | 4 | 6.0 | 6 | 3.1 | 3 | 6.0 | 6 |
| dT | 3.0 | 3 | 5.9 | 6 | 5.0 | 5 | 6.0 | 6 | 4.0 | 4 |
| dA | 7.1 | 7 | 1.0 | 1 | 5.1 | 5 | 4.1 | 4 | 4.0 | 4 |

**BASE COMPOSITION STUDIES: ACCURACY BETWEEN SIMILAR OLIGONUCLEOTIDES**

| | 49-mer | | 50-mer | | 50-mer | | 51-mer | |
|---|---|---|---|---|---|---|---|---|
| | Exp | Theory | Exp | Theory | Exp | Theory | Exp | Theory |
| dC | 16.9 | 17 | 17.0 | 17 | 16.9 | 17 | 16.9 | 17 |
| dG | 13.3 | 13 | 13.3 | 13 | 12.2 | 12 | 13.4 | 13 |
| dT | 8.9 | 9 | 9.7 | 10 | 9.8 | 10 | 10.7 | 11 |
| dA | 10.0 | 10 | 10.0 | 10 | 11.1 | 11 | 10.0 | 10 |

*Actual versus theoretical values of base-composition studies with (a) a number of 18-mers, and (b) a nested set of 49 through 51-mers.*

# 5′-END GROUP ANALYSIS

There is always concern that an isolated product is, in fact, the purified, desired species. Major impurities usually are the result of *n-1 mers* that are hard to separate from the desired product due to the high degree of similarity between them. These *n-1 mers* are primarily generated from two sources.

The first is a result of capping unreacted oligonucleotides. This *n-1 mer,* when the penultimate base is different from the 5′-base in the desired sequence, can be distinguished from a full length product by end group analysis[21]. In this process, the purified product is 5′-end labeled and subsequently degraded with snake venom phosphodiesterase to generate a labeled mononucleotide, the 5′-nucleotide of the individual molecules. The individual nucleotides can be easily separated by PEI-cellulose TLC and identified by autoradiography. If there is any nucleotide in the autoradiogram corresponding to the base at the penultimate base position (again, it must be different from the 5′-position), the presence of *n-1 mer* can be verified. See Appendix 2.13 for the protocols.

There can be a problem with the accuracy of this assay due to a second source of *n-1 mers.* They result from non-quantitaive reactions involving detritylation during the synthesis process. With incomplete detritylation, those molecules with a trityl group would not be coupled or capped during the next synthesis cycle and probably would be detritylated during the subsequent detritylation steps. This would cause an internal deletion in the sequence resulting in an *n-1 mer,* but with the *correct* 5′-base. Obviously, these contaminants can not be detected by this assay.

Since no reaction is fully quantitative at every step, the generation of multiple internal deletion *n-1 mers* is probable though their amount is probably quite low due to the high efficiency of detritylation. It is still important to know that they can exist in the crude reaction mixture and to be aware that they are due to incomplete detritylation.

# APPENDICES

## MATERIALS AND EQUIPMENT NEEDED FOR PURIFICATION AND ANALYSIS

Below are lists of instrumentation, general labware and chemicals needed for the methodologies described in this publication. These lists are guides for setting up new laboratories or determining what is needed to analyze and purify synthetic oligonucleotides. The lists do not include basic laboratory supplies.

### 1.1 Instrumentation

1. HPLC system.
2. UV-Vis spectrophotemeter.
3. Savant Speed-Vac.
4. 55°C heating block or bath.
5. Electrophoresis apparatus (sequencing type)
   - Three sets of plate spacers are desirable, they should be approximately 0.4 mm, 0.8 mm and 1.5 mm in thickness.
   - Power supply should have constant power capability with a maximum voltage of no less than 2000 volts.
   - Gel apparatus (Note: DNA sequencing gel apparatus Part No. SG-800-33 from CBS Scientific is highly recommended).
   - Gel sealing tape, cooling plate, metal spring clamps (to clamp plates together) and sample-well combs.

### 1.2 Labware

- Micropipettes (i.e., Pipetman).
- 1-cm Cuvette.
- Disposable microcentrifuge tubes (Eppendorf, polypropylene).
- C-18 Sep-Paks and/or Sephadex G-50 or G-75 cartridges.
- Short-wave (254 nm), hand-held UV lamp.
- Safety Glasses.
- Fluorescent TLC plates.
- Stainless steel tweezers/forceps.
- Plastic film.
- Clean single-edge razor blades.
- BioRad Econo-columns (Part No. 731-1550).
- Hamilton syringe (10 microliters with guide).
- Radioactive safetyware, i.e. shield, carrying box, waste container, etc.
- X-Ray film (Kodak BBL-5 or equivalent), film developing chemicals, film-holder and intensifying screens (Kodak Cronex, Lightning-PLUS).

### 1.3 Chemicals

- Concentrated, fresh ammonium hydroxide.
- Distilled, deionized water.
- Triethylamine.
- 0.1 M Toluene sulfonic acid monohydrate in acetonitrile.
- 95% Ethanol
- Reagent grade formamide.
- Mixed-bed ion exchange resin (Sigma Part No. MB-1A).
- Xylene cylanol blue.
- Bromophenol blue.
- Ammonium acetate.
- HPLC grade methanol.

# PROTOCOLS

## 2.1 Manual Detritylation of Oligonucleotides after Deprotection

1. Evaporate to dryness the trityl-bearing, deprotected sample.
2. Treat the evaporated sample, which could be an oil, with approximately 300 microliters of 80% acetic acid at room temperature for twenty minutes.

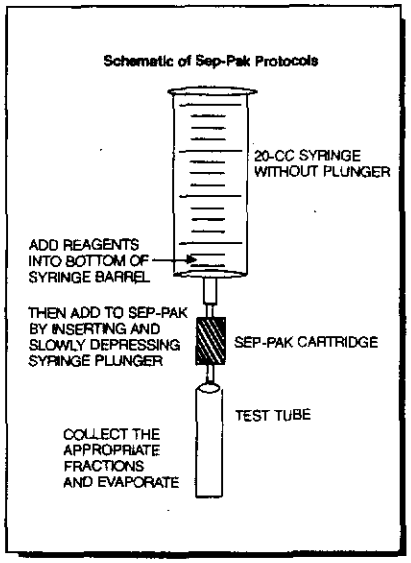
   NOTE: This acidic treatment does not cause depurination because the bases are much more stable to acid after deprotection.

3. Add an equal volume of ethanol and evaporate the solution (again the resulting sample could be an oil).

   NOTE: Several co-evaporations with distilled deionized water may be necessary to remove the remaining volatile salts apparent by the presence of white precipitate in the sample.

4. The hydrolyzed trityl group can be removed by organic aqueous extraction, ethanol precipitation, or de-salting on Sephadex or Sep-Pak chromatography columns.

*Figure 44*




*A schematic showing the protocol for using Sep-Pak cartidges.*

## 2.2 De-salting Procedure Using C-18 Sep-Pak Cartridges

This procedure is a general method used to de-salt oligonucleotides. It is very useful for the de-salting of crude reaction mixture prior to analysis and purification.

1. Flush a C-18 Sep-Pak cartridge with 10 mL of HPLC grade methanol using a syringe, then flush with 10 mL of distilled, deionized water.
2. Resuspend the dried trityl-on oligonucleotide in 3 mL of 50 mM triethylammonium acetate (TEA-Ac).
3. Connect a 20-cc sterile syringe, with the plunger removed, to the long end of the Sep-Pak cartridge (see Figure 44).
4. Add the oligonucleotide solution into the syringe, insert the plunger and slowly inject the solution onto the cartridge.
5. Elute the salts from the crude mixture by adding 20 mL of distilled, deionized water using the syringe.
6. Elute the oligonucleotide mixture with 3 mL of TEA-Ac/ methanol, 1:1 and collect as 1-mL fractions.
7. Evaporate the 1-mL fractions containing the de-salted oligonucleotide to dryness, rinse with 90% ethanol and re-dry.
8. The oligonucleotide can be quantified and analyzed.

## 2.3 Purification Procedure Using C-18 Sep-Pak Cartridges

This procedure is a quick method to purify trityl-on oligonucleotides. It does not resolve the trityl-bearing species. The trityl-on oligonucleotide is prepared exactly as described on page 29. This protocol has been used successfully with fragments up to 90 bases in length.

1. Flush a C-18 Sep-Pak cartridge with 10 mL of HPLC-grade methanol using a syringe, then flush with 10 mL of distilled, deionized water.
2. Resuspend 25 OD of dried trityl-on oligonucleotide in 3 mL of 50 mM triethylammonium acetate (TEA-Ac).
3. Connect a 20-cc sterile syringe, with the plunger removed, to the long end of the Sep-Pak cartridge (see Figure 44).
4. Add the oligonucleotide solution into the syringe, insert the plunger and slowly inject the solution onto the cartridge.
5. Elute the trityl-off oligonucleotides by adding 10 mL TEA-Ac/acetonitrile, 85:15. Save the eluant for quantification or analysis.
6. Elute the trityl-on oligonucleotide with 3 to 10 mL of TEA-Ac/acetonitrile, 70:30 and collect as 1-mL fractions.
7. Evaporate those fractions containing the trityl-on oligonucleotides to dryness, rinse with 90% ethanol and re-dry.
8. Detritylate the oligonucleotides (See Appendix 2.1) before 5'-end labeling for analysis or ligation.

## 2.4 Ethanol Precipitation

This procedure gives high yields of de-salted oligonucleotide longer than 20 bases in a quantity of greater than 0.1 OD. For compounds that are shorter or are in smaller quantity, the yield may be variable. For extremely small quantities of DNA, a carrier such as tRNA should be used.

1. Evaporate the oligonucleotide in an Eppendorf tube and dissolve in 200 microliters of ammonium acetate (2.5 M, pH 7.0).
2. Add 600 microliters of absolute ethanol (-20°C) and place at -70°C for 15 minutes (an ethanol and dry-ice bath works well).
3. Spin the sample in a microcentrifuge for 10 minutes at 4°C.
4. Remove as much supernatant as possible with a glass pasteur pipette without disturbing the pellet.
5. Rinse the pellet with 1 mL of ethanol (-20°C) by carefully pipetting the reagent down the side of the tube opposite the pellet.
6. Re-spin the sample for 30 seconds.
7. Carefully remove the ethanol and evaporate the pellet to dryness.
8. Store the oligonucleotide at -20°C.

## 2.5 Quantification of DNA by UV Absorption at 260nm

1. Evaporate the ammonia solution containing the sample to dryness. If the ammonia solution is transferred into a different vial or if an aliquot is taken, the solution should be thoroughly chilled prior to the transfer to reduce the pressure in the vial.
2. Reconstitute the evaporated oligonucleotide in 1 mL of 10 mM triethylamine bicarbonate (TEAB) buffer, pH 7.0 to 7.4.
3. Dilute the sample to give an absorbance reading of between 0.2 and 1.2 OD. Generally, if 1% of the total crude material from a one-micromole synthesis is dissolved in 1 mL of TEAB buffer, the absorbance reading will fall within this range. Another method of determining the dilution factor is to multiply the length of the sequence by 10. For example, a 50-mer, dissolved in 1.0 mL, could be diluted 1:500 by removing 4 microliters and diluting to 2.0 mL with TEAB buffer.
4. Measure the absorbance in a 1-cm quartz cell at 260nm. The cell should be cleaned after each use in order to prevent cross-contamination between samples.
5. OD = (original volume in mL)(dilution factor)(absorbance)

   Example : For a 50-mer

   Total OD = (1 mL) (500) (0.5) = 250

## 2.6 Manual Method for Ammonia Cleavage and Deprotection

1. Remove the synthesis column from the instrument and attach it to a Luer-tippped 1-mL syringe.
2. Attach the other end of the column to a male-to-male Luer connector fitted with a syringe needle.
3. Add concentrated ammonium hydroxide (29%) to the column, using the syringe, so that the column is completely filled and there is a minimum of reagent drawn into the syringe barrel.
4. Insert the syringe needle into a large (#10) rubber stopper.
5. After 30 minutes, expel the ammonium hydroxide into a small vial with a tight-fitting screw cap.
6. Repeat steps 3, 4 and 5 two additional times, adding the ammonium hydroxide solution into the same vial.

   NOTE: There may be positive pressure inside the syringe system during ammonia treatment. This can cause leakage of the ammonium hydroxide, now containing oligonucleotide, out of the needle after removal. This can be minimized by using ammonium hydroxide at room temperature and by slightly withdrawing the syringe barrel as the needle is removed from the stopper.

## 2.7 5′-End Labeling

This procedure can be used at different sample concentrations depending upon the quantity and length of oligonucleotide to be labeled. Variations to this methodology are also used for phosphorylating oligonucleotides for ligations and for use as probes in hybridization experiments.

1. Evaporate each sample to dryness in a clean Eppendorf tube, rinse with ethanol and redry.
2. Prepare a 10x 5′-kinase buffer, consisting of the following reagents:

   700 mM Tris-HCl, pH 7.6
   100 mM $MgCl_2$
   1 mM KCl
   50 mM Dithiothreitol (DTT)

   NOTE: This buffer can be prepared in quantity and stored in 1 mL aliquots at -20°C. To avoid poor results, individual aliquots should be discarded after they have been thawed.
3. Add to each of the samples, placed on ice, the following reagents:

   1 microliter 10x 5′-kinase buffer
   1 microliter unlabeled ATP, 660 M
   1 microliter spermidine, 10 mM
   1 microliter gamma $^{32}P$ labeled ATP, >1000 Ci/mmole
   5 microliters distilled, deionized water

   NOTE: A stock solution of 5′-labeling reaction solution can be made by multiplying the volume of each reagent by the number of samples to be labeled (plus one). Mix the solution before addition.
4. Vortex the individual samples and place on ice.
5. Add $T_4$ kinase (1 microliter, approximately 2 units) to each reaction mixture and spin in a microcentrifuge.
6. Incubate the samples at 37°C for 30 minutes.
7. Add 25 microliters of 98% deionized formamide, containing 0.1% bromophenol blue, 0.1% xylene cylanol blue and 10 mM EDTA to each sample prior to loading on a gel.

## 2.8 3′-End Labeling

1. Prepare a stock solution of 10x 3′-end-labeling buffer with the following reagents:

   1.4 M Sodium Cacodylate*
   10 mM Cobaltous Chloride
   1 mM Dithiothreitol
   Adjust to pH 7.5 with HCl

   *WARNING: This chemical is carcinogenic, handle with care.

2. Evaporate 2 pmol of oligonucleotide in an Eppendorf-type tube and rinse with 100 microliters of 95% ethanol and redry.

3. Add to each of the samples, placed on ice, the following reagents:

   1 microliter of 10X 3′end-labeling buffer
   1 microliter of [alpha-$^{32}$P]-cordycepin-5-triphosphate (New England Nuclear No. NEE-114, specific activity >5000 Ci/mmole)
   7 microliters of deionized water
   1 microliter of TDT (ENZO Biochemicals, 5 units/microliter)

4. Microcentrifuge the reaction to the bottom of the tube.

5. Incubate the reaction at 37°C for one hour.

6. Add 25 mL of a solution containing 80% deionized formamide, 1mM EDTA to each sample. Bromophenol blue and xylene cylanol may be added as marker dyes to *the formamide prior* to addition to the sample.

   NOTE: The 3′-label retards the migration of the oligonucleotide by approximately one-half of a base in the gel.

   NOTE: To minimize the possible interference that the marker dyes may cause to the electrophoresis of the sample, it is recommended that the dye-formamide mix be diluted (approximatley 1:10) with additional deionized formamide).

## 2.9 Gel Staining

This protocol has been used to stain gels that are between 0.4-mm to 1.0-mm thick. The detection level of the stain is approximately 100 picomoles per lane (with 1.5-cm wells). However, the detection of sample in the gel will be dependent on the quality of the synthesis. It is recommended that two or more lanes be loaded with different amounts of sample in each in order to get good visualization of the sample.

1. After electrophoresis, carefully remove the gel from the glass plates. This can be more easily achieved if the plates have been siliconized before the gel was poured (Pierce Surfasil works well).

2. Soak the gel with Stains-All solution in the dark for 30 minutes to 1 hour. The gel should be flat and completely submersed in the dye solution. It is best to use a glass dish for this. A polypropylene tub works although it stains with time.

3. Drain the Stains-All solution, then destain the gel by adding 400 mL of deionized water. Soak the gel until the purple background diminishes, leaving the stained bands visible (about 30 minutes).

4. Dry the gel using a conventional gel dryer.

## 2.10 Recovery of DNA from a Gel Slice Using a Sep-Pak Cartridge.

This procedure is used to recover purified oligonucleotides from gel slices after UV shadowing.

1. Place the gel slices in a fritted Econo-Column (BioRad) or a plugged 10 mL syringe.
2. Add 3 mL of Sep-Pak loading buffer (100 mM tris, 0.5 M NaCl, 5 mM EDTA, pH = 8.0) to the column. Seal the top of the column with a cap or paraffin film.
3. Vortex the sample vigorously and then incubate at 60°C overnight.
4. Flush a C-18 Sep-Pak cartridge with 10 mL of HPLC grade methanol using a syringe, then flush with 10 mL of distilled, deionized water.
5. Connect the Sep-Pak cartridge to the column (See Figure 44).
6. Attach a 30-mL syringe to the bottom of the Sep-Pak cartridge and draw the liquid out of the column onto the Sep-Pak cartridge.
7. Rinse the gel slices with additional loading buffer (3 x 1 mL), and add to the Sep-Pak cartridge.
8. Remove the salts by addition of 20 mL of distilled, deionized water.
9. Elute the oligonucleotide with 3.0 mL of 100mM TEA-Ac (pH 7.3) and methanol, 1:1, and collect in three Eppendorf tubes.
10. Evaporate the oligonucleotide, rinse with 90% ethanol, and redry.

NOTE: Samples which have been eluted from polyacrylamide often contain extraneous acrylamide. This can easily be removed by ethanol precipitation.

## 2.11 Procedure for Sequencing Oligonucleotides (5 to 150 bases)

Part 1. 5′-End Labeling of the Oligonucleotide

1. Add to 100 to 300 picomoles of evaporated, de-salted oligonucleotide the following set of reagents:

   2 microliters of 10x 5′-kinase buffer
   1 microliter of 10 mM Spermidine
   1 to 5 microliters of gamma-$^{32}$P-ATP, specific activity = 1000 Ci/mmole, concentration 1-5 pmol/ L
   deionized, distilled water to 19 microliters.

   NOTE: Unlabeled ATP is not required with purified compounds since preferential labeling of minor species is not a problem

2. Add 1 microliter of $T_4$ kinase solution containing at least 8 units of activity to the mixture.
3. Gently mix the sample and spin in a centrifuge very briefly to bring contents to the bottom of the tube.
4. Incubate the mixture at 37°C for 60 minutes.
5. Stop the reaction by ethanol precipitation using 1 microliter of 2 M NaCl and 100 microliters 95% ethanol.
6. Spin the sample in a microcentrifuge for seven minutes, decant, and check for the presence of radiolabeled oligonucleotide using a Geiger counter.
7. Dry the sample and reconstitute in 35 microliters of distilled, deionized water.

Part 2. The Sequencing Reactions

It is recommended that initially, each of the reactions be done separately. With experience, reactions may be performed simultaneously, however, timing of the various steps is extremely critical for good results.

*The dC/dT Specific Reactions*

1. Add 10 microliters of the radiolabeled oligonucleotide solution to 10 microliters of distilled, deionized water in an Eppendorf tube.
2. Add 30 microliters of hydrazine. To minimize the oxidation of hydrazine, aliquots can be sealed in small vials to prevent frequent opening of the bottle.
3. Vortex the sample and microcentrifuge for a few seconds.
4. Incubate the sample at 37°C for 20 minutes.
5. Stop the reaction with 200 microliters of 0.3 M sodium acetate, pH 6.0, 10 microliters of tRNA (1 mg/mL) and 1.25 mL of ethanol (-20°C).
6. Label the tube clearly "C + T".

*dG/dA Specific Reaction*

1. Add 10 microliters of the radiolabeled oligonucleotide to a Eppendorf tube which contains 50 microliters of 88% formic acid.
2. Vortex the sample and microcentrifuge for a few seconds.
3. Incubate the sample at 37°C for 20 minutes.
4. Stop the reaction with 100 microliters of 0.3 M sodium acetate, pH 6.0, 10 microliters of tRNA (1 mg/mL) and 850 microliters of 95% ethanol (-20°C).
5. Clearly label the tube "G + A".

*The dC Specific Reaction*

1. Add 5 microliters of the radiolabeled oligonucleotide to 15 microliters of 5 M NaCl.
2. Add 30 microliters of hydrazine.
3. Vortex the sample and microcentrifuge for a few seconds.
4. Incubate the sample at 25°C for 30 minutes.
5. Stop the reaction with 200 microliters of 0.3 M sodium acetate, pH 6.0, 10 microliters of tRNA (1mg/mL) and 1.25 mL of 95% ethanol (-20°C).
6. Clearly label the tube "C".

*The dG Specific Reaction*

1. Add 5 microliters of the radiolabeled oligonucleotide to 200 microliters of 50 mM sodium cacodylate buffer, pH 8.0 with 1 mM EDTA.
2. Add 1 microliter of reagent-grade dimethyl sulfate.
3. Vortex the sample and microcentrifuge for a few seconds.
4. Incubate the sample at 25°C for 20 minutes.
5. Stop the reaction with 50 microliters of a 1.5 M sodium acetate, (pH 7), 1.0 M mercaptoethanol buffer, 10 microliters of tRNA (1 mg/mL) and 1.25 mL of 95% ethanol.
6. Clearly label the tube "G".

Part 3. The Cleavage Reactions
(These steps are done with all four reactions.)

1. Remove the salts and reagents by ethanol precipitation (See Appendix 2.4).
   NOTE: The supernatants from the dC/dT and dC reactions should be put into a waste bottle containing 2M ferric chloride to destroy the hydrazine. The supernatant from the dG reaction should be put in a waste bottle containing 0.5 M NaOH to neutralize the dimethyl sulfate. It should be noted that at every decanting step, the tube containing the pellet should be measured by a geiger counter to confirm that the radiolabeled sample has remained in the tube.
2. Dissolve the samples in 100 microliters of 1 M (10%) freshly diluted piperidine.
3. Vortex the samples and securely cover the Eppendorf tube.
4. Incubate the samples at 90°C for 45 minutes. It may be necessary to place a weight on top of the tubes to keep them securely in the heating block.
5. Remove the tubes from the heating block and place in an ice bath.
6. Microcentrifuge the samples briefly to bring all the liquid to the bottom of the tube.
7. Freeze the samples with dry ice and then evaporate (This may take four hours or longer).
8. After centrifugation, remove the residual piperidine that coats the inside of the tubes by adding 50 microliters of distilled, deionized water to the bottom of each tube.
9. Dissolve the DNA gently by pipeting the sample once or twice and remove to a sterile, labeled Eppendorf tube. This rinsing process should be repeated until the pelleted DNA appears small, white and compact.
10. Prepare samples for loading on the sequencing gel by dissolving the material in each tube in 10 microliters of a solution containing 80% deionized formamide, 10 mM NaOH, 1 mM EDTA, 0.1% xylene cylanol and 0.1% bromophenol blue.
11. Heat the samples at 90°C for two minutes, chill briefly on ice and load immediately on the gel.
12. The sequencing gel used depends on the length of the oligonucleotide sequenced. As in the PAGE analysis of oligonucleotides, sequences that are 50 bases or shorter can be loaded on a 20% acrylamide gel. Sequences between 50 and 100 bases long should be loaded on a 12% gel. If longer sequences are to be run, an 8-10% gel may be used; these gels are hard to manipulate so care should be taken.
13. Pre-warm the gel at 30 to 40 milliamp for 30 minutes prior to loading.
14. Load 2 to 4 microliters of each sample per well.
15. Run the gel at 20 to 25 milliamp, 1200 volts until the bromophenol blue (darker blue dye) has migrated about 15 cm.
16. Load four more lanes and run the bromophenol blue from the first loading to approximately 25 cm from the origin (for a 40-cm long gel).
17. Visualize the gel by autoradiography.

## 2.12 Base Composition Analysis

1. Evaporate 0.1 OD of purified oligonucleotide to dryness.

2. Prepare the following stock digestion solution:

| | |
|---|---|
| 40 microliters | Distilled, deionized water |
| 1.2 microliters | 1 M $MgCl_2$ |
| 5 microliters | 0.5 M Tris pH 7.5 |
| 20 microliters | Bacterial alkaline phosphatase |
| 12 microliters | Snake venom phosphodiesterase |

NOTE: This mixture can be made as a stock solution and added to each sample after mixing. An extra volume of the reaction mixture should always be prepared as a safety precaution for dispensing the correct amount and to use as a control in the subsequent HPLC protocol.

3. Add 78.2 microliters of stock digestion solution to each oligonucleotide and incubate at 37°C overnight.

4. Add 10 microliters of 3 M NaOAc, pH 5.5 to each sample and mix.

5. Add 233 microliters of cold ethanol, mix, and place on dry ice for 15 minutes.

6. Spin the samples in a microcentrifuge at 4°C for 15 minutes.

7. Remove the supernatant *and* save.

8. Precipitate the remaining sample with 678 microliters of cold ethanol, centrifuge and add the supernatant to the first sample.

9. Evaporate the supernatants to dryness and reconstitute in 100 microliters of distilled, deionized water.

10. Inject 50 microliters of each sample onto two Applied Biosystems Spheri-5 reverse phase columns (4.6 mm x 23 cm) joined in series and chromatograph using the following gradient of (A) 50 mM NaPO4 in 5% MeOH, pH 7.0 and (B) 90% aqueous MeOH:

| Time (min) | % B |
|---|---|
| 00.0 | 00 |
| 10.0 | 00 |
| 70.0 | 20 |
| 90.0 | 45 |
| 100.0 | 100 |

## 2.13 5′-End Group Analysis

1. 5′-end label 10 picomoles of purified oligonucleotide.

2. Treat the reaction mixture with 5 units of snake venom phosphodiesterase in 100 mM Tris, pH 8.9, and then incubate at 37°C overnight.

3. Apply the reaction mixture to a PEI-cellulose TLC plate (20 x 20 cm) and chromatograph with 1 M LiCl as the mobile phase.

4. Dry the TLC plate and visualize by autoradiography (exposure time is very short; approximately 2 minutes).

5. Determine the respective bases by position on the TLC plate. The order is C>A>T>G.

# BUFFERS

**3.1** 10 x TBE (dilute to 1x as needed)

| | |
|---|---|
| 90 mM Tris | 107 g |
| 90 mM Boric acid | 55 g |
| 2 mM EDTA | 8.2 g |
| distilled water | to 1 liter of solution |

**3.2** 10x 5′-Kinase Buffer

700 mM Tris-HCl, pH 7.6
100 mM $MgCl_2$
1 mM KCl
50 mM Dithiothreitol (DTT)

**3.3** 10x 3′-End-Labeling Buffer

1.4 M Sodium Cacodylate*
10 mM Cobaltous Chloride
1 mM Dithiothreitol, pH 7.5 with HCl

*WARNING: This chemical is carcinogenic, handle with care.

**3.4** Stains-All Solution

| | |
|---|---|
| Stains-All dye | 50 mG, Aldrich (Part No. 23-096-0) |
| Deionized formamide | 200 mL |
| Deionized water | 200 mL |

Filter through Whatman 1 paper

**3.5** Sep-Pak Loading Buffer

100 mM TRIS
0.5 M NaCl
5 mM EDTA, pH = 8.0

**3.6** Sep-Pak Elution Buffer

50% 100 mM TEA-Ac, pH 7.3
50% MeOH

**3.7** Sample Loading Buffer for Gel Electrophoresis

80% deionized formamide
10 mM NaOH
1 mM EDTA
0.1% xylene cylanol blue
0.1% bromophenol blue

**3.8** Oligonucleotide Digestion Solution

| | |
|---|---|
| 40 microliters | Distilled, deionized water |
| 1.2 microliters | 1 M $MgCl_2$ |
| 5 microliters | 0.5 M Tris pH 7.5 |
| 20 microliters | Bacterial alkaline phosphatase |
| 12 microliters | Snake venom phosphodiesterase |

**3.9** Deionized Formamide

1. 5 g of mixed bed resin (Sigma Part No. MB-1A) was added to 100 mL of formamide.
2. Stir for 30 minutes.
3. Filter and store at room temperature.

**3.10** Stock Acrylamide — Bis Solution (dilute to the proper percentage of acrylamide as needed)

| | |
|---|---|
| Acrylamide | 380 g |
| Bis-acrylamide | 20 g |
| distilled water | to 1 liter of solution |

**3.11** TEAB

1. Bubble $CO_2$ through a 900 mL aqueous mixture containing 1.0 mole of triethylamine (TEA) prepared with distilled water. Chill the solution in an ice bath. The $CO_2$ is added by placing dry ice in a separate flask with a tube that extended into the TEA-water mixture.
2. This mixture forms two phases; the TEA on the top and water on the bottom. As the water soluble TEAB forms, the top layer decreases and eventually disappears.
3. Dilute the solution to 1.0 M, adjust to pH 7.0 (with TEA or $CO_2$), and store at 4°C.

**3.12** TEA-Ac

1. Prepare a stock solution of 1.0 M TEA-Ac by dropwise addition of 1 mole of triethylamine into a aqueous solution (500 mL) containing 1 mole of acetic acid in an ice bath.
2. Dilute the solution to 1.0 liter and adjust the pH to 7.0 (with TEA or HOAc). This stock can be diluted to a 0.1 M solution as needed.

# GLOSSARY

**ACETYLATION**–the process of adding an acetyl group ($CH_3C(0)$), which is labile to strong base. It is the capping reagent used to block oligonucleotide chains that have not coupled during synthesis, hence they are blocked from further reactions.

**AUFS**–Absorbance Units Full Scale.

**ATP (adenosine triphosphate)**–the phosphate donor in the enzymatic phosphorylation of the 5′-hydroxyl position of DNA by $T_4$ kinase.

**AMINOLINK 1**–the Applied Biosystems reagent for derivatization of the 5′-hydroxyl position of DNA, yielding a 5′-amino moiety.

**AMMONIOLYSIS**–the chemical process of DEPROTECTION of the base-labile protecting groups on the DNA strand.

**APURINIC SITE**–positions on a DNA strand where the purine base, guanine or adenine, has been chemically removed *and* the phosphate backbone is still intact.

**AUTORADIOGRAM**–a visualization of the separation of radiolabeled components in PAGE by using X-ray film.

**BASE MODIFICATION**–the process by which individual bases are chemically modified during synthesis *or* deprotection; can cause a significant loss in biological activity.

**BEER'S LAW**–the fundamental equation of spectrophotometry where the absorbance (A) at wavelength $\partial$, is proportional to the pathlength of the spectrophotometer cell (d), the concentration of the sample (c) and the extinction coefficient of the sample ( $\Sigma$ ) at $\partial$, by the equation $A = \Sigma dc$.

**β-CYANOETHYL**–a phosphate protecting group used in phosphoramidite chemistry that is efficiently removed by ammonium hydroxide.

**BIOLOGICAL ACTIVITY**–the ability of a synthetic piece of DNA to function in a biological system.

**CAPPING**–see ACETYLATION

**COMPETITIVE COUPLING**–the phenomenon of equivalent coupling of each base at a mixed-base site that results in stoichiometrically correct amounts of the individual DNA strands.

**CONTROLLED PORE GLASS (CPG)**–the solid support matrix used in DNA chemistry. Through a special process, the pores of this bead can be controlled with regard to diameter and structure.

**CORDECYPIN**–an analog of deoxy-ATP, where the 3′-position of the sugar has a hydrogen moiety, hence it cannot participate in further chain elongation by an enzyme after incorporation.

**CRUDE PRODUCT YIELD**–the amount of DNA cleaved from the CPG solid phase support after synthesis and deprotection.

**CRUDE REACTION MIXTURE**–the array of compounds present after cleavage of the DNA from the solid phase support.

**DELETION SEQUENCE**–those compounds in a crude reaction mixture that failed to couple in a specific synthesis cycle.

**DEMETHYLATION**–chemical cleavage of the internucleotide methyl phosphate protecting group by thiophenol.

**DENATURATION**–the physico-chemical process of breaking base-pairing, either inter- and intra-strand in nature. Can be accomplished by increased temperature, or chemically by urea or formamide.

**DEOXYADENOSINE, DEOXYGUANOSINE, DEOXYCYTIDINE, THYMIDINE**–the basic building blocks of DNA, the sequence of which carries the genetic information.

**DEPROTECTION**–chemical removal of the protecting groups used in the synthesis process, i.e., benzoyl, DMT, methyl, etc.

**DEPURINATION**–the chemical process in which purine bases are cleaved from the DNA strand by acid. Results in APURINIC SITES.

**DE-SALTING**–the process of removing extraneous salts from a mixture.

**DETRITYLATION**–the chemical removal, by acid, of the 5′-protecting group (DMT) of DNA during synthesis.

**DIMER**–two *mono*mers, in the case of DNA, dA, dG, dC or T, covalently bonded together.

**DMT** (4, 4′-dimethoxy-triphenylmethyl)–the acid-labile 5′-hydroxyl protecting group used in DNA synthesis.

**ELECTROPHORESIS GEL**–the porous matrix used to separate compounds based on charge and size.

**END-GROUP ANALYSIS**–an enzymatic and chromatographic process of identifying of the 5′-base of a DNA strand.

**ETHANOL PRECIPITATION**–a chemical process where salts and protein can be easily and quickly removed from DNA.

**EXOCYCLIC AMINES**–the amino groups bound to the C-4 position of deoxycytidine, the C-2 position of deoxyguanosine, and the C-6 position of deoxyadenosine.

**GLYCOSIDIC BOND**–the chemical bond between the deoxyribose sugar and its respective base.

**HOMODECAMERS**–10-mers containing only one base throughout the sequence.

**HOMOPURINE**–DNA consisting solely of the purines A and G.

**HOMOPYRIMIDINE**–DNA consisting solely of the pyrimidines C and T.

**HYDROPHOBIC**–literally, "water hating": that which is non-polar, i.e., alkyl, aromatic, etc., rather than polar.

**INTER-STRAND BASE PAIRING**–the phenomenon where two DNA strands base-pair by Watson-Crick rules (A-T and G-C).

**INTRA-STRAND BASE PAIRING**–the phenomenon where one DNA strand forms base pairs with itself, i.e., hairpin formation

**ION-EXCHANGE CHROMATOGRAPHY**–an HPLC method in which the 5′-DMT group is removed. Separation is based on the differences in the net charge of the individual components in the crude reaction mixture.

**KINASED**–the enzymatic process of phosphorylation of the 5′-hydroxyl position of DNA.

**LIGATION**–the enzymatic process of joining two double-stranded DNA's.

**METHYL**–a phosphate protecting group used in phosphoramidite chemistry that is efficiently removed in thiophenol.

**METHYLATION**–the covalent attachment of methyl groups to DNA, primarily on the bases.

**MIXED-BASE SITES**–specific sites on the DNA strand where more than one base is purposefully added to result in the synthesis of a nested set of DNA strands of similar DNA sequence and identical length.

**MOBILE PHASE**–the liquid phase associated with HPLC.

***n-MER* OLIGONUCLEOTIDE**–the desired synthetic piece of DNA.

***n-1 MER***–a contaminant of synthetic DNA that is one base shorter than the desired piece.

**OLIGONUCLEOTIDE**–a short piece of single-stranded DNA, usually less than 20 bases. See note on inside front cover.

**OD (optical density unit)**–that amount of a substance dissolved in 1.0 mL which will give an absorbance reading of 1.00 in a spectrophotometer with a 1-cm path length. See BEER'S LAW.

**PALINDROMIC SEQUENCES**–a DNA sequence whose complement is itself, i.e., "GGAATTCC".

**PENULTIMATE BASE**–the base on a DNA strand immediately before the 5′-position.

**PHOSPHATE GROUPS**–the internucleotide bonds of DNA that is a component of its backbone.

**PHOSPHORAMIDITE**–a trivalent phosphorus compound containing a phosphorus-nitrogen bond.

**POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)**–a process that separates compounds based on charge and size.

**POLYNUCLEOTIDE**–a DNA strand longer than 20 bases. See note on inside front cover.

**POST-SYNTHESIS STRATEGY**–a designed set of protocols to analyze, purify and characterize a specific piece of synthetic DNA.

**PURINE**–a polyaromatic, nitrogenous base found in DNA, i.e., adenine and guanine.

**PYRIMIDINE**–a nitrogenous, aromatic base found in DNA, i.e., cytosine and thymine.

**REVERSE PHASE CHROMATOGRAPHY**–in HPLC, where the solid support is bonded with hydrophobic groups.

**SECONDARY STRUCTURES**–see INTRASTRAND BASE PAIRING.

**SELF-COMPLIMENTARY**–the ability of single-stranded DNA to base-pair with itself. See INTRA-STRAND BASE-PAIRING.

**SEQUENCE**–to ascertain the linear order of the bases in a DNA strand.

**SEQUENCER, DNA**–an automated instrument for DNA sequencing.

**SIZE EXCLUSION CHROMATOGRAPHY**–a chromatographic process where separation is based on size.

**SOLID PHASE DNA SYNTHESIS**–synthesis of DNA where the growing chain is bound to an insoluble support and the reagants are added in solution.

**SOLUTION PHASE DNA SYNTHESIS**–synthesis of DNA where all the components of each reaction are in solution. Requires purification after each reaction.

**SPONTANEOUS DETRITYLATION**–a phenomenon that occurs with CPG during storage in which the DMT group on the support-bound nucleoside is cleaved.

**STEPWISE YIELD**–the yield at each step, or nucleotide (base) addition, in the synthesis of an oligonucleotide.

**STERIC**–pertaining to size.

**STRAND SCISSION**–the cleavage of oligonucleotides at apurinic sites by ammonium hydroxide during deprotection.

**SUPPORT-BOUND NUCLEOSIDE**–the nucleoside bound to the CPG support. Becomes the 3′-base of the synthetic oligonucleotide.

**SYNTHESIS CYCLE**–a specific set of automated operations that carry out the chemistry required to efficiently add one nucleotide *and* to prepare that coupled nucleotide for the next cycle.

**TEMED**–the polymerizing agent used in preparing electrophoresis gels.

**TETRAZOLE**–the reagent that activates phosphoramidites for coupling to the growing oligonucleotide chain.

**$^{32}$P KINATION**–the enzymatic attachment of a radioactive $^{32}$P phosphate moiety to the 5′-hydroxyl position of an oligonucleotide by $T_4$ kinase and ATP.

**TLC (thin layer chromatography)**–chromatography in which the solid support is bound to a glass plate and the mobile phase is transported by capillary action.

**THYMIDINE DIMERIZATION**–a phenomenon that occurs in UV light where the bases of adjoining dT residues on the same DNA strand are covalently bound to each other.

**TCA (trichloroacetic acid)**–a reagent that removes the DMT group during synthesis.

**TRITYL**–See DMT.

**TRITYL-OFF CHROMATOGRAPHY**–a reverse phase HPLC method in which the 5′-DMT group is removed. Separation is based on the slight hydrophobicity differences of the individual components in the crude reaction mixture.

**TRITYL-ON CHROMATOGRAPHY**–a reverse phase HPLC method in which the 5′-DMT group is still attached. Separation is based on the large hydrophobicity differences between DMT- and non-DMT-bearing compounds in the crude reaction mixture.

**TRIVALENT PHOSPHORUS INTERNUCLEOTIDE TRIESTER**–the acid-labile internucleotide bond prior to oxidation to its normal pentavelant form.

**TRUNCATED SEQUENCES**–those shorter compounds in a crude reaction mixture formed from non-quantitatve coupling and subsequent capping.

**UV SHADOWING**–a visualization process in which the DNA in the gel after PAGE is identified and excised for purification.

**VOLATILE SALTS**–salts that can be removed by evaporation or lyophilization.

**"WET" ACETONITRILE**–acetontrile that contains amounts of water >300 ppm and is detrimental to DNA synthesis.

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In addition to the above, please refer to DNA Synthesis *User Bulletin 17-Revised January 17, 1987* for a more comprehensive bibliography. *User Bulletin 17-Revised January 17, 1987* is also available from your Applied Biosystems representative or from any of the offices shown on the back cover of this Bulletin.

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# USER BULLETIN

Model No. 380
Issue No. 17 (Revised)
June 30, 1987

SUBJECT: Annotated Bibliography of Publications Involving Oligonucleotides made with Applied Biosystems DNA Synthesizers and/or Chemicals

## Introduction

Enclosed you will find an updated annotated bibliography of papers that use oligonucleotides from Applied Biosystems DNA synthesizers and/or chemicals. As this document is in its second revision and has grown larger, we have decided to reformat it based on specific use of the oligonucleotides in each paper. The bibliography was divided by the following categories of use: 1) primers, 2) probes, 3) gene constructs, 4) chemistry/biochemistry, 5) site-directed mutagenesis, and 6) physical studies. The new publications are denoted by _____. Also, we have included an index of authors by page number. Hopefully, this format will make it easier to find the publications of greater interest.

An interesting trend appeared regarding probes when reviewing the new publications. Seven out of the fourteen new publications useed primer extension with Klenow and radiolabeled nucleotides to produce a higher specific-activity probe. One (Kaul, *et al.*) used a double-stranded probe where both strands were extended from oligonucleotides that were internally complimentary.

Some other new publications of interest were 5'-chemical phosphorylation procedures, new biotinylation procedures, a new electrophoresis concept, three describing gene synthesis and one on non-enzymatic sequence-specific cleavage of DNA.

The electrophoresis publication (Myers, *et al.*) decsribes the use of denaturant gradient gels that can resolve homoduplexes of wild-type and mutant DNA's *and* the wild-type-mutant heteroduplex, where the mutant is a single base substitution.

The sequence-specific cleavage publication (Chu and Orgel) decsribes the attachment of and EDTA moiety to the 5'-end of a synthetic oligonucleotide. After hybridization of the EDTA-oligonucleotide to its compliment, ferrous ions are added to the solution that are chelated by the EDTA. In the presence of dithiothreitol, singlet oxygen is produced, which cleaves the complimentary strand. Although the cleavage is random, the concept is a very interesting one if the cleavage can be controlled to yield DNA products that can be manipulated, i.e., bearing 5'-phosphates and 3'-OH's.

This document is testimony to the quality of DNA made on Applied Biosystems DNA synthesizers. If you have any published work using DNA from our synthesizers, please send them to Bob Ellis, Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, California, 94404. Also, if you have any published work on applications of synthetic DNA, we have an ongoing Application Note program where we regularly publish approved works. Again, send them to Bob.

## PRIMERS

Amstutz, H., Muz, P., Heyer, W.-D., Leupold, U., Kohli, J., Cell 40, 879-886 (1985). *Concerted evolution of tRNA genes: intergenic conversion among three unlinked serine tRNA genes in S. pombe.* (From University of Bern, Institute of General Microbiology, Baltzer-Strasse 4, CH-3012 Bern, Switzerland.)

Summary

A 14-mer was used as a specific sequencing primer which binds to the 5'-flanking sequence of the *sup 3* gene for serine tRNA in *Schizosaccharomyces pombe.*

... ... ... ... ... ...

Arden, B., Klotz, J. L., Siu, G., Hood, L. E., Nature 316, 783-787 (1985). *Diversity and structure of genes of the family of mouse T-cell antigen receptor.* (From California Institute of Technology, Division of Biology, Pasadena, CA, 91125, U.S.A.; City of Hope Medical Center, Division of Cytogenetics, Duarte, CA, 91010, U.S.A.)

Summary

Synthetic DNA was used as primers for dideoxy sequencing.

... ... ... ... ... ...

Concino, M. F., Lee, R. F., Merryweather, J. P., Weinmann, R., Nucleic Acids Research 12, 7423-7433 (1984). *The adenovirus major late promoter TATA box and initiation site are both necessary for transcription in vitro.* (From The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, PA, 19104, and Chiron Corporation, Emeryville, CA, 94608, U.S.A.)

Summary

A 15-mer was used as a primer for confirming the sequence of DNA altered by site-directed mutagenesis. Sequencing was performed by the Sanger dideoxy terminator method. A 21-mer was used as a primer for assaying the extension of cDNA following hybridization to various RNA's.

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Docherty, A.J.P., Lyons, A., Smith, B. J., Wright, E. M., Stephens, P. E., Harris, J. R., Murphy, G., Reynolds, J. J., Nature 318, 66-69 (1985). *Sequence of human tissue inhibitor of metalloproteinases and its identity to erythroid-potentiating activity.* (From Celltech Limited, 244-250 Bath Road, Slough SL1 4DY, U.K.; Cell Physiology Department, Strangeways Research Laboratory, Cambridge CB1 4RN, U.K.)

Summary

A synthetic 69-mer was used to probe a human fetal diploid lung cell genome for the collagenase regulator tissue inhibitor for metalloproteinases. Positive clones were screened with an 18-mer used to prime dideoxy sequencing to find the longest clone.

... ... ... ... ... ...

Edman, J., Ellis, L., Blacher, R., Roth, R., Rutter, W., Nature 317, 267-270 (1985). *Sequence of protein disulphide isomerase and implications of its relationship to thioredoxin.* (From Hormone Research Laboratory, University of California, San Francisco, CA, 94143, U.S.A.; Chiron Corporation, Emeryville, CA, 94608, U.S.A.; Stanford University School of Medicine, Department of Pharmacology, Stanford, CA, 94305, U.S.A.)

Summary

Specific oligonucleotide primers were used for dideoxy sequencing.

... ... ... ... ... ...

Elion, E. A., Warner, J. R., Cell 39, 663-673 (1984). *The major promoter element of rRNA transcription in yeast lies 2 kb upstream.* (From Albert Einstein College of Medicine, Departments of Biochemistry and Cell Biology, Bronx, N.Y., 10461, U.S.A.)

Summary

The transcription of ribosomal RNA was studied using a series of artificial rRNA genes containing a fragment of DNA from the *E. coli* bacteriophage T7 which were introduced into the yeast *Saccharomyces cerevisiae.* The T7 rRNA was detected by primer extension using a labeled 18-mer which was complimentary to a portion of the T7 rRNA as the primer.

... ... ... ... ... ...

NEW

Episkopou, V., Murphy, A. J. M., Efstratiadis, A., Proc. Natl. Acad. Sci. USA, 4657-4661 (1984). *Cell-specified expression of a selectable hybrid gene.* (From Department of Human Genetics and Development, Columbia University, 701 W. 168th St., N.Y., N.Y., 10032.)

Summary

A synthetic oligonucleotide was used as a primer in an experiment to produce chimeric genes that are selectable in a cell specific expression system. The chimera were selectable bacterial genes linked to the promoters of the herpes virus thymidine kinase gene and the rat preproinsulin II gene. This system can be placed into systems for easy assay of cell specific expression such as regulatory sequences and transcriptional factors.

... ... ... ... ... ...

Gritz, L., Abovich, N., Teem, J.L., Rosbash, M., Molecular and Cellular Biology 5, 3436-3442 (1985). *Posttranscriptional regulation and assembly into ribosomes of a Saccharomyces cerevisiae ribosomal protein-galactosidase fusion.* (From Brandeis University, Waltham, MA, 02254, U.S.A.)

Summary

An 18-mer was used to prime a reverse transcription of fusion RNA. The rate of fusion was studied to investigate the regulation of ribosomal protein genes.

... ... ... ... ... ...

Hagblom, P., Segal, E., Billyard, E., So, M., Nature 315, 156-158 (1985). *Intragenic recombination leads to pilus antigenic variation in Neisseria gonorrheae.* (From University of Umea, Department of Microbiology, S-901 87 Umea, Sweden; Scripps Clinic and Research Foundation, 10666 N. Torrey Pines Road, La Jolla, CA, 92037, U.S.A.)

Summary

Synthetic primers were used in the sequencing of several variants of the pilus genes of *Neisseria gonorrheae.*

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NEW

Kobori, J.A., Strauss, E., Minard, K., Hood, L., Science 234: 173-179 (1986). *Molecular Analysis of the Hotspot of Recombination in the Murine Major Histocompatibility Complex.* (From the California Institute of Technology, Pasadena, CA, 91125.)

Summary

Primers were used to sequence the I-B and I-J subregions of the I region of the major histocompatibility complex. The I-J region was especially important to acquire sequence information because of its presumed encoding of the elusive T-cell suppressor factors.

... ... ... ... ... ...

Liu, X.-Y., Maeda, S., Collier, K., Pestka, S., Gene Anal Techn 2, 83-88 (1985). *Rapid identification of genomic interferon genes by sequencing on phage DNA templates.* (From Roche Research Center, Nutley, N.J., U.S.A.; Kumamoto University, Japan.)

Summary

A 20-mer, common to all known α-IFN sequences, was used to prime dideoxy sequencing reactions. It was used in a novel approach in screening and identifying genes or closely related genes from a genomic library by directly sequencing λ-phage DNA.

... ... ... ... ... ...

Luck, D.N., Ngsee, J.K., Rottman, F.M., Smith, M., DNA 5, 21-28 (1986). *Synthesis of bovine prolactin in Escherichia coli.* (From Department of Biochemistry, Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5.)

Summary

Oligonucleotides were used in various experiments to aid in the expression of bovine prolactin in *E. coli.*

... ... ... ... ... ...

Noda, Masaharu, Ikeda, T., Kayano, T., Suzuki, H., Takeshima, H., Kurasaki, M., Takahashi, H., Numa, S., Nature 320, 188-192 (1986). *Existence of distinct sodium channel messenger RNAs in rat brain.* (From Departments of Medical Chemistry and Molecular Genetics, Kyoto University Faculty of Medicine, Kyoto 606, Japan.)

Summary

Synthetic primers were used in the sequencing of cDNAs to investigate the existence of distinct sodium channel mRNAs in rat brains.

... ... ... ... ... ...

Power, M.D., Marx, P.A., Bryant, M.L., Gardner, M.B., Barr, P.J., Luciw, P.A., Science 231, 1567-1572 (1986). *Nucleotide sequence of SRV-1 , a type D simian acquired immune deficiency syndrome retrovirus.* (From Chiron Corporation, Emeryville, CA, 94608, U.S.A.)

Summary

Synthetic primers were used in the sequencing of the SRV-1 virus, a type D simian AIDS retrovirus.

... ... ... ... ... ...

Ratner, L., et al, Nature 313, 277-283 (1985). *Complete nucleotide sequence of the AIDS virus, HTLV-III.* (From National Cancer Institute, Laboratory of Tumor Cell Biology, Developmental Therapeutics Program, Division of Cancer Treatment, Bethesda, MD, 20205, U.S.A.; and Central Research and Development Department, Experimental Station, E. I. DuPont de Nemours and Co., Wilmington, DE, 19898, U.S.A.)

Summary

Synthetic DNA was used as specific primers for dideoxy sequencing.

... ... ... ... ... ...

Rhodes, P.R., Vodkin, L.O., PNAS 82, 493-497 (1985). *Highly structured sequence homology between an insertion element in the gene in which it resides.* (From Plant Genetics and Germplasm Institute, U.S. Department of Agriculture, Beltsville, MD, 20705, U.S.A.)

Summary

Oligonucleotide primers were used to extend the reading of large stable cloned fragments of *Tgm1*, a mobile genetic element found in the recessive allele for the soybean seed lectin. To reduce the likelihood of multiple priming, new primer sites that had less than 60% homology between *Tgm1* and M13 were identified and synthesized.

... ... ... ... ... ...

Sanchez-Pescador, R., Urdea, M.S., DNA 3, 339-343 (1984). *Use of unpurified synthetic deoxynucleotide primers for rapid dideoxy-nucleotide chain termination sequencing.* (From Chiron Research Laboratories, Chiron Corporation, 4560 Horton St., Emeryville, CA, 94608, U.S.A.)

Summary

Synthetic oligonucleotides made with Applied Biosystems phosphoramidites were used as primers for sequencing by the dideoxynucleotide chain terminator method of Sanger. This paper shows that the synthetic oligonucleotides can be used without purification.

... ... ... ... ... ...

Segal, E., Billyard, E., So, M., Storzbach, S., Meyer, T.F., Cell 40, 293-300 (1985). *Role of chromosomal rearrangement in N. gonorrhoeae pilus phase variation.* (From Scripps clinic and Research Foundation, Department of Molecular Biology, La Jolla, CA, 92037, U.S.A.; Max Planck Institute, Department of Molecular Biology, Jahnstrasse 29, D6900 Heidelberg, Federal Republic of Germany.)

Summary

Synthetic oligonucleotides were used as specific primers for dideoxy sequencing.

... ... ... ... ... ...

Seidman, C.E., Bloch, K., Klein, K., Smith, J.A., Seidman, J.G., Science 226, 1206-1209 (1984). *Nucleotide sequences of the human and mouse atrial natriuretic factor genes.* (From Massachusetts General Hospital, Boston, MA, 02114, U.S.A.; Harvard Medical School, Boston, MA, 02115, U.S.A.)

Summary

A synthetic oligonucleotide was used to prime subcones encoding the 3'-end of murine and human atrial natriuretic factor genes. These genes code for a potent vasoactive peptide found in mammalian atria. It was found, with the aid of the oligonucleotide, that each gene consists of 3-coding blocks separated by non-conserved intervening sequences.

... ... ... ... ... ...

Zagursky, R.J., Baumeister, K., Lomax, N., Berman, M.L., Gene Anal. Techn. 2, 89-94 (1985). *Rapid and easy sequencing of large linear double-stranded DNA and supercoiled plasmid DNA.* (From E. I. DuPont de Nemours & Co., Central Research and Development Department, Wilmington, DE; and Bionetics Research, Inc., Rockville, MD, U.S.A.)

Summary

An oligonucleotide primer was used in a novel sequencing method employing supercoiled plasmid DNA and bacteriophage DNA.

## PROBES

Bushman, F.D., Anderson, J.E., Harrison, S.C., Ptashne, M., Nature 316, 651-653 (1985). *Ethylation interference and X-ray crystallography identify similar interactions between 434 repressor and operator.* (From Harvard University, Fairchild Biochemistry Bldg, 7 Divinity Ave., Cambridge, MA 92138, U.S.A.)

Summary

A synthetic oligodeoxynucleotide 14 bases long representing a symmetrical concensus operator for phage *434* was used to probe repressor-operator interaction. The operator sequence was cloned into pEMBL8 and ethylated such that each DNA molecule had an average of no more than one ethyl-phosphate. The *434* repressor was bound and scission of the ethylated DNA was accomplished by heating in alkali. Phosphate residues necessary for protein-DNA interaction were identified as 5' to positions 1, 9, and 10.

... ... ... ... ... ...

Chu, B.C.F., Orgel, L.E., DNA 4, (4), 327-331 (1985). *Detection of specific DNA sequences with short biotin-labeled probes.* (From The Salk Institute for Biological Studies, San Diego, CA, 92138, U.S.A.)

Summary

A simple, general synthesis of nonradioactive DNA probes in which biotin is attached to the 5'-terminal phosphate of an oligodeoxyribonucleotide 16 bases long via an ethylenediamine or hexamethylenediamine linker. The products are stable under normal hybridization conditions. They hybridize to target DNA as efficiently as the underivatized oligodeoxyribonucleotide. Color development, using a commercially available kit, is complete within 3 hours using the biotin-detected method. The sensitivity of detection of homologous DNA with a probe to which biotin was attached via a hexamethylenediamine linker is about one-tenth of that achieved overnight by autoradiography with the corresponding $^{32}$P-labeled probe.

... ... ... ... ... ...

NEW

DasSarma, S., Tischer, E., Goodman, H.M., Science 232, 1242-1244, 1260 (1986). *Plant Glutamine Synthetase Complements a* glnA *Mutation in Escherichia coli.* (From Department of Molecular Biology, Massachusetts General Hospital and Department of Genetics, Harvard Medical School, Massachusetts General Hospital, Boston, MA, 02114.)

Summary

Several oligonucleotides were used as probes and primers in the expression of the glutamine synthase gene from alfalfa in an *E.coli* strain deficient in the *gln* gene. The strain will be used for further protein engineering experiments.

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Docherty, A.J.P., Lyons, A., Smith, B.J., Wright, E.M., Stephens, P.E., Harris, J.R., Murphy, G., Reynolds, J.J., Nature 318, 66-69 (1985). *Sequence of human tissue inhibitor of metalloproteinases and its identity to erythroid-potentiating activity.* (From Celltech Limited, 244-250 Bath Road, Slough SL1 4DY, U.K.; Cell Physiology Department, Strangeways Research Laboratory, Cambridge CB1 4RN, U.K.)

Summary

A synthetic 69-mer was used to probe a human fetal diploid lung cell genome for the collagenase regulator tissue inhibitor for metalloproteinases. Positive clones were screened with an 18-mer used to prime dideoxy sequencing to find the longest clone.

... ... ... ... ... ...

NEW

Domdey, H., Apostol, B., Lin, R., Newman, A., Brody, E., Abelson, J., Cell 39, 611-621 (1984). *Lariat Structures Are In Vivo Intermediates in Yeast Pre-mRNA Splicing.* (From Div. of Biology, California Institute of Technology, Pasadena, CA, 91125, U.S.A.)

Summary

A number of intron-specific and mRNA-specific synthetic oligonucleotides were synthesized to aid investigation of pre-mRNA splicing in yeast. One of the probes was constructed to hybridize with a hypothetical circular RNA from an intron in the ADC-1 actin gene. Analysis of the major species of the intron revealed the presence of a branch RNA linked at the 3`-5` bond and a 2`-5` bond to yield an RNA species in the form of a lariat.

... ... ... ... ... ...

Finn, C.W., Silver, R.P., Habig, W.H., Hardegree, M.C., Zon, G., Garon, C.F., Science 224, 881-884 (1984). *The structural gene for tetanus neurotoxin is on a plasmid.* (From Office of Biologics Research and Review, FDA, Bethesda, MD, 20205, U.S.A.)

Summary

Synthetic DNA probes were used to determine whether the structural gene for the tetanus toxin resides in the *Clostridium tetani* plasmid or the nucleus. A section of six amino acids of the toxin suggested 96 possible 18-mer sequences for the gene. These were prepared in two pools of 32 and 64 sequences and used as probes.

... ... ... ... ... ...

NEW

Garriga, G., Lambowitz, A.M., Cell 38, 631-641 (1984). *RNA Splicing in Neurospora Mitochondria: Self-Splicing of a Mitochondrial Intron In Vitro.* (From The Edward A. Doisy Department of Biochemistry, Saint Louis University School of Medicine, St. Louis, MO, 63104, U.S.A.)

Summary

An oligonucleotide was used in a primer extension experiment to analyze RNA products of in vitro splicings of cytochrome *b* introns. The experiment used a Neurospora mutant which accumulates unspliced mitochondrial precursor mRNA's to identify mitochondrial introns that are self-splicing. Results in this system with the *cyt-b* introns show that these must be facilitated by a protein in vivo.

... ... ... ... ... ...

Gill, P., Jeffreys, A.J., Werrett, D.J., Nature 318, 577-579 (1985). *Forensic application of DNA 'fingerprints'.* (From Central Research Establishment, Home Office Forensic Science Service, Aldermaston, Reading, Berkshire RG 7 4PN, U.K.; University of Leicester, University Rd., Leicester LE1 7RH, U.K.)

Summary

Synthetic oligonucleotides are used as probes in human genomes to detect certain polymorphic loci. The DNA "fingerprints" produced by Southern blot analysis are used by forensic biologists in identifying individuals in crime related areas.

... ... ... ... ... ...

Gottschling, D.E., Cech, T.R., Cell 38, 501-510 (1984). *Chromatin structure of the molecular ends of Oxytricha macronuclear DNA: phased nucleosomes and a telomeric complex.* (From the Department of Chemistry, University of Colorado, Boulder, CO, 80309, U.S.A.)

Summary

The synthetic probe $C_4A_4C_4A_4$ was used to detect repeating sequences which are thought to be important in maintenance of linear chromosomes.

... ... ... ... ... ...

Graham, J.S., Pearce, G., Merryweather, J., Titani, K., Ericsson, L., Ryan, C.A., The Journal of Biological Chemistry 260, 6555-6560 (1985). *Wound-induced proteinase inhibitors from tomato leaves.* (From Washington State University, Pullman, WA 99164, U.S.A.; Chiron Corporation, Emeryville, CA, 99608, U.S.A.; University of Washington, Seattle, WA, 98195, U.S.A.)

Summary

A synthetic 26-base mixed oligonucleotide probe with 64-fold degeneracy was used to identify clones of Inhibitor 1 from a wound-induced tomato leaf cDNA library. The probe coding for the N-terminus of the protein was capable of selecting only for those cDNAs with full length or near full length copies of the open reading frame of Inhibitor I. The probe also hybridized very strongly to 2 of 130 colonies selected by mRNA probes that were isolated from both wounded and unwounded plants.

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Graham, J.S., Pearce, G., Merryweather, J., Titani, K., Ericsson, L.H., Ryan, C.A., The Journal of Biological Chemistry 260, 6561-6564 (1985). *Wound-induced proteinase inhibitors from tomato leaves.* (From Washington State University, Pullman, WA, 99164, U.S.A.; Chiron Corporation, Emeryville, CA, 94608, U.S.A.; University of Washington, Seattle, WA, 98195, U.S.A.)

Summary

A synthetic 17-base mixed oligonucleotide probe with 64-fold degeneracy was used to identify clones of Inhibitor II from a wound-induced tomato leaf cDNA. The probe hybridized to 58 out of the 130 colonies selected by mRNA probes that were isolated from both wounded and unwounded plants. One was further identified by more stringent hybridization conditions after hybrid select translations had identified this clone as hybridizing to the Inhibitor II more strongly.

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NEW

Hill, W.E., Wentz, B.A., Payne, W.L., Jagow, J.A., Zon, G., J. Assoc. Off. Anal. Chem 69, 531-536 (1986). *DNA Colony Hybridization Method Using Synthetic Oligonucleotides to Detect Enterotoxigenic Escherichia coli: Collaborative Study.* (From FDA, Division of Microbiology, Washington, D.C., 20204, U.S.A.; FDA, Minneapolis Center for Microbiological Investigations, Minneapolis, MN, 55401, U.S.A.; FDA, Division of Biochemistry and Biophysics, Bethesda, MD, 20205, U.S.A.)

Summary

Detection of eneterotoxigenic *E.coli* was accomplished by DNA colony hybridization using synthetic oligonucleotides. The paper describes the technique used which was labeled as the official method of the AOAC.

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NEW

Kaul, R.K., Hildebrand, B., Roberts, S., Jagadeeswaran, P., Gene 41, 311-314 (1986). *Isolation and characterization of human blood-coagulation factor X cDNA.* (From Center for Genetics, University of Illinois College of Medicine, 808 South Wood Street, Chicago, IL, 60612, U.S.A.)

Summary

Primer extended, radiolabeled oligonucleotide probes were used to identify the human blood coagulation factor X cDNA from a human liver cDNA library. Two 30-mers, with a 13-bp region of complementarity, were synthesized, annealed, and extended with Klenow and radiolabeled triphosphates to yield a high specific activity double-stranded probe. Sequence analysis of Factor X DNA showed a 60% homology to Factor IX and a 40% homology to prothrombin, which supports the idea of gene evolution by gene duplication followed by divergence.

... ... ... ... ... ...

Krainer, A.R., Maniatis, T., Cell 42, 725-736 (1985). *Multiple factors including the small nuclear ribonucleoproteins U1 and U2 are necessary for pre-mRNA splicing in vitro.* (From Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA, 02138, U.S.A.)

Summary

Several oligonucleotides were used in the identification of ribonucleoproteins that are necessary for pre-mRNA splicing in vitro.

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NEW

Kubo, T., Fukuda, K., Mikami, A., Maeda, A., Takahasi, H., Mishina, M., Haga, T., Haga, K., Ichiyama, A., Kangawa, K., Kojima, M., Matsuo, H., Hirose, T., Numa, S., Nature 323, 411-416 (1986). *Cloning, sequencing and expression of complementary DNA encoding the muscarinic acetylcholine receptor.* (From Departments of Medical Chemistry and Molecular Genetics, Kyoto University Faculty of Medicine, Kyoto 606, Japan; Department of Biochemistry, Hamamatsu University School of Medicine, Hamamatsu 431-31, Japan; Department of Biochemistry, Miyazaki Medical College, Miyazaki 889-16, Japan; Pharmaceutical Institute, Keio University School of Medicine, Tokyo 160, Japan.)

Summary

Oligonucleotides were used as probes and primers in the cloning, sequencing, and expression of a porcine muscarinic acetylcholine receptor gene from a porcine brain mRNA bank. The receptor was found to be homologous with the ß-adrenergic receptor and rhodopsin in both amino acid sequence and suggested transmembrane topography.

... ... ... ... ... ...

NEW

Kwiatkowski, D.J., Stossel, T.P., Orkin, S.H., Mole, J.E., Coltens, H.R., Yin, H.L., Nature 323, 455-458 (1986). *Plasma and cytoplasmic gelsolins are encoded by a single gene and contain a duplicated actin-binding domain.* (From Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, 02114, U.S.A.; Department of Pediatrics, Children's Hospital, Harvard Medical School, Boston, MA, 02115, U.S.A.; Department of Biochemistry, University of Massachusetts Medical School, Worcester, MA, 01605, U.S.A.; Department of Pediatrics, St. Louis Children's Hospital, Washington University School of Medicine, St. Louis, MO, 63110, U.S.A.)

Summary

Oligonucleotides were used as probes in the isolation and characterization of plasma and cytoplasmic gelsolins, a class of actin-modulating proteins which sever actin filaments.

... ... ... ... ... ...

Lee, D.C., Rose, T.M., Webb, N.R., Todaro, G.J., Nature 313, 489-491 (1985). *Cloning and sequence analysis of a cDNA for rat transforming growth factor-α.*(From Oncogen, 3005-1st Ave., Seattle, WA, 98121, U.S.A.; University of Washington, Department of Pathology, School of Medicine, Seattle, WA, 98195, U.S.A.)

Summary

Two 17-mers of 32 sequences each were used as hybridization probes to identify a rat transforming growth factor-α cDNA clone.

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NEW

Lei, K., Liu, T., Zon, G., Soravia, E., Liu, T., Goldman, N.D., Journal of Biological Chemistry 260, 13377-13383 (1985). *Genomic DNA Sequence for Human C-reactive Protein.* (From the Division of Biochemistry and Biophysics, Office of Biologics Research and Review, Center for Drugs and Biologics, FDA, Bethesda, MD 20205.)

Summary

Several oligonucleotides were used to screen a human liver genomic library for the presence of the prototype acute phase reactant, the C-reactive protein (CRP). CRP is involved in the initial response of the body to infection, inflammation or tissue injury. Multiple probing was done with three different oligonucleotides coding for unique areas of the gene to aid in identification of full length DNA's from the genomic library.

... ... ... ... ... ...

Linnane, A.W., Beilharz, M.W., McMullen, G.L., Macreadie, I.G., Murphy, M., Nisbet, I.T., Novitski, C.E., Woodrow, G.C., Biochemistry International 8, 725-732 (1984). *Nucleotide sequence and expression in E.coli of a human interferon- αgene selected from a genomic library using synthetic oligonucleotides.* (From Department of Biochemistry, Monash University, Clayton, Victoria, 3168, Australia.)

Summary

A human genomic library was screened for the presence of α–INF genes with synthetic oligonucleotide probes. The sequence of these probes corresponds to a number of different highly conserved segments within published α-IFN coding regions. Using the probes individually, 3 X $10^5$ recombinant phages (λ Charon 4A) were screened. This resulted in the identification of 297 putative α -IFN clones. This number of clones was reduced to 28 by using combinations of the probes. One clone, M1, that hybridized to all five probes, was selected and subcloned using several oligonucleotides. Four subclones were identified and the oligonucleotides that were used as probes were then used as primers for dideoxy sequencing.

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Luck, D.N., Ngsee, J.K., Rottman, F.M., Smith, M., DNA 5, 21-28 (1986). *Synthesis of bovine prolactin in Escherichia coli.* (From Dept. of Biochemistry, Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5.)

Summary

Oligonucleotides were used in various experiments to aid in the expression of bovine prolactin in *E.coli.*

... ... ... ... ... ...

Meier, J.T., Simon, M.I., Barbour, A.G., Cell 41, 403-409 (1985). *Antigenic variation is associated with DNA rearrangements in a relapsing fever Borrelia.* (From National Institutes of Health, Department of Human Services, Public Health Service, National Institute of Allergy and Infectious Diseases, and Laboratory of Microbial Structure and Function and Laboratory of Pathobiology, Rocky Mountain Laboratories, Hamilton, MT, 59840, U.S.A.; California Institute of Technology, Division of Biology, Pasadena, CA, 91125, U.S.A.)

Summary

Two mixed-sequence 17-mers were used as probes for both Southern and Northern hybridization. One probe was specifically for the gene coding a serotype-specific antigen designated, Serotype 7. The other probe sequence was derived from a portion of the protein sequence common to both Serotypes 7 and 21. This probe was used to detect genes for both proteins.

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Murakami, H., Yabusaki, Y., Ohkawa, H., DNA 5, 1-10 (1986). *Expression of rat NADPH-cytochrome P-450 reductase cDNA in Saccharomyces cerevisiae.* (From Biotechnology Laboratory, Takarazuka Research Center, Sumitomo Chemical Co., Ltd., Takarazuka, Hyogo 665, Japan.)

Summary

A 17-base oligonucleotide was used as a mixed base probe to identify rat NADPH-dependent cytochrome P-450 reductase in *S. cerevisia.*

... ... ... ... ... ...

Murre, C., Waldmann, R.A., Morton, C.C., Bongiovanni, K.F., Waldmann, T.A., Shows, T.B., Seidman, J.G., Nature 316, 549-552 (1985). *Human α-chain genes are rearranged in leukemic T-cells and map to the short arm of chromosome 7.* (From Harvard Medical School, Department of Genetics, Boston, MA, 02115, U.S.A.; National Cancer Institute, National Institutes of Health, Bethesda, MD, 20205, U.S.A.; Roswell Park Memorial Institute, Department of Human Genetics, Buffalo, N.Y., 14263, U.S.A.)

Summary

A 14-mer was used as a probe to screen a cDNA library which had been constructed from MD26 (a murine cytolytic T-cell) poly (A)+ mRNA. The probe was used to isolate the C-1 constant region of the human α-chain gene from leukemic T-cells.

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NEW

Myers, R.M., Lumelsky, N., Lerman, L.S., Maniatis, T., Nature 313, 495-498 (1985). *Detection of single base substitutions in total genomic DNA.* (From Dept. of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Ave., Cambridge, MA, 02138, U.S.A.; Dept. of Biological Sciences, State University of New York, Albany, N.Y., 12222, U.S.A.)

Summary

An oligonucleotide, complementary to part of a restriction fragment known to code for the human ß-globin gene, was used in primer extension experiments to produce a high specific activity probe for site-specific ß-thallasemia mutations. These mutations, which are single base substitutions, can be detected in cloned DNA homoduplexes by polyacrylamide gel electrophoresis containing a gradient of DNA denaturants parallel to the direction of electrophoresis. Since separation is based on differences in the melting behavior of the wild-type homoduplexes is significantly different from the separation of wild-type and mutant homoduplexes.

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NEW

Nemer, M., Chamberland, M., Sirois, D., Argentin, S., Drouin, J., Dixon, R.A.F., Zivin, R.A., Condra, J.H., Nature 312, 654-656 (1984). *Gene structure of human cardiac hormone precursor, pronatriodilatin.* (From Laboratoire de Biologie Moleculaire, Institut de Recherches Cliniques de Montreal, 110 ouest, Avenue de Pins, Montreal, Canada H2W 1R7; Virus and Cell Biology Research, Merck Sharp & Dohme Research Laboratories, West Point, PA, 19486, U.S.A.)

Summary

Oligonucleotides were used as probes in the identification of clones for the human cardiac hormone, pronatriodilatin. A 20-mer primer, complementary to a known amino acid sequence in the hormone, was used in a primer extension experiment with the resulting polynucleotide used as the probe for the gene in a human genomic library.

... ... ... ... ... ...

NEW

Nguyen, N.Y., Suzuki, A., Cheng, S., Zon, G., Liu, T., Journal of Biological Chemistry 261, 10450-10455 (1986). *Isolation and Characterization of Limulus C-reactive Protein Genes.* (From the Division of Biochemistry and Biophysics, Office of Biologics Research and Review, Center for Drugs and Biologics; Food and Drug Administration, Bethesda, MD, 20892, U.S.A.)

Summary

Mixed base oligonucleotides were used to screen a *Limulus polyphemus* (horseshoe crab) genomic library for the presence of three homologous genes coding for C-reactive protein (CRP), a plasma protein expressed in acute phase response to infection in mammals. The CRP protein in *L. polyphemus* is constitutively expressed and represents a major component of the horseshoe crab's hemolymph.

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Ooi, B.G., McMullen, G.L., Linnane, A.W., Nagley, P., Novitski, C.E., Nucleic Acids Research 13, 1327-1339 (1985). *Biogenesis of mitochondria: DNA sequence analysis of mit-mutations in the mitochondrial ATPase subunit 9 in Saccharomyces cerevisiae.* (From Department of Biochemistry, Monash University, Clayton, Victoria 3168, Australia.)

Summary

Synthetic oligonucleotides were used as probes in the identification of clones for the *oli1* gene of yeast mitochondrial DNA. The *oli1* gene was found to be subunit 9 of the oligomycin-sensitive mitochondrial membrane. This complex is responsible for electron transport and a concommitant protein gradient across the inner membrane which subsequently is utilized to drive the conversion of ADP to ATP. Using synthetic oligonucleotide primers, the first wild type oligomycin-sensitive sequence of the *oli1* gene was elucidated.

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Parker, K.L., Chaplin, D.D., Wong, M., Seidman, J.G., Smith, J.A., Schimmer, B.P., Proc. Natl. Acad. Sci. USA, 7860-7864 (1985). *Expression of murine 21-hydroxylase in mouse adrenal glands and in transfected Y1 adrenocortical tumor cells.* (From Harvard Medical School, Boston, MA, 02115, U.S.A.; Washington University School of Medicine, St. Louis, MO, 63110, U.S.A., University of Toronto, Toronto, ON M5G 1L6, Canada.)

Summary

Synthetic 15-mers, specific for 21-hydroxylase A and B genes, were used to probe mouse BALB/c adrenal RNA for detection of mRNA specific to the hydroxylase genes. By use of the probes it was concluded that all 21-hydroxylase mRNA is derived from the 21-hydroxylase A gene.

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Prusiner, S.B., Scientific American 251, (4) 50-59 (1984). *Prions.* (From University of California, School of Medicine, San Francisco, CA, U.S.A.)

Summary

Synthetic probes were used to detect the natural DNA which encodes for PrP, a protein which is the major constituent of the prion.

... ... ... ... ... ...

Ravetch, J.V., Feder, R., Pavlovec, A., Blobel, G., Nature 312, 616-620 (1984). *Primary structure and genomic organization of the histidine-rich protein of the malaria parasite Plasmodium lophurae.* (From The Dewitt Wallace Research Laboratory, Memorial Sloan-Kettering Cancer Center, New York, N.Y., 10021, U.S.A.; The Rockefeller University, Laboratory of Cell Biology, New York, N.Y., 10021, U.S.A.)

Summary

Two probes were used to detect multiple occurrences of the signal exon sequence in three restriction enzyme digest of the *Plasmodium lophural* genome.

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Rupp, G., Acha-Orbea, H., Hengartner, H., Zinkernagel, R., Joho, R., Nature 315, 425-427 (1985). *Identical $V_B$ T-cell receptor genes used in alloreactive cytotoxic and antigen plus I-A specific helper T-cells.* (From Universitatsspital, Abteilung fur Experimentelle Pathologie, Sternwartstrasse 2, 8091 Zurich, Switzerland.)

Summary

A 28-mer was used as a probe to screen a cDNA library.

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NEW

Rupp, F., Frech, G., Hengartner, H., Zinkernagel, R.M., Joho, R., Nature 321, 876-878 (1986). *No functional γ-chain transcripts detected in an alloreactive cytotoxic T-cell clone.* (From Institut fur Pathologie der Universitat Zurich, Universitatsspital, CH-8091 Zurich, Switzerland.)

Summary

A synthetic 23-mer probe was used to isolate three different γ-gene transcripts of an alloreactive cytotoxic T-cell clone from a human genomic library. These γ-transcripts were found to be non-functional rearrangements of T-cell clones due to translational stops in their expression.

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Samson, S.M., Belagaje, R., Blankenship, D.T., Chapman, J.L., Perry, D., Skatrud, P.L., VanFrank, R.M., Abraham, E.P., Baldwin, J.E., Queener, S.W., Ingolia, T.D., Nature 318, 191-194 (1985). *Isolation, sequence determination and expression in Escherichia coli of the isopenicillin N synthetase gene from Cephalosporium acremonium.* (From Eli Lilly & Co., Indianapolis, IN, 46285, U.S.A.; Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, U.K.; University of Oxford, South Parks Road, Oxford OX1 3QY, U.K.)

Summary

A mixed set of oligonucleotides (18-mer) with a 32-fold degeneracy was used to probe a cosmid genome library for the gene coding for isopenicillin N synthetase from *Cephalosporium acremonium.* The IPS enzyme catalyzes the central reaction in the pathway to clinically important penicillins and cephalosporins.

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Seidman, C.E., Duby, A., Choi, E., Graham, R., Haber, E., Homcy, C., Smith, J.A., Seidman, J.G., Science 225, 324-326 (1984). *The structure of rat preproatrial natriuretic factor as defined by a complementary DNA clone.* (From General Hospital, Boston, MA, 02114, U.S.A.; Harvard Medical School, Boston, MA 02115, U.S.A.)

Summary

Two mixed oligonucleotide probes were synthesized to identify the clone for rat preproatrial natriuretic factor. The probes corresponded to ANF regions located near the $NH_2$- terminus and the carboxy terminus. the $NH_2$- terminus probe that had a 96-fold degeneracy was used first to identify 15 clones from the ANF cDNA library. These 15 clones were rescreened with the -COOH terminus probe that had a 384-fold degeneracy. Eleven of the 15 clones were positive and were found to contain some portion of the ANF sequences derived from mRNA.

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NEW

Shah, D.M., Horsch, R.B., Klee, H.J., Kishore, G.M., Winter, J.A., Tumer, N.E., Hironaka, C.M., Sanders, P.R., Gasser, C.S., Aykent, S., Siegel, N.R., Rogers, S.G., Fraley, R.T., Science 233, 478-481 (1986). *Engineering Herbicide Tolerance in Transgenic Plants.* (From Plant Molecular Biology, Division of Biological Sciences, Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO, 63198, U.S.A.)

Summary

Mixed base oligonucleotides were used as probes in the isolation of a glyphosate tolerant strain of transformed petunia cells. The probes hybridized to a 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase gene in a genomic library from a petunia strain known to be tolerant to glyphosate. Chimeric EPSP synthase genes were transformed into petunia cells. Regenerate transgenic plants were found to be tolerant to glyphosate.

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NEW

Soares, M.B., Ishii, D.N., Efstratiadis, A., Nucleic Acids Research 14, (4) 1119-1134 (1985). *Development and tissue-specific expression of a family of transcripts related to rat insulin-like growth factor II mRNA.* (From Columbia University, Department of Human Genetics and Development and Pharmacology, 701 W. 168th Street, New York, N.Y., 10032, U.S.A.)

Summary

The structure of pre-pro-rIGF-II mRNA was determined from a cDNA library. The 3.4-kb mRNA was found to have a 464-bp 5'-noncoding region, a 69-bp signal region, 134-bp of coding sequence, and 89-bp trailer peptide and approximately 2.7-kb of 3'-noncoding region. A labeled synthetic oligonucleotide probe was used to isolate the cDNA. A unique methodology was used to generate a high specific activity probe. First, a message-like DNA 39-mer was synthesized containing five two-fold degeneracies and random choices (favoring G-U base-pairing) for the rest of the codons. A complementary 10-mer was annealed to the 39-mer and extended with labeled triphosphates and Klenow fragment of DNA polymease. This approach is superior due to the high specific activities obtained and the high concentration of exact matches obtained in the middle of the probe.

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Takahashi, N., Roach, A., Templow, D.B., Prusiner, S.B., Hood, L., Cell 42, 139-148 (1985). *Cloning and characterization of the myelin basic protein gene from mouse: one gene can encode both 14-kd and 18.5-kd MBPs by alternate use of exons.* (From California Institute of Technology, Division of Biology, Pasadena, CA, 91125, U.S.A.; University of California San Francisco, Department of Biochemistry and Biophysics School of Medicine, San Francisco, CA, 94143, U.S.A.)

Summary

Two 17-mer mixtures of 32 sequences each were used to probe the gene for myeline basic protein from mouse.

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Toole, J.J., Knopf, J.L., Wozney, J.M., Sultzman, L.A., Buecker, J.L., Pittman, D.D., Kaufman, R.J., Brown, E., Shoemaker, C., Orr, E.C., Amphlett, G.W., Foster, W.B., Coe, M.L., Knutson, G.J., Fass, D.N., Hewick, R.M., Nature 312, 342-347 (1984). *Molecular cloning of a cDNA encoding human antihaemophilic factor.* (From Genetics Institute, Inc., 225 Longwood Avenue, Boston, MA, 02115, U.S.A.; and Hematology Research Section, Mayo Clinic/Foundation, Rochester, MN, 55905, U.S.A.)

Summary

Thrombin cleavage fragments of porcine factor VIII:c were purified by gel electrophoresis and sequenced with the Applied Biosystems 470A Protein/Peptide Sequencer. Using these sequences, two sets of mixed DNA probes were constructed. The sequence of the first, a set of six 45-mers, was derived from a section of one polypeptide which had the least amount of codon ambiguity. The number of sequences produced was also minimized by using eukaryotic codons, taking into account the relative stability G/T versus A/C mismatches and the infrequency of the dinucleotide CG in eukaryotic genes. These 45-mers were used to screen a genomic library from BamH1 digested porcine DNA under nonstringent conditions. Sequence analysis later confirmed that this probe had an 11% (5 out of 45) mismatch. The second set of probes included sixteen 15-mers. The recombinants were screened with this probe at a lower temperature than used for the 45-mers. Recombinants which hybridized to both probes were purified and used to provide a 6.6-kb probe for locating the corresponding human genomic clone. Another mixed probe of sixty-four 56-mers was used to identify a recombinant containing the code for one of the polypeptide fragments of factor VIII:C.

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Wallner, B.P., Mattaliano, R.J., Hession, C., Cate, R.L., Tizard, R., Sinclair, L.K., Foeller, C., Chow, E.P., Browning, J.L., Ramachandran, K.L., Pepinsky, R.B., Nature 320, 77-81 (1986). *Cloning and expression of human lipocortin, a phospholipase $A_2$ inhibitor with potential anti-inflammatory activity.* (From Biogen Research Corp., 14 Cambridge Center, Cambridge, MA, 02142, U.S.A.)

Summary

Oligonucleotides were used as mixed-base probes in the cloning of human lipocortin, a phospholipase $A_2$ inhibitor with potential anti-inflammatory activity.

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Wickstrom, E., Simonet, W.S., Medlock, K., Ruiz-Robles, I. Biophysical Society 49, 15-17 (1986). *Complementary oligonucleotide probe of vesicular stomatitis virus matrix protein mRNA translation.* (From University of South Florida, Tampa, FL, 33620, U.S.A.)

Summary

A 15-mer complementary to predicted single-stranded regions of a mRNA for vesicular stomatitis virus was used in an experiment to attempt to stop translation of the mRNA. By competing with initiation factors and ribosomes for sites on the mRNA, the oligonucleotide caused inhibition of translation ranging from 23 to 88% in concentrations of 5 to 50 M.

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Winoto, A., Mjolsness, S., Hood, L., Nature 316, 832-836 (1985). *Genomic organization of the genes encoding mouse T-cell receptor α-chain.* (From California Institute of Technology, Division of Biology, Pasadena, CA, 91124, U.S.A.)

Summary

A synthetic probe was used to detect joining gene segments in T-cell receptor α-chains.

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Wolfson, B., Manning, R.W., Davis, L.G., Arentzen, R., Baldino, F., Nature 315, 59-61 (1985). *Co-localization of corticotropin releasing factor and vasopressin mRNA in neurons after adrenalectomy.* (From E. I. du Pont de Nemours & Co., Central Research & Development Department, Experimental Station, Wilmington, DE, 19898, U.S.A.)

Summary

A 48-mer complementary to the glycoprotein region of vasopressin mRNA was prepared by linking two 24-mers with a 17-mer holding fragment and T4 DNA ligase. The resulting probe was used to localize vasopressin in te parvo-cellular para-ventricular nucleus of rat hypothalmus.

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Wong, W.W., Klickstein, L.B., Smith, J.A., Weis, J.H., Fearon, D.T., Proc. Natl. Acad. Sci. USA, 7711-7715 (1985). *Identification of a partial cDNA clone for the human C3b/C4b receptor.* (From Brigham and Women's Hospital, Harvard Medical School, Boston, MA, 02115, U.S.A.; Massachusetts General Hospital, and Department of Pathology, Harvard Medical School, Boston, MA, 02114, U.S.A.)

Summary

Three mixed probes were used to screen a human tonsilar DNA genomic library for human complement fragments C3b/C4b. A single positive clone that hybridized to all 3 probes was identified.

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Zeitlin, S., Efstrdiadis, A., Cell 39, 589-602 (1984). *In vivo splicing products of the rabbit γ-globin pre-mRNA.* (From Columbia University, Departments of Human Genetics and Development, 701 W. 168th Street, New York, N.Y., 10032, U.S.A.)

Summary

The mecnanism of rabbit γ—globin pre-mRNA processing was studied to determine the order of removal of two introns. The excised introns were found to exist in both linear and lariat forms. Synthetic oligodeoxynucleotides were used as probes to distinguish between lariat and Y-form structures by RNase H digestion. Synthetic oligodeoxynucleotides were also used as primers for reverse transcriptase to probe for covalent crosslinks along the IVS.

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Zinn, A.R., Butow, R.A., Cell 40, 887-895 (1985). *Nonreciprocal exchange between alleles of the yeast mitochondrial 21S rRNA gene: kinetics and the involvement of a double-strand break.* (From the University of Texas, Health Science Center, Dept. of Biochemistry/Div. of Molecular Biology, Dallas, TX 75235, U.S.A.)

Summary

15-mers were used as probes to detect $w^+$ and $w^-$ alleles of the yeast mitochondrial 215 rRNA gene.

# CONSTRUCTS

NEW

Brent, R., Ptashne, M., Nature, 612-615 (1984). *A bacterial repressor protein or a yeast transcriptional terminator can block upstream activation of a yeast gene.* (From Department of Biochemistry and Molecular Biology, 7 Divinity Ave., Harvard University, Cambridge, MA, 02138, U.S.A.)

Summary

Synthetic *lexA* operators, complementary 26-mers with XhoI sticky ends, were used in an experiment to show that bacterial repressor proteins (Lex A), when placed between the TATA box and the upstream activator sequence in yeast *S cerevisiae*, block transcription of the gene.

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Busby, S., Kumar, A., Joseph, M., Halfpap, L., Insley, M., Berkner, K., Kurachi, K., Woodbury, R., Nature 316, 271-273 (1985). *Expression of active human factor IX in transfected cells.* (From ZymoGenetics, Inc., 1212 N. 35th St., Seattle, WA, 98103, U.S.A.; University of Washington, Department of Biochemistry, Seattle, WA, 98195, U.S.A.)

Summary

Two oligonucleotides were used to create an adaptor which aided the insertion of factor IX cDNA into pBR322.

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Davis, N.G., Model, P., Cell 41, 607-614 (1985). *An artificial anchor domain: Hydrophobicity suffices to stop transfer.* (From the Rockefeller University, New York, NY, 10021, U.S.A.)

Summary

Oligonucleotides were used to construct hydrophobic and polar regions of a coliphage F1 gene III protein (pIII) in experiments to determine the role of hydrophobicity in the anchoring of a protein to the cytoplasmic membrane. A complementary 12-mer which was synthesized and inserted into a plasmid results in the translation frameshift mutant pIII proteins that either have hydrophobic or polar regions. Hydrophobic regions convert secretory forms of pIII to anchored forms; the location of the hydrophobic domain in pIII alters its position in the membrane. PIII products containing the polar domain seem to be unable to anchor to the membrane and are therefore secretory.

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Ferretti, L., Karnik, S.S., Khorana, H.G., Nassal, M., Oprian, D.D., Proc. Natl. Acad. Sci. USA 83, 599-603 (1986). *Total synthesis of a gene for bovine rhodopsin.* (From Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA, 02139, U.S.A.)

Summary

The structural gene for bovine rhodopsin, a 1057-bp fragment, was synthesized from 72 synthetic oligonucleotides, 15 to 40 residues long. The total gene was assembled from three synthetic fragments that were cloned.

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Gearing, D.P., McMullen, G.L., Nagley, P., Biochemistry International 10, 907-915 (1985). *Chemical synthesis of a mitochondrial gene designed for expression in the yeast nucleus.* (From Centre for Molecular Biology and Medicine, Monash University, Clayton, Victoria 3168, Australia.)

Summary

A 170-bp DNA molecule was enzymatically constructed from 12 synthetic oligonucleotides (12 to 45 bases in length) in a single annealing/ligation mixture. This fragment constitutes a synthetic gene encoding the subunit 8 of the mitochondrial ATPase of *Saccharomyces cerevisiae*. This *NAP1* gene will provide a route for manipulation through site specific mutagenesis of the structure of the protein normally encoded by mitochondrial DNA.

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Giniger, E., Varnum, S.M., Ptashne, M., Cell 40, 767-774 (1985). *Specific DNA binding of GAL4, a positive regulatory protein of yeast.* (From Harvard University, Department of Biochemistry and Molecular Biology, Cambridge, MA, 02138, U.S.A.)

Summary

Two complementary 17-base oligodeoxynucleotides, which represent a putative upstream activating sequence (UAS), were synthesized. The fragments were inserted upstream of fusions of *E.coli lacz* to the yeast *CYC1* or *GAL1* genes. Such plasmids produced 50-70% of the ß-galactosidase activity of plasmids containing the wild-type UASg. Thus, GAL4 is shown to be a positive regulatory protein which acts by binding to four related 17bp sequences and inducing transcription of a downstream gene.

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Hutchinson, III, C.A., Nordeen, S.K., Vogt, K., Edgell, M.H., Proc. Natl. Acad. Sci. USA 83, 710-714 (1986). *A complete library of point substitution mutations in the glucocorticoid response element of mouse mammary tumor virus.* (From Curriculum in Genetics, and Program in Molecular Biology and Biotechnology, The University of North Carolina, Chapel Hill, NC, 27514, U.S.A.)

Summary

Complementary oligonucleotides were synthesized with 5.0% "impurities", 1.5% of each of the other three amidites in each amidite, to give random mixed base oligonucleotides. The oligonucleotides were cloned and the mutant library was screened for each mutant, most of which were identified by sequence analysis.

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NEW

Inaoka, T., Miura, K., Ohtsuka, E., Nucleic Acids Research Symposium Series No.17, 105-108 (1986). *Chemical synthesis of the T4 endonuclease V gene and its expression in Escherichia coli.* (From Faculty of Pharmaceutical Science, Hokkaido University, Sapporo 060, Japan.)

Summary

The T4 endonuclease V gene was chemically synthesized from 26 oligonucleotides. The gene, 422-bp, was synthesized using oligonucleotides 22 to 36 nucleotides in length and ligated in fragments, 4 to 8 oligonucleotides per individual ligation. The resulting DNA was cloned into *E.coli* and expressed through the trp promoter. A protein of correct molecular weight was isolated on SDS polyacrylamide gel electrophoresis after translation of the gene.

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NEW

Jones, P.T., Dear, P.H., Foote, J., Neuberger, M.S., Winter, G., Nature, 522-525 (1986). *Replacing the complementarity-determining regions in a human antibody with those from a mouse.* (From Laboratory of Molecular Biology, Medical Research Council, Hills Road, Cambridge CB2 2QH, U.K.)

Summary

The genetic construct for the V(H) domain of the human myeloma protein was synthesized from 28 oligonucleotides, 14 to 59 bases in length. The duplexes were assembled from eight single-stranded blocks. The sub-fragments were then ligated together to form the full length gene fragment. The gene fragment, constructed as a *PstI-HindIII* fragment, is a complementarity determining region (CDR) of a heavy chain of an antibody. When this CDR was placed in conjunction with a mouse light chain corresponding to the mouse antibody B1-8, thus substituting for the heavy chain of B1-8, the resulting antibody still had substantial hapten affinity.

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Keegan, L., Gill, G., Ptashne, M., Science 231, 699-704 (1986). *Separation of DNA binding from the transcription-activating function of a eukaryotic regulatory protein.* (From Sherman Fairchild Biochemistry Building, 7 Divinity Ave., Harvard University, Cambridge, MA, 02138, U.S.A.)

Summary

A synthetic 17-mer was used to show how transcription of eukaryotic genes is dependent on sites that are far from the gene for activation/repression. Results show that DNA binding and the activation/repression process are two separate functions.

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Lally, C., Lorper, T., Groger, W., Antoine, G., Winnacker, E.L., EMBO Journal 3, 333-337 (1984). *A size analysis of the adenovirus replicon.* (From Institut fur Biochemie, Universitat Munchen, Karlstrasse 23, D-8000 Munchen 2, F.R.G.)

Summary

Synthetic oligonucleotides which represent 17-bp, 19-bp, 21-bp, and 38-bp terminal DNA sequences of adenovirus genome were used to investigate initiation and limited elongation. It was concluded that a DNA sequence as short as the terminal 19-bp but longer than 17-bp is necessary and sufficient to initiate adenovirus-specific DNA replication in vitro.

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Luck, D.N., Ngsee, J.K., Rottman, F.M., Smith, M., DNA 5, 21-28 (1986). *Synthesis of bovine prolactin in Escherichia coli.* (From Department of Biochemistry, Faculty of Medicine, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.)

Summary

Oligonucleotides were used in various experiments to aid in the expression of bovine prolactin in *E.coli.*

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McNeil, J.B., Smith, M., Molecular and Cellular Biology 5, 3545-3551 (1985), . (From Faculty of Medicine, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.)

Summary

A 54-mer double-stranded oligonucleotide homologous to the 5'-end of a CYC-1 gene in *Saccharomyces cerevisiae* was synthesized with a low level of random base pair mismatches. These oligos were used in constructs with CYC1 mutants without 5' ends to examine the role of the 5' ends in expression.

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Nikaido, T., Shimizu, A., Ishida, N., Sabe, H., Teshigawara, K., Maeda, M., Uchiyama, T., Yodoi, J., Honjo, T., Nature 311, 631-635 (1984). *Molecular cloning of cDNA encoding human interleukin-2 receptor.* (From Kyoto University, Faculty of Medicine, Sakyo-ku, Kyoto 606, Japan.)

Summary

After the interleukin-2 receptor was sequenced with the Applied Biosystems 470A Protein/Peptide Sequencer, a mixture of 32 possible oligonucleotides (14-mers) was constructed and used to screen a cDNA library complementary to poly(A)+ mRNA of MT-1 cells. The interleukin-2 receptor was identified and sequenced.

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NEW

Noda, M., Ikeda, T., Suzuki, H., Takeshima, H., Takahashi, T., Kuno, M., Numa, S., Nature 322, 826-828 (1986). *Expression of functional sodium channels from cloned cDNA.* (From Departments of Medical Chemistry and Molecular Genetics and Physiology, Kyoto University Faculty of Medicine, Kyoto 606, Japan.)

Summary

A synthetic linker was used to bridge the 5'-flanking region and the coding sequence of the transmembrane protein gene (voltage-gated sodium channel) essential for the generation of action potentials in excitable cells. This linker aided in the expression of a rat brain sodium channel gene isolated from cDNA's.

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Simons, A., Tils, D., von Wilcken-Bergmann, B., Muller-Hill, B., Proc. Natl. Acad. Sci. USA 81, 1624-1628 (1984). *Possible ideal lac operator: Escherichia coli lac operator-like sequences from eukaryotic genomes lack the Central G -C Pair.* (From Institut fur Genetik der Universitat zu Koln, Weyertal 121, 5000 Koln 41, F.R.G.)

Summary

An unpurified 24-base synthetic oligonucleotide was ligated into a pBR211-derived plasmid. The sequence of this oligonucleotide includes a 22-bp perfectly palindromic sequence which was derived from a comparison of *lac* repressor binding sites with the objective of making a new synthetic *lac* operator. Subsequent studies showed that this synthetic *lac* operator binds the *lac* repressor eight times more tightly than does the natural *lac* operator.

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NEW

Theriault, N.Y., Tomich, C.C., Wierenga, W., Nucleosides & Nucleotides, 15-32 (1986). *Synthesis of a gene coding for human vasoactive intestinal peptide (VIP).* (From Biopolymer Chemistry, Molecular Biology and Cancer & Viral diseases Research, the Upjohn Company, Kalamazoo, MI, 49001, U.S.A.)

Summary

A synthetic gene was constructed as a 99-bp fragment coding for human vasoactive intestinal peptide. The gene was constructed from sixteen oligonucleotides that were annealed and ligated. The resulting fragment was cloned into pBR322 and sequenced with perfect fidelity. The strategy was designed around codon frequencies in yeast, elimination of regions of self-complementarily, placement of unique restriction sites and introduction of translation stop codons.

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Wang, K., Herrera-Estrella, L., Von Montagu, M., Zambryski, P., Cell 38, 455-462 (1984). *Right 25-bp terminus sequence of the nopaline T-DNA is essential for and determines direction of DNA transfer from Agrobacterium to the plant genome.* (From Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000, Gent, Belgium.)

Summary

A pair of complementary 25-mers were incorporated into pGV3852, a mutant *Ti* plasmid which contains a deletion of only the right T-DNA border region and is unable to produce gall tumors. When this altered plasmid was introduced into *Agrobacterium*, it produced tumors in plant cells. Since only this 25-bp terminus sequence is required, it appears that *Ti* plasmid-derived vectors can be simplified.

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Willson, T.A., Oakley, K.M., Nagley, P., Gene Anal Techn 2, 77-82 (1985). *A simple method for construction directly repeated multimeric DNA segments.* (From Monash University, Clayton, Victoria, Australia, and Carlton and United Breweries, Carlton, Victoria, Australia.)

Summary

Oligonucleotide linkers for *SalI &XhoI* were added into clones so that one pair was added per clone. These linkers were selected because they generate the same sticky ends when cut; their specificity for their particular restriction enzyme depends on the flanking bases adjacent to the central common sequence. The clones were ligated at a high DNA concentration which resulted in a mixture of linear aggregates. One of these aggregates, which is a directly repeated multimeric segment of the clone, was resistent to cutting by the *XhoI/SalI* enzymes because the junctions of this piece contained hybrid *SalI/XhoI* sites.

## CHEMISTRY/BIOCHEMSTRY

NEW

Agrawal, S., Christodoulou, Gait, M.J., Nucleic Acids Res. 14, 6227-6245 (1986). *Efficient methods for attaching non-radioactive lubels to the 5'-ends of synthetic oligodeoxyribo-nucleotides.* (From MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.)

Summary

The synthesis of two chemical linkers for the attachment of non-radioactive labels to the 5'-terminus of synthetic oligonucleotides was presented. The linkers were designed for use as the *5'-base* in a scheme of attachment on most DNA synthesizers. The linkers described were diisopropylaminophosphoramidites that had aminoethyl groups where the amino moiety was blocked by an Fmoc group (the amino group could be derivated), and a 2'-3'-pixyl-Uridine-5'-diisopropylphosphoramidite, which after condensation could be labeled by a hydrazine activited species after treatment of the ribose sugar with periodate to glycolize the 2',3'-moieties.

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Becker, C.R., Efcavitch, J.W., Heiner, C.R., Kaiser, N.F., Journal of Chromotography 326, 293-299 (1985). *Use of a $C_4$ column for reverse-phase high performance liquid chromatographic purification of synthetic oligonucleotides.* (From Applied Biosystems, Inc., 850 Lincoln Centre Dr., Foster City, CA, 94404, U.S.A.)

Summary

The small-pore $C_{18}$ column materials commonly used for reverse phase high performance liquid chromatographic purification of synthetic oligodeoxyribonucleotides do not yield DNA sufficiently pure for some applications. Separation and purification on large-pore $C_4$ columns was investigated. Model studies presented demonstrate excellent resolution of oligonucleotides of similar sequence. Also presented are the results of purification of larger compounds.

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Caruthers, M.H., Science 230, 281-285 (1985). *Gene synthesis machines; DNA chemistry and its uses.* (From University of Colorado, Boulder, CO, 80309, U.S.A.)

Summary

A review of state-of-the-art DNA chemistry methodologies, automated DNA synthesis instruments, and the biochemical applications of synthetic DNA.

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NEW

Chollet, A., Kawashima, E.H., Nucleic Acids Research 13, (5) 1529-1540 (1985). *Biotin-labeled synthetic oligodeoxyribonucleotides: chemical synthesis and uses as hybridization probes.* (From BIOGEN S.A., Department of Chemistry, 3 route de Troinex, CH-1227 Carougc, Geneva, Switzerland.)

Summary

The attachment of biotin to the 5'-end of synthetic oligonucleotides was achieved by the activation of a 5'-phosphate with imidazole and ethylenediamine, or N-methylimidazole and ethylenediamine. The activated phosphate was then derivatized with a N-hydroxysuccinamide activated biotin molecule.

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NEW

Chu, B.C.F., Orgel, L.E., Proc. Natl. Acad. Sci. USA, 963-967 (1985). *Nonenzymatic sequence-specific cleavage of single-stranded DNA.* (From the Salk Institute, P.O. Box 85800, San Diego, CA, 92138.)

Summary

Ethylenediaminetetraacetic acid and diethylenetriamine-pentaacetic acid were covalently attached to the 5'-terminus of a synthetic 16-mer via an ethylenediamine linker. In the presence of ferrous ions and dithiothreitol, these reagents brought about the hybridization dependent, radical-mediated cleavage of a 39-mer which contains an internal complementation site to the modified 16-mer. The position of attack seems to be dependent upon the length of the linker that binds the EDTA moiety. These sequence dependent phenomena could have restriction enzyme-like applications when made sufficiently efficient.

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NEW

Drager, R.R., Regnier, F.E., Analytical Biochemistry 145, 47-56 (1985). *High performance anion-exchange chromatography of oligonucleotides.* (From Purdue University, Department of Biochemistry, West Lafayette, IN, 47907, U.S.A.)

Summary

High performance ion-exchange chromatography was enhanced for separation of synthetic oligonucleotides by the optimization of a polyethyleneimine bonding chemistry. Quarternization of the ion-exchange matrix was found to increase resolution of oligonucleotides ranging from 35 to 50 bases in length. Separation was achieved on small columns (0.42 x 5.0 cm) using a 60 to 120 minute ammonium sulfate gradient.

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NEW

Jirieny, J., Martin, D., Nucleic Acids Research 14, 1943-1949 (1986). *Restriction endonucleases* HindIII and TaqI *cleave DNA with mismatched nucleotides within their recognition sequences.* (From Friedrich Miescher Institut, P.O. Box 2543, CH-4002 Basel, Switzerland.)

Summary

Synthetic 16-mers, with single base mismatches (A/C or G/T) in the restriction site determinant region d[GTCGAC], were synthesized to ascertain the stringency of certain restriction enzymes for sequence fidelity. The core sequence can be recognized by *HindIII*, *SalI*, and *TaqI* enzymes. Results showed that only *SalI* would not hydrolyze the two strands that contained the mentioned mismatches. Efficiencies were greater than 75%. Also, *HindIII* and *SalI* were shown to hydrolyze the correct strands with different rates which suggests differences in sequence recognition mechanisms.

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NEW

Kwiatkowski, M., Sandstrom, A., Balgobin, N., Chattopadhyaya, J., Acta Chemica Scandinavica, 721-733 (1984). *Use of Reverse Phase Ion Pair Chromatography to Fractionate and Purify DNA Fragments and Monomeric Components of RNA.* (From Bioorganic Chemistry Laboratory, Department of Microbiology, Uppsala University, Biomedical Center, Box 581, S-751 23 Uppsala, Sweden.)

Summary

The paper describes the comparison of several countercations as possible candidates for the formation of ion-pairs with oligonucleotides in order to obtain optimum separation on a C-18 RP column. The effect of the countercation concentration was also explored. It was shown that 0.0075 M tetrabutylammonium sulfate (pH 6.9) brings about the best separation of oligonucleotides up to 16-mers. It was also found that with the use of tetrapentylammomium phosphate and acetonitrile separation of the following reagents could be achieved: four ribonucleosides and their 2'-3'-cyclic phosphates; four ribonucleosides and their 5'-monophosphates; and, four ribonucleosides and their 2'- and 3'-monophosphates.

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NEW

McBride, L.J., Kierzek, R., Beaucage, S.L., Caruthers, M.H., J.Amer. Chem. Soc., 2040-2048 (1986). *Amidine Protecting Groups for Oligonucleotide Synthesis.* (From the Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO, 80309, U.S.A.)

Summary

The synthesis of oligonucleotides by the phosphoramidite approach was achieved using amidine protection of the exocyclic amino groups of the bases in yields similar to the classical acylated bases. Amidine protection was very easy to synthesize and was shown to have certain advantages over amide protection: 1) amidine protected dA was shown to be about 20-fold more resistant to depurination than N-6-benzoyl dA under certain acidic conditions; 2) amidine protected dC are not susceptible to attack on C-4 by primary amines; and 3) high yield synthesis of completely protected nucleoside using amidines makes these reagents ideal for synthesis with modified bases such as 5-methyl deoxycytidine.

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Riley, C.T., Liquid Chromatography 3, 948-952 (1918). *Reverse phase columns for oligonucleotide purification.* (From Howard Hughes Medical Institute, the Johns Hopkins University School of Medicine, Maryland, U.S.A.)

Summary

The use of a 10 cm x 4.6 mm, 5µ, fully end-capped C-18 silica column is the best column for reversed phase HPLC purification of crude oligonucleotide mixtures from automated synthesis. Also the use of 4-ethyl morpholine acetate works well as an aqueous buffer in the purification process using acetonitrile as the organic phase in the gradient purification process.

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NEW

Schott, H., Eckstein, H., Journal of Chromatography, 363-368 (1984). *High-Performance Liquid Chromatographic Separations of Isomeric Pyrimidine Oligodeoxynucleotides.* (From Institute of Organic Chemistry, University of Tubingen, Auf der Morgenstelle 18, D-7400 Tubingen-1, F.R.G.)

Summary

Mixtures of sequence-isomeric pyrimidine oligonucleotides, up to pentamers, were synthesized in a one-pot approach and purified either by reverse phase or ion-exchange chromatography. Of the 47 theoretically possible isomers, 40 could be separated and characterized by snake-venom phosphodiesterase or wandering spot analysis.

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NEW

Sheardy, R.D., Seeman, N. C., J. Amer. Chem. Soc. 110, 4301-4303 (1986). *The Synthesis of a Deoxyoligonucleotide Incorporating 5-Iododeoxyuridine.* (From Department of Chemistry, Pennsylvania State University, Hazelton Campus, Hazelton, PA, 18201; and Department of Biological Sciences, State University of New York at Albany, Albany, N.Y., 12222.)

Summary

A 16-mer was successfully synthesized on a large scale (10 µmole) using the modified base 5-iodouridine in two sites. The iodouridine was prepared as a diisopropyl phosphoramidite and reacted in yields adequate for synthesis (app. 98%). The iodouridine derivative was used to act as an aid in X-ray scattering experiments for the study of macromolecular DNA.

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Smith, L.M., Fung, S., Hunkapiller, M.W., Hunkapiller, T.J., Hood, L.E., Nucleic Acids Research 13, 2399-2412 (1985). *The Synthesis of Oligonucleotides Containing an Aliphatic Amino Group at the 5' Terminus: Synthesis of Fluorescent DNA Primers for Use in DNA Sequence Analysis.* (From California Institute of Technology, Division of Biology, Pasadena, CA, 91125, U.S.A.; Applied Biosystems, Inc., Foster City, CA, 94404, U.S.A.)

Summary

A rapid and versatile method has been developed for the synthesis of oligonucleotides which contain an aliphatic amino group at their 5'-terminus. This amino group reacts specifically with a variety of electrophiles, thereby allowing other chemical species to be attached to the oligonucleotide. This chemistry has been utilized to synthesize several fluorescent derivatives of an oligonucleotide primer used in DNA sequence analysis by the dideoxy (enzymatic) method. The modified primers are highly fluorescent and retain their ability to specifically prime DNA synthesis. The use of these fluorescent primers in DNA sequence analysis will enable DNA sequence analysis to be automated.

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NEW

Stec, W.J., Zon, G., Egan, W., Byrd, R.A., Phillips, L.R., Gallo, K.A., Journal of Organic Chemistry 50, 3908-3913 (1985). *Solid-Phase Synthesis, Separation, and Stereochemical Aspects of P-Chiral Methane- and 4,4'-Dimethoxytriphenylmethanephosphate Analogues of Oligodeoxyribonucleotides.* (From Division of Biochemistry and Biophysics, FDA, 8800 Rockville Pike, Bethesda, MD, 20205, U.S.A)

Summary

The paper describes the automated solid phase synthesis, separation and stereochemical aspects of P-chiral methane and 4,4'-dimethoxytriphenylmethane-phosphonate analogs of oligonucleotides.

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Stec, W.J., Zon, G., Egan, W., Stec, B., Journ. of the Amer. Chem. Soc., 6077-6079 (1984). *Automated solid phase synthesis, separation, and stereochemistry of phosphorothioate analogues of oligodeoxyribonucleotides.* (From Food and Drug Administration, Bethesda, MD, 20205, U.S.A.)

Summary

A procedure for the automated synthesis of oligonucleotides labeled with $^{17}O$ or $^{18}O$ at the phosphate moiety and for phosphorothioates and selenium analogues is given. These compounds are useful as substrates for studying phosphorolytic and phosphoryl-transfer enzymes and are also employed in the stereospecific synthesis of P-chiral nucleoside phosphates. The diastereomers produced can be separated by reverse phase $C_{18}$ HPLC. Self-complementary octamers containing the *EcoRI* endonuclease site were synthesized as sulfur analogues: GGpSAATTCC (compound 1) and GpSGAATTCC (compound 2). Neither diastereomer of compound 1 is a good substrate for the endonuclease, however, the $R_p$ form of compound 2 is cleaved while the $S_p$ was not. This suggests the possibility of using this method to *protect* restriction sites.

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Stec, W.J., Zon, G., Tetrahedron Letters 25, 5275-5278 (1984). *Synthesis, separation, and stereochemistry of diastereomeric oligodeoxy-ribonucleotides having a 5'-terminal internucleotide phosphorothioate linkage.* (From Food and Drug Administration, Division of Biochemistry and Biophysics, Office of Biologics Research and Review, 8800 Rockville Pike, Bethesda, MD, 20205, U.S.A.)

Summary

This paper describes synthesis and HPLC separation of several oligonucleotides having the general formula NpSR where N is one of the four nucleotides, PS is the sulfur analog of the phosphate linkage and R is the rest of the sequence with 2 to 15 bases. Retention data for both diastereomers in both the 5'-dimethoxytrityl and 5'-OH form are given with the results of digestion with snake venom phosphodiesterase and nuclease P1.

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Stec, W.J., Zon, G., Tetrahedron Letters 25, 5279-5282 (1984). *Stereochemical studies of the formation of chiral internucleotide linkages by phosphoramidite coupling in the synthesis of oligodeoxyribonucleotides.* (From Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD, 20205, U.S.A.)

Summary

The mechanism of tetrazole-catalyzed phosphoramidite coupling was investigated in order to determine if stereochemical integrity could be maintained at the phosphorus in $^{17}O$, $^{18}O$, S and Se analogs. Since the rate of epimerization is several orders of magnitude faster than the rate for coupling, it was concluded that this maintenance was not possible.

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Stec, W.J., Zon, G., Gallo, K.A., Byrd, R.A., Uznanski, B., Guga, P., Tetrahedron Letters 26, 2191-2194 (1985). *Synthesis and absolute configuration of P-chiral O-isopropyl oligonucleotide triesters.* (From FDA, Division of Biochemistry and Biophysics, 8800 Rockville Pike, Bethesda, MD, 20205, U.S.A.; Polish Academy of Sciences, Centre of Molecular and Macromolecular Studies, 90-362 Lodz, Poland.)

Summary

Mixed triester/diester oligonucleotides were produced by the use of selective removal of the methoxy phosphate protecting group under conditions which do not remove the isopropoxy group. Two stage (trityl-on and trityl-off) purification was used to separate various diastereomers produced from these selectively phosphate-protected compounds. The presence of the isopropoxy group was shown to give resistance to cleavage by nuclease *P1* and *EcoRI* endonuclease.

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Stec, W.J., Zon, G., Journal of Chromatography 326, 263-280 (1985). *Reverse phase high-performance liquid chromatographic separation of diastereomeric phosphorothioate analogues of oligodeoxyribonucleotides and other back-bone-modified congeners of DNA.* (From FDA (HFN-870), 8800 Rockville Pike, Bethesda, MD, 20205, U.S.A.)

Summary

Diastereomeric phosphorothioate analogs of DNA, separated by HPLC, were used for exploratory studies of stereochemistry and phosphorolytic enzymes.

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NEW

Thompson, J.A., BioChromatography 1, 16-20 (1986). *A Review of High-Performance Liquid Chromatography in Nucleic Acids Research.* (From the Laboratory of Molecular Hematology, National Heart, Lung, and Blood Institute, National Institute of Health, Bethesda, MD, 20892, U.S.A.)

Summary

The paper is a brief overview of the chronological development of chromatography matrices for HPLC applications in nucleic acids research.

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NEW

Uhlmann, E. and Engels, J., Tetrahedron Letters, 1023-1026 (1986). *Chemical 5'-Phosphorylation of Oligonucleotides Valuable in Automated DNA Synthesis.* (From Hoechst AG, Postfach 80 03 20, D-6230 Frankfurt am Main 80, West Germany.)

Summary

A new method for the chemical phosphorylation of oligonucleotides during automated oligonucleotide synthesis was developed. The reagent, a monofunctional phosphoramidite, was protected by methyl or ß-cyanoethyl and 2-(p-nitrophenyl)-ethyl (NPE) which can be deprotected by ß-elimination. The hydrophobic NPE group, at the phosphodiester level, can serve as a purification handle in reverse phase HPLC.

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NEW

Werntges, H., Steger, G., Riesner, D., Fritz, H., Nucleic Acids Research 14, 3773-3790 (1986). *Mismatches in DNA double strands: thermodynamic parameters and their correlation to repair efficiencies.* (From Institut fur Physikalische Biologie, Universitat Dusseldorf, 4000 Dusseldorf; and Max-Planck-Institut fur Biochemic, Abteilung Zellbiologie 8033 Martinsried bei Munchen, F.R.G.)

Summary

The helix-coil transitions of the sixteen18-mer DNA duplexes of d[CGTCGTTTXACAACGTCG] x d[CGACGTTGTX'AAACGACG] were measured by UV-absorption. The possible mismatches were classified into three classes: wobble base pairs, open base pairs, and weak base pairs. These classes show an interesting relationship to the biological repair efficiencies of different mismatches. Open base pairs show the lowest efficiency, whereas mismatches with high repair efficiency always belong the wobble base pair class.

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Zaug, A.J., Cech, T.R., Science 231, 470-475 (1986). *The intervening sequence RNA of Tetrahymena is an enzyme.* (From University of Colorado, Boulder, CO, 80309, U.S.A.)

Summary

A dC pentamer was used as a competitive inhibitor in an experiment to show that an intervening RNA sequence is in itself an enzyme that cleaves RNA.

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Zon, G., Gallo, K.A., Samson, C.J., Shao, K., Summers, M.F., Byrd, R.A., Nucleic Acids Research 13, 8181-8196 (1985). *Analytical studies of "mixed sequence" oligodeoxyribonucleotides synthesized by competitive coupling of either methyl- or ß-cyanoethyl-N,N-diisopropylamino phosphoramidite reagents, including 2'-deoxyinosine.* (From FDA, 8800 Rockville Pike, Bethesda, MD, 20892, U.S.A.)

Summary

This study was made to determine the effective competitive coupling yields of methyl- versus ß-cyanoethyl-N,N-diisopropylamino phosphoramidite reagents used in mixtures on automated DNA synthesizers. When used as freshly prepared solutions, these phosphoramidite mixtures resulted in ratios of products that indicated negligibly small differences among the rates of the various competitive coupling reactions employed. These studies looked at deoxyinosine/deoxycytidine mixtures used in mixed probe oligonucleotides as well. However, studies of reagent stability in solution revealed that both types of the N-isobutyryl deoxyguanosine phosphoramidite decompose faster than their corresponding dA, dC, or dT reagents. This problem was attenuated for the ß-cyanoethyl reagents due to their slower rate of decomposition.

## SITE-DIRECTED MUTAGENESIS

NEW

Burke, D.T., Olson, M.V., DNA 5, 325-332 (1986). *Laboratory methods: Oligodeoxynucleotide-directed mutagenesis of Escherichia coli and yeast by simple co-transformation of the primer and template.* (From Department of Genetics, Washington University, School of Medicine, St. Louis, MO, 63110, U.S.A.)

Summary

*Oligonucleotides* were used to develop a method of site-directed mutagenesis which requires no *in vitro* DNA synthesis. Co-transformation of the synthetic primer and single-stranded template into competent spheroplasts of *E.coli* generates the directed mutation. The event is detected using the mutant oligonucleotide as the probe. The mutation arises at a frequency of about 0.1% and produces fewer untargeted mutations than conventional protocols.

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Cohn, L.E., Glimcher, L.H., Waldman, R.A., Smith, J.A., Ben-Nun, A., Seidman, J.G., Choi, E., Proc. Natl. Acad. Sci. USA 83, 747-751 (1986). *Identification of functional regions on the I-A$^b$ molecule by site-directed mutagenesis.* (From Departments of Cancer biology, Harvard School of Public Health, The Massachusetts General Hospital, Boston, MA, 02114, U.S.A.)

Summary

Three oligonucleotides were used in site-directed mutagenesis experiments to identify functional regions of T-cell receptor protein DNA.

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Davis, C.G., Lehrman, M.A., Russell, D.W., Anderson, R.G.W., Brown, M.S., Goldstein, J.L., Cell 45, 15-24 (1986). *The J.D. Mutation in familial hypercholesterolemia: Amino acid substitution in cytoplasmic domain impedes internalization of LDL receptors.* (From Departments of Molecular Genetics, Cell Biology, and Internal Medicine, University of Texas Health Science Center, 5323 Harry Hines Blvd., Dallas, TX 75235, U.S.A.)

Summary

Oligonucleotides were used in site-directed mutagenesis experiments to investigate the terminal exons of human LDL receptor genes.

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Fouser, L.A., Friesen, J.D., Cell 45, 81-93 (1986). *Mutations in a yeast intron demonstrate the importance of specific conserved nucleotides for the two stages of nuclear mRNA splicing.* (From Department of Medical Genetics, Medical Sciences Bldg., University of Toronto, Toronto, Ontario, Canada, M5S 1A8.)

Summary

Various oligonucleotides were used in site-directed mutagenesis experiments to show the importance of conservation of 5'-regions of exon-intron junctions.

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Kramer, W., Drutsa, V., Jansen, H.W., Kramer, B., Pflugfelder, M., Fritz, H.J., Nucleic Acids Research 12, (24) 9441-9456 (1984). *The gapped duplex DNA approach to oligonucleotide-directed mutation construction.* (From Institut fur Genetik, Universitat zu Koln, Weyertal 121, D-5000 Koln 41, and Max Planck Institut fur Molekulare Genetik, Ihnestrasse 63, D-1000 Berlin 33, F.R.G.)

Summary

A simple and efficient method is described for introduction of structurally pre-determined mutations into recombinant genomes of filamentous phage M13. The method uses a gapped duplex DNA (gdDNA) molecule of the phage M13 genome as the key intermediate. In this gdDNA, the (+) and the (shorter) (-) strand carry different genetic markers in such a way, that a rigorous selection can be applied for phage carrying the markers of the (-) strand. For introduction of the mutation, a synthetic oligonucleotide with partial homology to a target site within the single-stranded DNA region is annealed to the gdDNA. The oligonucleotide subsequently becomes part of the (-) strand by enzymatic DNA gap filling and sealing. The physical linkage is preserved at the genetic level after transfection of a recipient *E.coli* strain deficient in DNA mismatch correction, so that the synthetic marker can be selected from the phage progeny independent from its potential phenotype. This method demonstrates that mutants can be constructed with marker yields in excess of 70%.

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NEW

Mead, D.A., Szczesna-Skorupa, E., Kemper, B., Protein Engineering 1, 67-74 (1986). *Single-stranded DNA "blue" T7 promoter plasmids: a versatile tandem promoter system for cloning and protein engineering.* (From Department of Physiology and Biophysics and College of Medicine at Urbana-Champaign, University of Illinois, Urbana, IL, 61801, U.S.A.)

Summary

Site-directed mutagenesis was done using several oligonucleotides to eliminate an efficient transcriptional terminator of T7 RNA polymerase in a cDNA clone of bovine preproparathyroid hormone. The mutation was needed to destroy the terminator because the experiment was done in an efficient promoter plasmid that contained the T7 promoter. Genes under control of T7 permit expression of single proteins *in vitro* or *in vivo* by translation of T7 transcripts. This promoter, used in the ß-gal gene, permits a simple blue-to-white color assay.

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Newman, A.J., Lin, R.J., Cheng, S.C., Abelson, J., Cell 42, 335-344 (1985). *Molecular consequences of specific intron mutations on yeast mRNA splicing in vivo and in vitro.* (From California Institute of Technology, Division of Biology, Pasadena, CA, 91125, U.S.A.)

Summary

Two synthetic oligonucleotides were used to introduce specific mutations into the yeast CYH2$^m$ gene intron using single-stranded phage DNA as the template. The first mutation changed the highly conserved 5'-splice site hexanucleotide GTATGT to ATATGT. The second converted the essential intron-conserved sequence TACTAAC to TACTACC. In this intron, the A residue, which normally forms the crux of the intramolecular branch, is now a C. Another 24-mer was used as a reverse transcription primer.

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Nisbet, I.T., Beilharz, M.W., Gene Anal Techn 2, 23-29 (1985). *Simplified DNA manipulations based on in vitro mutagenesis.* (From Centre for Molecular Biology and Medicine, Monash University, Clayton, Victoria, Australia.)

Summary

A modified oligonucleotide-directed mutagenesis procedure is described that gives frequencies of single-base substitutions of 5 to 30%. The oligonucleotide extensions are performed at 30°C, enzymes and substrates replenished after 1 hour and total reaction time limited to 4 hours. This procedure allows the insertion of long nucleotide sequences at predetermined positions. To illustrate the technique, an insertion of 12 bases immediately 5'- to the cysteine 1 codon of IFN-14 has been accomplished with a single 37-base oligonucleotide. With the increase in the efficiency of longer insertions, the inserted sequence can have multiple functions that simplify complex DNA manipulations.

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Nisbet, I.T., Beilharz, M.W., Hertzog, P.J., Tymms, M.J., Linnane, A.W., Biochemistry International 11, 301-309 (1985). *Single amino acid substitutions at conserved residues of human α-interferon can affect antiviral specific activity.* (From Centre for Molecular Biology and Medicine, Monash University, Clayton, Victoria 3168, Australia.)

Summary

Synthetic oligonucleotides were used in site-specific mutagenesis experiments to investigate conserved residues of human α-interferon. Results showed that single amino acid substitutions can have marked effects on antiviral specific activity. Substitution at different sites causes different changes in antiviral activities of both human and bovine cells. It was shown by these experiments that the disulfide bridge between residues 29 and 139 is critical for the antiviral activity of α-interferons.

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Porter, S.D., Smith, M., Nature 320, 766-768 (1986). *Homoeo-domain homology in yeast MAT 2 is essential for repressor activity.* (From Department of Biochemistry, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5.)

Summary

Complementary oligonucleotides, 56 bases in length, were used in oligonucleotide-directed "cassette mutagenesis" experiments to investigate whether the conserved region of the MAT 2 locus of *S.cerevisiae*, a negative regulator of A-cell type-specific genes. In cassette mutagenesis, a wild type region is replaced by a synthetic duplex fragment containing a low level of mutant nucleotides at designated positions. These mutant nucleotides are generated at specific positions in the oligonucleotide by the coupling of a mixture of 96% wild-type base and 4% of an equimolar mixture of the remaining three bases. The oligonucleotides are purified, mixed together, and then annealed very slowly to allow correct "mutant strands" to aggregate. Ligation of the oligonucleotide mixture yields the randomly mutated cassette that can be screened for specific activities wanted. The sites for mixed synthesis are chosen to avoid generation of nonsense mutations, and the fraction of mutant bases was that required to yield the maximum number of molecules containing a single base-pair substitution.

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Rettenmier, C.W., Roussell, M.F., Quinn, C.O., Kitchingman, G.R., Look, A.T., Sherr, C.J., Cell 40, 971-981 (1985). *Transmembrane orientation of glycoproteins encoded by the V-fms oncogene.* (From St. Jude Children's Research Hospital, Memphis, TN, 38101, U.S.A.)

Summary

An 18-mer was used for deletion mutagenesis. Seventy-eight base pairs were removed in a single loop flanked by two runs of nine base pairs each. The sequence removed is that believed to code for a membrane-spanning peptide.

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NEW

Roberts, S., Rees, A.R., Protein Engineering 1, 59-65 (1986). *The cloning and expression of an anti-peptide antibody: a system for rapid analysis of the binding properties of engineered antibodies.* (From Laboratory of Molecular Biophysics, Department of Zoology, University of Oxford, OX1 3QU, U.K.)

Summary

Several oligonucleotides were used in the cloning and expression of an anti-peptide antibody developed for a system of rapid screening of binding properties of engineered antibodies. Oligonucleotide-directed mutagenesis was used for reconstruction of complete cDNA's from incomplete cDNA clones, which were screened for alternate binding properties.

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Russell, M., Model, P., Proc. Natl. Acad. Sci. USA 82, 29-33 (1985). *Thioredoxin is required for filaments phage assembly.* (From the Rockefeller University, New York, N.Y., 10021, U.S.A.)

Summary

Oligonucleotides were used in mutagenesis experiments to recover the mutant phenotype of the *fip* gene in *E.coli*. This gene codes for thioredoxin and is required for filamentous phage assembly. A synthetic 21-mer primer complementary to a wild-type *fip* fragment was extended using a temperature sensitive *fip* template. Single strands were converted to double strands and used to transfect cells. *Fip*+ phase were recovered at a frequency of 20%.

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Saffran, W.A., Cantor, C.R., Nucleic Acids Research 12, (24) 9237-9248 (1984). *The complete pattern of mutagenesis arising from the repair of site-specific psoralen crosslinks: analysis by oligonucleotide hybridization.* (From Department of Human Genetics and Development, College of Physicians and Surgeons, Columbia University, New York, N.Y., 10032, U.S.A.)

Summary

Although mutants will hybridize at low temperatures where binding is not very specific, hybridization will not occur at higher temperatures where the selectivity causes mismatches to be significant. This is the basis of a screening procedure which detects mutants without regard to their phenotype.

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NEW

Singh, M., Heaphy, S., Gait, M.J., Protein Engineering 1, 75-76 (1986). *Protocol: Oligonucleotide-directed misincorporation mutagenesis on single-stranded DNA templates.* (From MCR Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.)

Summary

Oligonucleotides were used in oligonucleotide-directed mutagenesis on single stranded DNA templates to investigate the structure-function relationship of T4 RNA ligase.

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NEW

Topal, M.D., Eadie, J.S., Conrad, M., Journal of Biological Chemistry 261, (21) 9879-9885 (1986). *O6-methylguanine mutation and repair is nonuniform: selection for DNA most interactive with O6-methylguanine.* (From the Lineberger Cancer Research Center, and the Depts of Pathology & Biochemistry, The University of No. Carolina Medical School, Chapel Hill, N.C. 27514, U.S.A.)

Summary

Analysis of several site-directed mutagenesis experiments with oligonucleotides containing $O^6$ methyl deoxyguanosine in *E.coli* indicated a significantly greater number of unmutated sites than expected, whereas the mutated sites generally fit a Poisson distribution. A concensus of DNA sequences surrounding the unmutated mutation sites was derived and had striking similarity to the region of the rat Harvey *ras* oncogene containing the N-methyl-N-nitrosourea activated site for transformation.

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Wertman, K.F., Mount, D.W., Journal of Bacteriology 163, 376-384 (1985). *Nucleotide sequence binding specificity of the LexA repressor of Escherichia coli K-12.* (From Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ, 85721, U.S.A.)

Summary

A synthetic 20-mer was used in a site-directed mutagenesis experiment to find the DNA binding specificity of the LexA repressor of *E.coli* K-12.

## PHYSICAL STUDIES

Borah, B., Roy, S., Zon, G., Cohen, J.S., Biochemical and Biophysical Research Communications 133, 380-388 (1985). *Unique pyrimidine 2D-COSY aromatic cross-peaks as monitors of pyrimidine environments and mobility in oligo- and polynucleotides.* (From Clinical Pharmacology Branch, NCI, and Lab of Molecular Pharmacology, DBB/FDA, National Institutes of Health, Bethesda, MD, 20892, U.S.A.)

Summary

Self-complementary oligonucleotides were used in model studies with 2D-COSY proton NMR to show the effects of drug binding to DNA. Measuring the intensity of the cross-peaks of the cytosine, adjacent aromatic protons (C5, C6) that are spin-coupled is a sensitive monitor of local mobility. Upon addition of the intercalating drug Daunomycin, selective intensity losses of the cross-peaks were observed, indicating binding to specific GC base pairs. Also observed was the mobility as a function of temperature by monitoring the 2D-COSY cross-peaks.

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Broido, M.S., James, T.L., Zon, G., Keepers, J.W., Eur. J. Biochem. 150, 117-128 (1985). *Investigation of the solution structure of a DNA octamer [d(GGAATTCC]2 using two-dimensional nuclear Overhauser enhancement spectroscopy.* (From Hunt College and Graduate Center of the City University of New York, NY; Schools of Pharmacy and Medicine, University of California, San Francisco, CA; Food and Drug Administration, Bethesda, MD; Rutgers University, New Brunswick, NJ, U.S.A.)

Summary

Proton 2-D nuclear Overhauser enhancement spectra were obtained for the self-complementary 8-mer GGAATTCC. Data suggest there are local variations in the mobility within the 8-mer duplex structure in solution.

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Broido, M.S., Zon, G., James, T.L., Biochemical and Biophysical Research Communications 119, (2) 663-670 (1984). *Complete assignment of the non-exchangeable proton NMR resonances of [d-(GGAATTCC)]2 using two-dimensional nuclear Overhauser effect spectra.* (From the University of California, San Francisco, CA, 94143, and National Center for Drugs and Biologics, Food and Drug Administration, Bethesda, MD, 20205, U.S.A.)

Summary

Proton two-dimensional nuclear Overhauser effect (2D NOE) spectra have been obtained for [d-(GGAATTCC)]2 at a series of mixing times. By performing the experiment at 500 MHz and obtaining pure absorption phase spectra, sufficient resolution was achieved to be able to assign all seventy of the non-exchangeable proton resonances in the self-complimentary octamer duplex, including the H3', resonances which overlap with the solvent peak.

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NEW

Gallo, K.A., Shao, K., Phillips, L.R., Regan, J.B., Koziolkiewicz, M., Uznanski, B., Stec, W. J., Zon, G., Nucleic Acids Research 14, 7405-7420 (1986). *Alkyl phosphotriester modified oligodeoxyribonucleotides. V. Synthesis and absolute configuration of Rp and Sp diastereomers of an ethyl phosphotriester (Et) modified EcoRI recognition sequence, d[GGAA(Et)TTCC]. A synthetic approach to regio- and stereospecific ethylation-interference studies.* (From Molecular Pharmacology Laboratory, Division of Biochemistry and Biophysics, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD, 20892, U.S.A.)

Summary

The oligonucleotide d[GGAA(Et)TTCC] was synthesized and its diastereoisomers purified by reversed phase HPLC. NMR studies on these oligonucleotides, in conjunction with other studies with analogs of the phosphate internucleotide bond, showed that the ApT bond is a contact point for the restriction enzyme, *EcoR1*.

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Jamin, N., James, T.L., Zon, G., Eur. J. Biochem. 152, 157-166 (1985). *Two-dimensional nuclear Overhauser enhancement investigation of the solution structure and dynamics of the DNA octamer [d(GGTATACC)].* (From School of Pharmacy and Medicine, University of California, San Francisco, CA 94143 USA; Center for Drugs and Biologics, Food and Drug Administration, Bethesda, MD, 20205 U.S.A.)

Summary

A self-complementary 8-mer oligonucleotide (GGTATACC) was used in a conformational investigation of duplex DNA in solution by 2-D nuclear Overhauser enhancement. The resonances of nearly all 70 of the non-exchangeable protons in the 8-mer were assigned using this technique. Experimental and theoretical spectra were compared using two B-DNA structures: standard B-form and an energy-minimized form. Both the 5'- and 3'- ends of the duplex are well represented by the regular B-DNA structure but large discrepancies from both B-DNA models are observed for the 'TATA' box. This suggests that there are some local differences in mobility within the duplex structure in solution.

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Jordan, S.R., Whitcombe, T.V., Berg, J.M., Pabo, C.O., Science 230, 1383-1385 (1985). *Systematic Variation in DNA Length Yields Highly Ordered Repressor-Operator Crystals.* (From Department of Biophysics, Johns Hopkins University School of Medicine, Baltimore, MD, 21205, U.S.A.)

Summary

Synthetic oligonucleotides specific to the operator site were co-crystalized with the I repressor to investigate the interaction between repressors and the related recognition site on an operator.

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Koo, H. S., Wu, H.M., Crothers, D.M., Nature 320, 501-506 (1986). *DNA bending at adenine-thymine tracts.* (From Department of Chemistry, Yale University, New Haven, CT, 06511, U.S.A.)

Summary

Twenty duplex sequences ranging from 9 to 20 bases were synthesized and used to create model systems for studying anomalous electrophoretic mobilities. The data suggests the molecules bend at specific sequence regions.

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NEW

Shindo, H., Ohkubo, S., Matsumoto, U., Zon, G., Nucleic Acids Research Symposium Series No.17, 211-214 (1986). *Total assignments of the $^1H$ NMR of decanucleotide duplexes containing the recognition sites of restriction enzymes.* (From Tokyo College of Pharmacy, Hachioji, Tokyo 192-03, Japan and Division of Biochemistry and Biophysics, FDA, Bethesda, MD, 20205, U.S.A.)

Summary

Self-complimentary 10-mers were synthesized and analyzed upon annealing by NOESY NMR protocols. The $^1H$ NMR analysis resulted in all assignments of imino and non-exchangeable protons in the duplex, which corresponded to restriction sites of the enzymes ScaI, XbaI, Bg1II, EcorI, HpaI, and EcorV.

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Stec, W.J., Zon, G., Egan, W., Stec, B., Journal of the American Chem. Soc., 6077-6079 (1984). *Automated solid phase synthesis, separation, and stereochemistry of phosphorothioate analogues of oligodeoxyribonucleotides.* (From FDA, Bethesda, MD, 20205, U.S.A.)

Summary

A procedure for the automated synthesis of oligonucleotides labeled with $^{17}O$ or $^{18}O$ at the phosphate moiety and for phosphorothioates and selenium analogues is given. These compounds are useful as substrates for studying phosphorolytic and phosphoryl-transfer enzymes and are also employed in the stereospecific synthesis of P-chiral nucleoside phosphates. The diastereomers produced can be separated by reserve phase $C_{18}$ HPLC. Self-complementary octamers containing the *EcoRI* endonuclease site were synthesized as sulfur analogues: GGPSAATTCC (compound 1) and GPSAATTCC (compound 2). Neither diastereomer of compound 1 is a good substrate for the endonuclease, however, the $R_P$ form of compound 2 is cleaved while the $S_P$ was not. This suggests the possibility of using this method to "protect" restriction sites.

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NEW

Stollar, B.D., Zon, G., Pastor, R.W., Proc. Natl. Acad. Sci. USA, 4469-4473 (1986). *A recognition site on synthetic helical oligonucleotides for monoclonal anti-native DNA autoantibody.* (From Department of Biochemistry and Pharmacology, Tufts University Health Science Campus, Boston, MA, 02111; and Division of Biochemistry and Biophysics, Office of Biologics Research and Review, FDA, Bethesda, MD, 20892, U.S.A.)

Summary

Oligonucleotides containing internucleotide analogs at key sites in the sequence were used to investigate the binding site in native DNA for a murine monoclonal anti-DNA antibody. The antibody bound to helical oligonucleotides at the (dG-dC)3 or (dG-dC)4 core in the center of the base-paired 18-mers. Results indicated that the antibody bound to a portion of cytosine and guanine in the major groove, a limited region of the backbone, and the 2-amino group of one guanine in the minor groove. For these interactions to occur, the antibody binding site would straddle the backbone of one of the helical strands of DNA.

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Summers, M.F., Byrd, R.A., Gallo, K.A., Samson, C.J., Zon, G., Egan, W., Nucleic Acids Research 13, 6375-6386 (1985). *Nuclear magnetic resonance and circular dichroism studies of a duplex - single-stranded hairpin loop equilibrium for the oligodeoxyribonucleotide sequence d(CGCGATTCGCG).* (From Division of Biochemistry and Biophysics, FDA, 8800 Rockville Pike, Bethesda, MD, 20205, U.S.A.)

Summary

NMR and CD studies on the mismatch DNA sequence d(CGCGATTCGCG) show that the duplex of this sequence exists as two conformational isomers. This equilibrium mixture shows slow interconversion between a B-type DNA duplex and a single-stranded hairpin loop structure.

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NEW

Summers, M.F., Powell, C., Egan, W., Byrd, R.A., Wilson, W.D., Zon, G., Nucleic Acids Research 14, 7421-7436 (1986). *Alkyl phosphotriester modified oligodeoxyribonucleotides. VI. NMR and UV spectroscopic studies of ethyl phosphotriester (Et) modified $R_P$-$R_P$ and $S_P$-$S_P$ duplexes, d[GGAA(Et)TTCC]$_2$.* (From Biophysics Laboratory and Molecular Pharmacology Laboratory, Division of Biochemistry and Biophysics, FDA, 8800 Rockville Pike, Bethesda, MD, 20892; and Department of Chemistry and Laboratory for Microbial and Biochemical Sciences, Georgia State University, Atlanta, GA, 30303-3053, U.S.A.)

Summary

The oligonucleotide d[GGAA(Et)TTCC] was synthesized and its diastereoisomers were purified by reverse phase HPLC. $^1H$ NMR chemical shifts for the duplex were made using 2-D NOE data. Unambiguous assignment of the phosphotriester $^{31}P$ signals was achieved in a novel way using selective insensitive nucleus enhancement by polarization transfer. The 2D-NOE data suggested possible steric interactions between the ethyl group and the H3`-flanking A residue.

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NEW

Wilson, W.D., Jones, R.L., Zon, G., Scott, E.V., Banville, D.L., Marzilli, L.G., J. Am. Chem. Soc., 7113-7114 (1986). *Actinomycin D Binding to Oligonucleotides with 5`d(GCGC)3` Sequences. Definitive $^{1}H$ and $^{31}P$ NMR Evidence for Two Distinct d(GC) 1:1 Adducts and for Adjacent Site Binding in a Unique 2:1 Adduct.* (From Department of Chemistry, Georgia State University, Atlanta, GA, 30303-3083; Molecular Pharmacology Laboratory, Division of Biochemistry and Biophysics, FDA, Bethesda, MD, 20892; and Department of Chemistry, Emory University, Atlanta, GA, 30322, U.S.A.)

Summary

$^{1}H$ and $^{31}P$ NMR experiments were done on Actinomycin D - oligonucleotide complexes to ascertain a concensus sequence of efficient binding of ActD to DNA. Results showed that there is no significant length dependence in the observed effects of ActD binding, ActD binds to GC sequences with a much higher preference than to CG or any other sequence in the oligonucleotides analyzed, spectral information contain the first direct evidence for formation of two 1:1 complexes by the unsymmetric phenoxazone ring of ActD, and that the exclusion limit of ActD is quite small (it can intercalate at adjacent GC sites).

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# USER BULLETIN

Model 380B
User Bulletin No. 49
August 1988

## Aminolink 2™

Aminolink 2 is a valuable new DNA synthesis reagent offered by Applied Biosystems. This reagent affords a convenient method for the introduction of an aliphatic primary amine at the 5' end of oligonucleotides. This amine can react to form oligonucleotide conjugates with a variety of substrates such as biotin[1,2], fluorescent dyes[3], EDTA[4], or alkaline phosphatase[5]. Applications include:

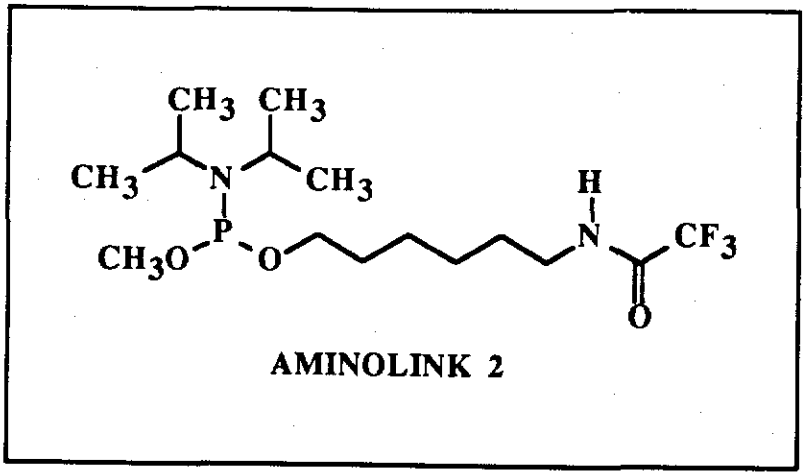
- Non radio-labeled hybridization probes[1,2,5,6]
- Sequence specific cleavage of single stranded DNA[4]
- automated DNA sequencing - affinity chromatography

Aminolink 2 offers many advantages when used in applications like those above. Because it is a phosphoramidite, it reacts like conventional nucleoside phosphoramidites, i.e., the compound is dissolved in anhydrous acetonitrile and placed on any of the extra phosphoramidite ports. It is activated with tetrazole and couples with the same high efficiency found in standard oligonucleotide synthesis.

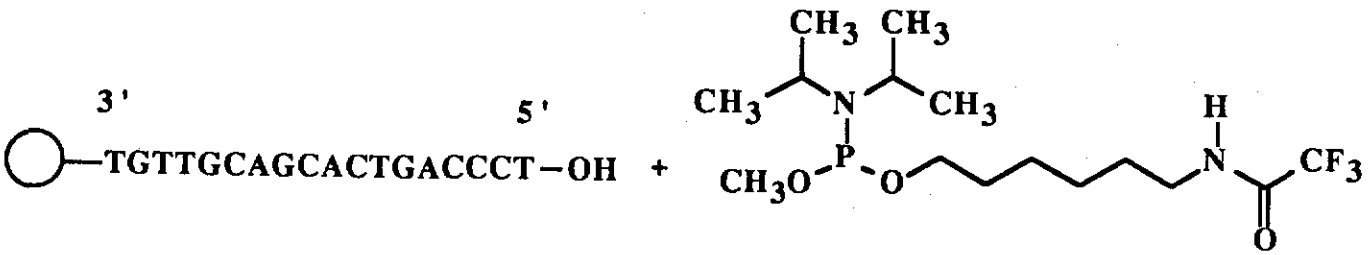
* Solution stability is comparable to nucleoside phosphoramidites (at least 2 weeks)
* No special cycles or protocols required regardless of instrument model
* Labeling at the 5'-terminal amine does not interfere with hybridization
* Aminolinked oligonucleotides can be easily labeled with a variety of commercially available reagents such as fluorescein isothiocyanate and biotin NHS ester.

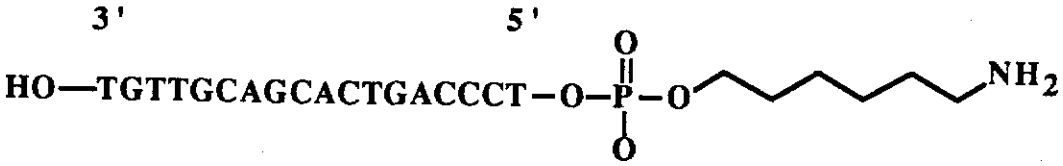
### CHEMICAL DESCRIPTION

Aminolink 2 (structure shown on the following page) generates an amino hexyl phosphate linker with a methoxy phosphate protecting group. Prior to deprotection, the phosphite triester is oxidized to the phosphate triester using standard oxidizing reagents. The amine group is protected by a



AMINOLINK 2





AMINOLINK 2 —— OLIGONUCLEOTIDE

trifluoroacetyl group, which is removed in the course of standard ammonia deprotection procedures.

Aminolink 2 (Part Number 400808) is supplied in 250 mg quantities. The compound is diluted with 3.3 mL of anhydrous acetonitrile following the procedures in your user manual. We recommend that the unopened linker be stored desiccated away from heat and moisture. Once diluted and placed on the instrument, the compound is stable for at least 2 weeks.

Use of Aminolink 2

The synthesis of an oligonucleotide derivatized with Aminolink 2 does not require a modified cycle.

Simply place Aminolink 2 on one of the extra base positions on your DNA Synthesizer (Numbers 5, 6, or 7 on models 380A or 380B and Number 5 on the Model 381.) Designate the Aminolink bottle position as the 5'-end of the sequence being synthesized. This will be the first base selected, using the sequence editor.

Configure the synthesis "Trityl ON" since detritylation is not necessary after Aminolink 2 addition.

If syntheses are performed using methoxy phosphoramidites, we recommend the use of thiophenol phosphate deprotection. Syntheses using cyanoethyl phosphoramidites can be deprotected without thiophenol. Ammonia alone is adequate to demethylate a single methyl phosphotriester group in an oligonucleotide. Product purity will not be adversely affected.

## ANALYSIS AND PURIFICATION OF AMINOLINK-OLIGONUCLEOTIDES

Aminolink oligonucleotides can be analyzed and purified by the same methods used for conventional oligonucleotides. Since the 5'-hydroxyl, usually available for phosphorylation by kinase and ATP, is blocked by the linker, an alternate 3'-end labeling procedure must be used to radiolabel aminolink-oligonucleotides. To effect a 3'-end label, the enzyme Terminal Deoxynucleotidyl Transferase (TDT) is used. Because the enzyme would continue propagation beyond the first nucleotide addition, a dideoxynucleotide analogue of [alpha-$^{32}$P]-ATP is used. The analogue, [alpha-$^{32}$P]-Cordycepin-5'-triphosphate, does not have a 3'-hydroxyl and, therefore, further addition is prevented. Below is a protocol for the labeling procedure.

## 3'-END LABELING

1) Prepare a stock solution of 10X 3'-end-labeling buffer:
   1.4 M Sodium Cacodylate*
   10 mM Cobaltous Chloride
   1.0 mM Dithiothreitol

pH 7.5 with HCl

* This chemical is carcinogenic, handle with care.

2) Aliquot 2 pmols of oligonucleotide into an Eppendorf-type tube (1.5 mL), dry in a Speed-vac and ethanol rinse with 10 uL of 95% Ethanol, dry.

3) Add the following to the tube, while on ice:

   1 µL of 10X 3'-end-labeling buffer
   1 µL of [alpha-$^{32}$P]-Cordycepin-5' Triphosphate (New England Nuclear #NEN-114, specific activity > 5000 Ci/mmol)
   7 µL of deionized water
   1 µL of TDT (ENZO Biochemicals, 5 units per microliter)

4) Spin the tube very briefly in a microcentrifuge to bring contents to the bottom. Incubate sample at 37°C for one hour.

5) Add 25 µL of 1.0 mM EDTA in deionized formamide loading buffer to the sample. Bromophenol Blue and Xylene Cyanol may be added as marker-dyes to the formamide prior to addition to the sample. The sample can be loaded onto an analytical gel in the same manner as described in Applied Biosystems DNA Synthesis User Bulletin 13, Revised. The linker retards the migration of the oligonucleotide by approximately one half of a base in the gel.

There is occasionally a radioactive contaminant which is a by-product of the labeling procedure. This contaminant is most likely a type of enzyme-Cordycepin complex, which co-electrophoreses with the sample and may appear on the autoradiogram as a fuzzy band in the region of a 16-mer. It is recommended that a blank sample containing the constituents of the reaction but without DNA be loaded on the gel so that the contaminant can be compared with the DNA sample. It should be noted that since phosphorylation of the 5'-hydroxyl by T4 kinase should be blocked by the presence of the linker, this procedure can be used as a qualitative analysis of the efficiency of Aminolink 2 addition.

Anion-exchange HPLC, described in User Bulletin 13, Revised, can be used for Aminolink-oligonucleotide analysis or purification. The inherent problem with using reverse-phase HPLC for purification is that the oligonucleotide and the aminolink-oligonucleotide are not well resolved.

## APPLICATIONS

### General Protocol for Tag/Labeling Substrate

As was described in the beginning of this bulletin, there are many applications for this linker. Below is a "generic" protocol from which to extrapolate individual application protocols. It should be noted that most commercially available biotinylation reagents come with usage protocols.

1) Prepare a solution of Aminolink oligonucleotide at a concentration of 1 mM or greater in a 50 mM-200 mM $NaHCO_3/Na_2CO_3$ buffer, pH 9.

2) Add an approximately 50-molar excess of the solid tag/labeling substrate (NHS-Biotin, Dye, etc.) and vortex to dissolve. If the tag or DMSO is insoluble in aqueous solutions, it may be dissolved in dimethylformamide before addition to the aminolink-oligonucleotide. The final concentration of organic solvent in the reaction mixture should not exceed 30%.

3) After several hours reaction time at room temperature, the mixture can be purified by gel-exclusion in a G-50 or G-25 Sephadex column to remove the excess tag. Use 100 mM TEAA as the eluent.

NOTE: Reactions with fluorescent dyes should be protected from light.

## CONCLUSION

This product increases the utility of your DNA Synthesizer by allowing the addition of a versatile linker to synthetic DNA. Please contact an Applied Biosystems' DNA Product Specialist if you have any questions or comments about this or any other DNA Synthesis Reagent.

Aminolink is a registered trademark of Applied Biosystems.

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# USER BULLETIN

User Bulletin No. 50
August 26, 1988

**Subject: HPLC Purification and Processing of 10 Micromole Synthetic Oligonucleotides**

This bulletin describes procedures for isolation and purification of milligram quantities of oligonucleotides using HPLC. HPLC represents the most rapid and convenient means of purifying oligonucleotides produced with typical 10-micromole syntheses. Included are procedures for preparing the crude DNA for HPLC purification, representative chromatograms from typical and anomalous analyses, and protocols for processing and quantifying the oligonucleotide product following chromatography.

These procedures have been used with phosphoramidite and H-phosphonate-derived oligonucleotides. They should facilitate your research requiring large quantities of oligonucleotide, regardless of application.

1. Prepare the base-deprotected, crude 5'-dimethoxytrityl (DMT) DNA for initial concentration as follows:

To the DNA ammonia solution (contained in 13 x 100 mm culture tube), add ~100 µL of triethylamine (to prevent loss of the acid-labile DMT) and vortex. Concentrate the sample under a stream of $N^2$ (or air) for 20-30 minutes in a fume hood to remove most of the $NH_3$. Reduce to 1/4 of the original volume. Alternatively, use a vacuum centrifuge connected to a water aspirator. Add another 100 µL of triethylamine and vortex briefly. Remove a small aliquot (5 µL is sufficient, but more may be removed if desired) and set aside to be used for an analytical HPLC injection. Concentrate the remaining sample to dryness in a vacuum centrifuge (e.g. Savant).

2. Prepare the crude 5'-DMT DNA for HPLC purification as follows:

To the tube containing the dried DNA sample, add 100 µL of triethylamine (to protect the acid labile DMT groups) followed by 1.2 mL of 0.1M triethylammonium acetate (pH 7 TEAA), vortex, and sonicate (if needed) to dissolve the DNA. Syringe filter the solution by attaching a Millipore filter (Miller-HV, 0.45 µm, Catalog No. SLHV025NS) to a 1 mL disposable syringe barrel. Allow the DNA-solution to gravity filter. Wash the test tube that contained the DNA-solution with 1 mL of 0.1M TEAA and add to the syringe filter set-up. Purge the filter by rapidly inserting the plunger (it is not necessary to allow the 1 mL of 0.1M TEAA wash to gravity filter). This will yield a final volume of ~2.0 mL (~300 µL filter hold-up).



Applied Biosystems

3. Perform the HPLC Purification of the crude 5'-DMT as follows:

Depending on the oligonucleotide sequence, one of two different HPLC purification methods should be used. The first method yields good results with DNA that is unlikely to form secondary structure [e.g., duplex and/or hairpin formation]. It employs an Applied Biosystems Aquapore (Prep -10) Octyl column, catalog number. 0711-0166, 10 x 250 mm.

The second method is used for oligonucleotides that are likely to form secondary structure (e.g. self-complimentary or partially self-complimentary sequences, and especially those with high G-C content). With this method it is advantageous to heat the column to melt-out any higher-order structure which otherwise would lead to a more difficult HPLC purification evidenced by giving multiple peaks. For this reason the second method takes advantage of a polymeric reverse phase column (Polypore PRP, Applied Biosystems catalog number 0712-0053, 7 x 250 mm column), which has shown very high capacity for its size. It can also be heated repeatedly without apparent damage to the column as indicated by its performance.

**Analytical injection:**
After choosing one of these methods, inject the 5 µL aliquot onto an Aquapore prep-10 octyl column at room temperature or a Polypore PRP equilibrated to 60°C (Waters column heater, Model 1122). Use the conditions shown in Figure 1 and set the detector absorbance units full scale (AUFS) [see Trace A].

| Time (min) | %Acetonitrile | %Triethylammonium Acetate, 0.1M, pH 7 |
|---|---|---|
| 0 | 20 | 80 |
| 10 | 30 | 70 |
| 25 | 30 | 70 |

* Flow rate for 7 x 250 mm Polypore PRP column is 3 mL/min.
* Flow rate for 10 x 250 mm Aquapore column is 11 mL/min.

Figure 1: Conditions for initial HPLC analysis of crude 5'-DMT oligonucleotide.

**Preparative injections:**
Set the UV detector to 280 nm (it is also recommended to use a preparative flow cell) and 2.0 AUFS. When using the Aquapore column at room temperature (see above gradient, flow rate = 11 mL/min), one-half of a 10 µmol synthesis can be injected without experiencing "overloading". The Polypore PRP column has enough capacity to purify about one-third of a 10 µmol synthesis (the Polypore PRP column can be used at room temperature in place of the Aquapore). If time is not a factor, better results may be obtained by injecting smaller amounts and by diluting each injection with as much 0.1M TEAA as possible, depending on injection loop size.

Although the purpose of heating the Polypore PRP column is to melt-out secondary structure, multiple peaks which usually are not present in the analytical trace, may appear when performing preparative injections. Such multiple peaks should be individually collected [see Trace B] and re-analyzed under the same conditions by directly injecting 25-30 µL of the collectate at 254 nm, 0.1 AUFS [see Traces C and D]. Often these multiple peaks are the same desired product and will give essentially identical retention times [see C and D]. However, one or more of these fractionated peaks may be undesired material [see Trace E-H].

Concentrate the collectate (pooled if warranted) under a stream of $N^2$ (or air) for ~30 minutes to remove most of the acetonitrile. Transfer the solution to a large test tube(s) and concentrate to dryness in a vacuum centrifuge.

Dissolve the resultant DNA residue in several mL's of $H_2O$ and transfer to a "Pyrex" or "Kimex" test tube (13 x 100 mm). Concentrate to dryness in a vacuum centrifuge.

4. Remove the 5'-DMT as follows:

Dissolve the resultant DNA residue in 3 mL 80% glacial acetic acid. Let stand at room temperature for 10 minutes. Freeze the reaction mixture on dry-ice and concentrate to dryness in a vacuum centrifuge.

5. Extract the 5'-HO-DNA with ethyl acetate to remove DMT-OH and other organic impurities as follows:

Dissolve the resultant DNA residue in 1 mL of $H_20$. Add 1 mL of ethyl acetate and vortex. Allow the organic layer to separate from the aqueous layer containing the DNA and possible HPLC column particulates (centrifuge if necessary). Remove the organic layer with a Pasteur pipet and discard. Repeat the extraction procedure two more times. During the third and final extraction, centrifuge for 5-10 minutes to separate organic and aqueous phases well enough to spin down particulates in the aqueous portion. Remove the organic layer, then carefully remove the aqueous, DNA-containing layer from any particulates spun to the bottom. Transfer to a clean test tube. Concentrate to dryness in a vacuum centrifuge.

6. *Precipitate the 5'-HO-DNA with ethanol to remove triethylammonium acetate and residual organic impurities as follows:

Dissolve the resultant DNA by sonicating and/or warming (50-60°C) in 300 µL of 1.0 M NaCl. If the DNA fails to dissolve, check the pH and, if necessary, adjust to either neutral or slightly basic by adding a small amount of NaOH (5 µL portions of 5N). Precipitate the DNA by adding 1 mL of 200 proof ethanol drop-wise while vortexing. Chill on ice for ~30 minutes. Centrifuge for ~5 minutes. Carefully remove supernatant and save for later OD measurement. Repeat the precipitation procedure using double the amounts of 1 M NaCl and ethanol. Bring the sample to dryness in a vacuum centrifuge.

* For tetramers and smaller oligos precipitation may not be possible. In these cases run a size exclusion column using Bio-Gel™ P-2, 200-400 mesh (Bio-Rad Laboratories, catalog no. 150-0150) instead of precipitation.

7. OD measurement:

Dissolve the resultant DNA residue in 2 mL of $H_20$. Remove a 10 µL aliquot for OD measurement. If any particulates remain, centrifuge for 5-10 minutes and carefully transfer the supernatant to a clean test tube (syringe filtering may lead to contamination due to lubricants used on the syringe plunger). Bring the 10 µL aliquot to 1 mL with $H_20$ and measure $OD_{260}$. Multiply the number obtained by 200 to determine the OD-units contained in the bulk sample. Concentrate the bulk sample to dryness in a vacuum centrifuge. Discard the OD aliquot or use for HPLC compositional analysis[1] after digestions with snake venom phosphodiesterase and alkaline phosphatase.

Check the supernatant of the precipitation procedure (obtained in step 6) for non-recovered DNA by bringing the volume to 3 mL with $H_20$ and removing a 150 µL aliquot. Dry the aliquot using a stream of $N^2$. Dissolve in 1 mL of $H_20$ and measure $OD_{260}$. Usually there is very little material (a few OD's) in the supernatant, which is then discarded.

Typically, about 50% yield (not corrected coupling efficiency) is obtained. For example, a 10 µmol synthesis of 5'-DMT (AT)5 afforded 500 $OD_{260}$ units of purified product, (AT)5,

which has a mM extinction coefficient given by (5 x 14)+(5 x 7) = 105. [Note: For purines A and G use 14, and for pyrimidines C and T use 7]. Since 1 µmol in 1 mL is 1 mM, 10 µmol OD (AT)5 in 1 mL would give 10 x 105 = 1,050 $OD_{260}$ units. Thus, the yield of product was (500/1,050) x 100 = 48%.

References

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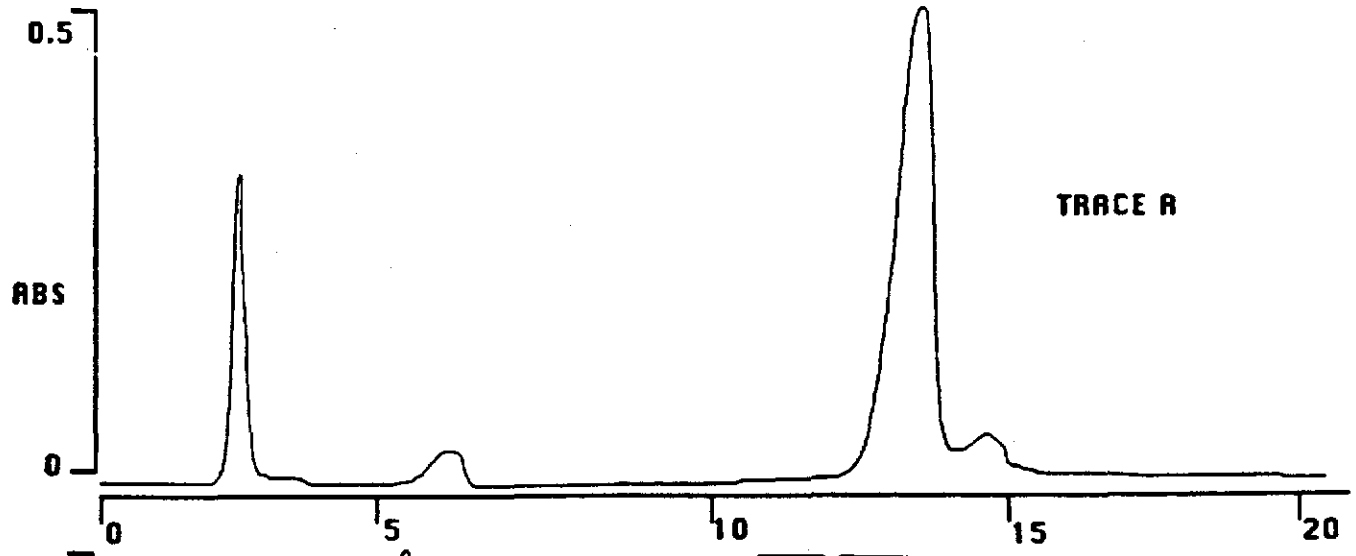
Bio-Gel is a registered trademark of Bio-Rad Laboratories.
Polypore is a registered trademark of Applied Biosystems, Inc.
Aquapore is a registered trademark of Applied Biosystems, Inc.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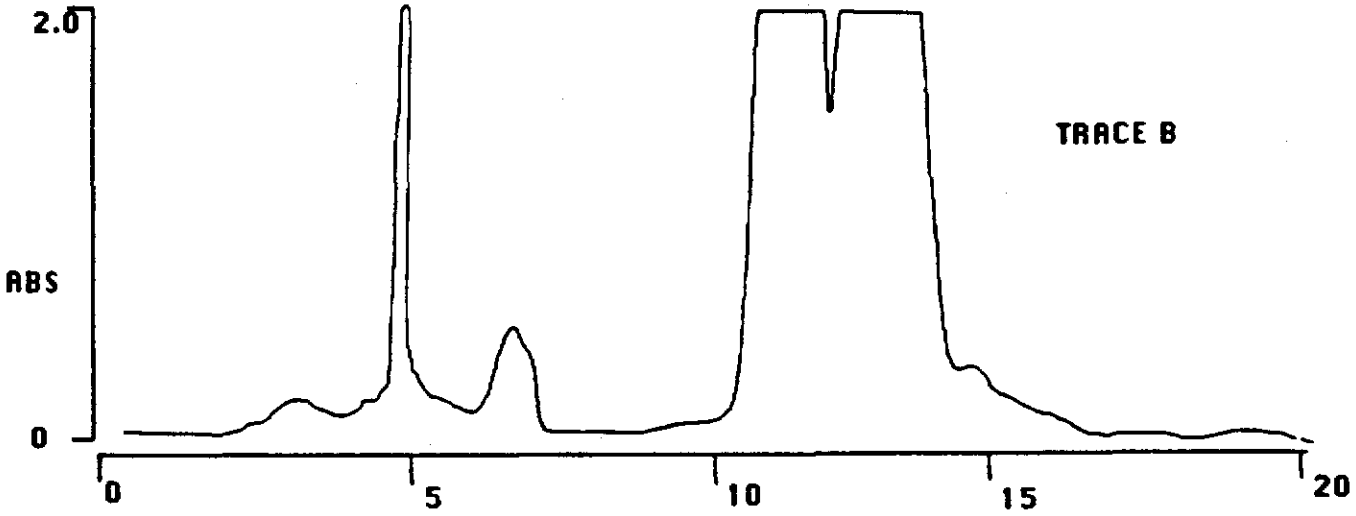
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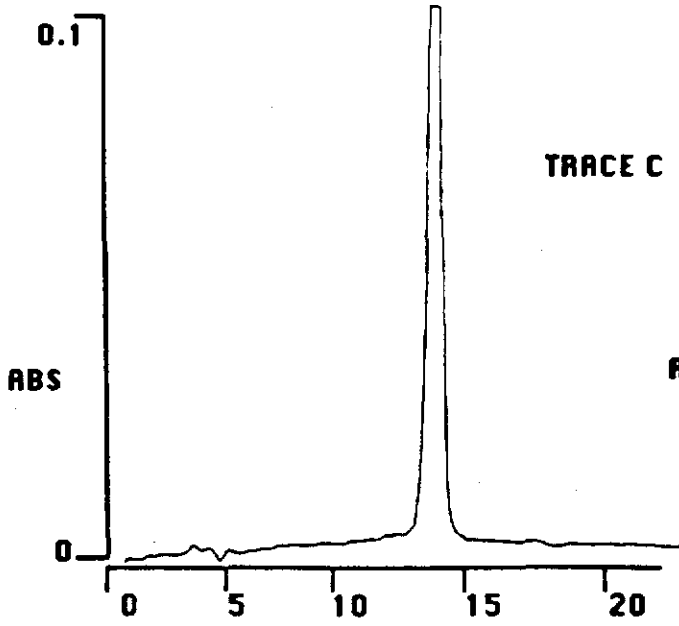
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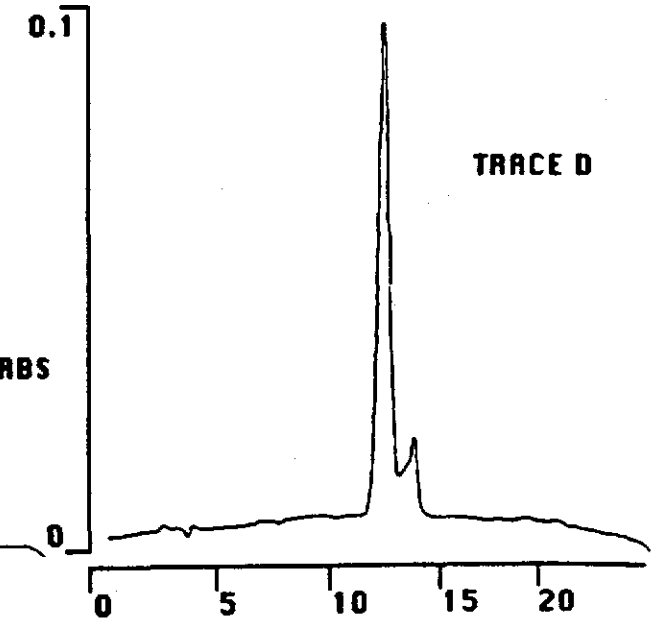
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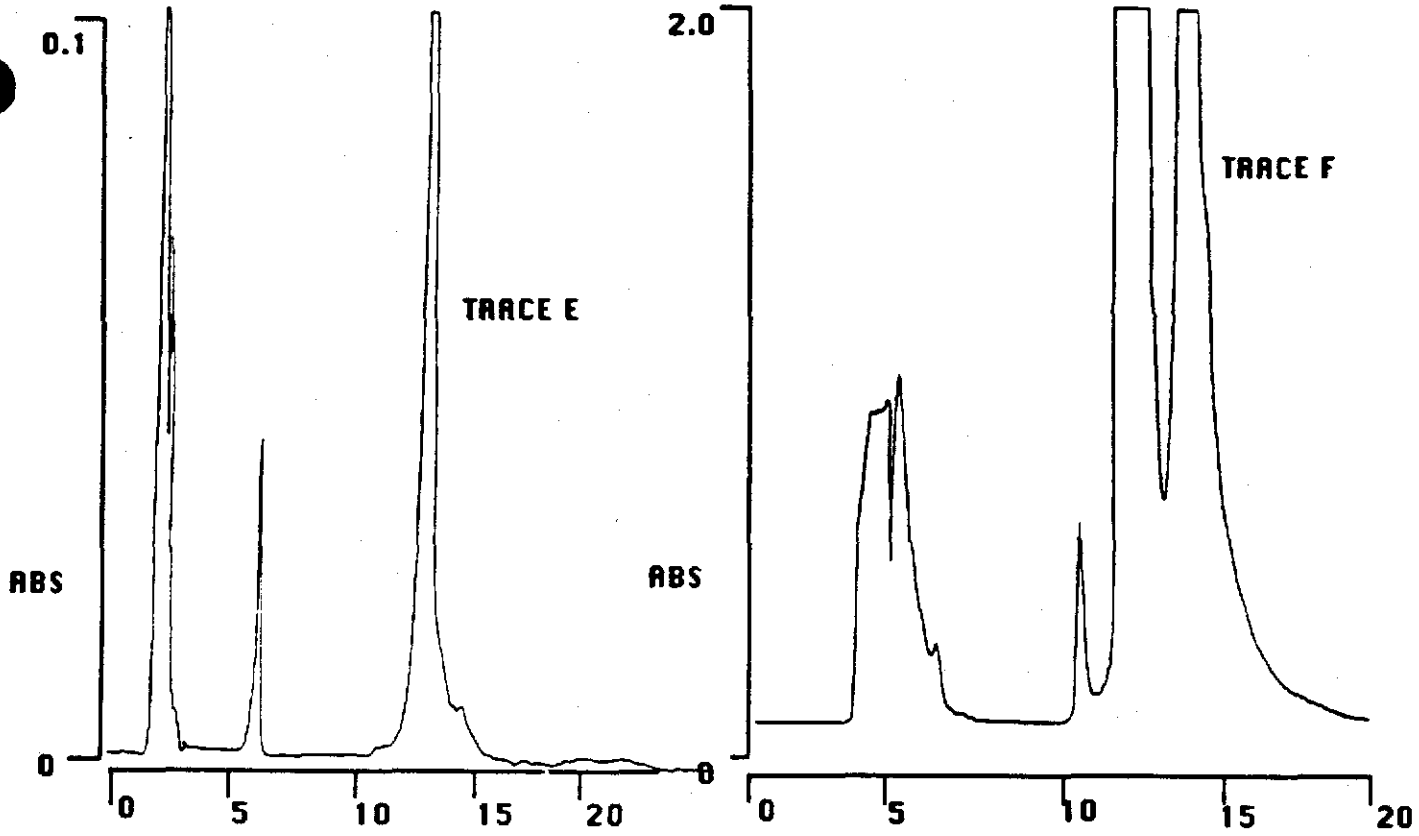
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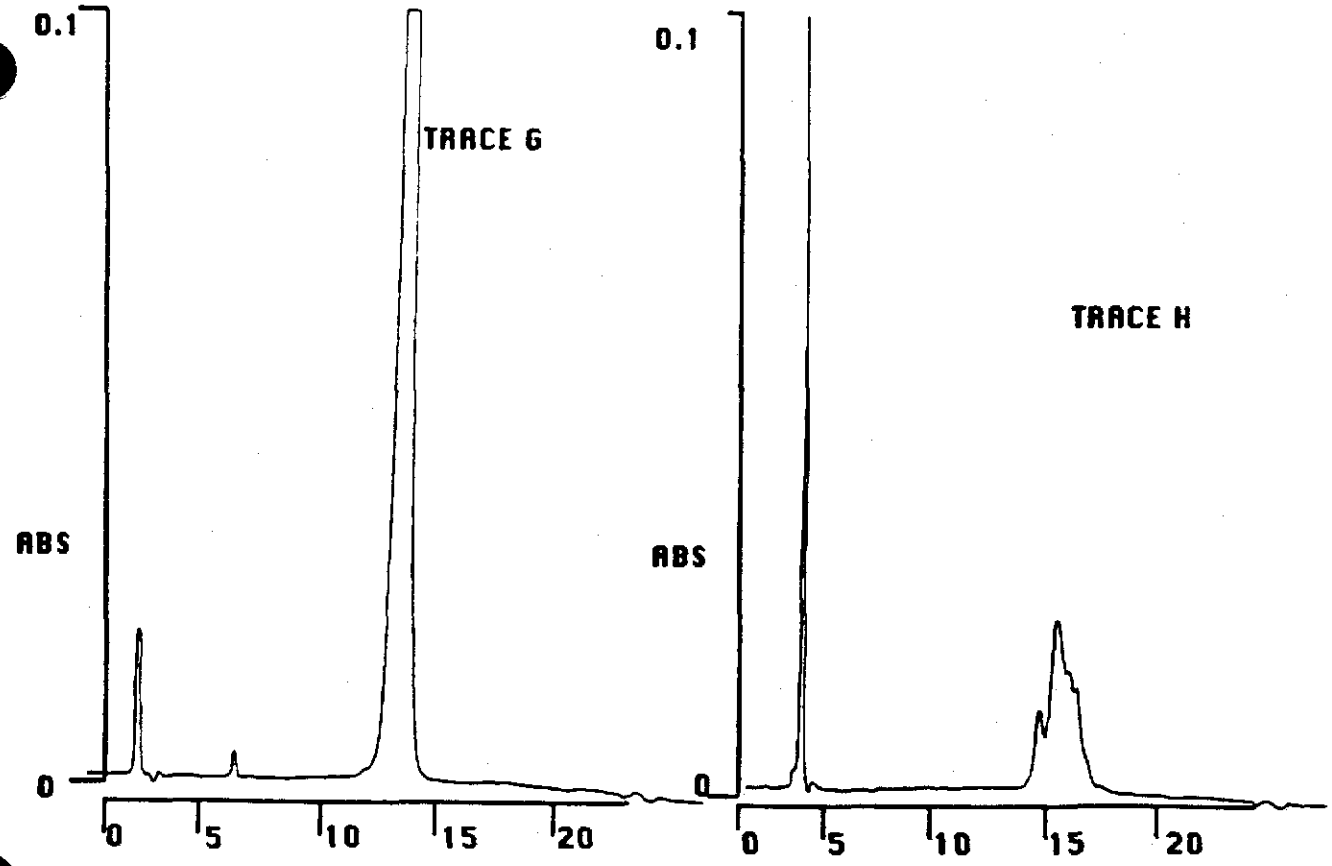
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# USER BULLETIN

Issue No. 51
November 1988

## SUBJECT: OPC™ Purification of Long Oligonucleotides

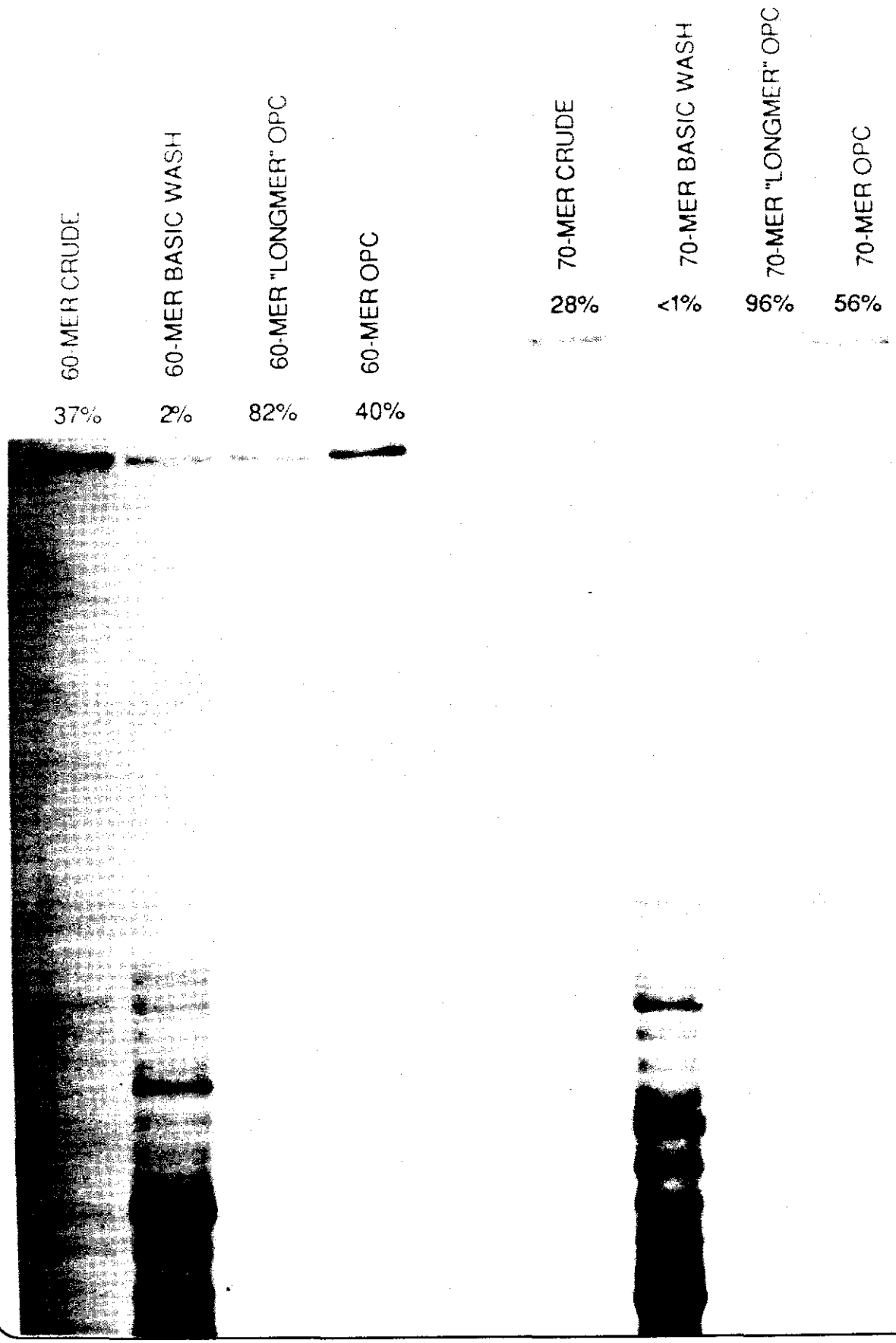
OPC (Part No. 400771) is a rapid purification cartridge used specifically for synthetic DNA. It was designed to provide the level of purity required for the common applications of synthetic DNA. Using OPC eliminates the need to purify by time-consuming and labor-intensive polyacrylamide gel electrophoresis (PAGE) or HPLC.

OPC is fast, is easy to use and delivers consistent results. The time required to go from the deprotected, crude oligonucleotide to its use in an experiment is greatly reduced. Complete purification requires only 15 minutes. After deprotection, an aliquot of the crude sample, still in ammonia, is loaded directly onto the OPC cartridge. This eliminates the need to evaporate the sample prior to purifying. The remaining portion of the crude synthesis can then be stored in ammonia at -20°C without harming the sequence. In fact, it is preferable to storing it as an evaporated sample or dissolved in water. The ammonia protects the sample from acidic by products present in the crude. Detritylation is also accomplished on the cartridge, eliminating the need for post-purification work-up. Quantification of the oligonucleotide can be easily accomplished and only the volume containing the amount required for the experiment need be evaporated. The remainder can be stored at -20°C for later use. The product obtained from OPC is high-purity DNA that can be used with complete confidence.

The existing OPC protocol can successfully purify sequences that are ≤40 bases. This limit is due to the synthesis chemistry, not OPC. As the length of the oligonucleotide increases beyond 40 bases, the trityl-bearing (n-minus)-mers resulting from the inherent trityl heterogeneity of the synthesis chemistry becomes statistically significant. These trityl-bearing side products are co-purified during the OPC process and result in sub standard purities. In response to this problem, we have devised a simple modification to the existing OPC protocol for the purification of sequences in to 40 to 70 base range.

This new protocol (see back page) incorporates additional washes with dilute ammonia after the detritylation step with 2% trifluoroacetic acid. The extra basic wash removes preferentially any shorter sequences that had trityl protecting groups attached and thus had co-purified with the n-mer sequence. After washing with water, the product can be eluted in the normal manner. The additional wash steps do not add significant time to the process.

*Figure 1*

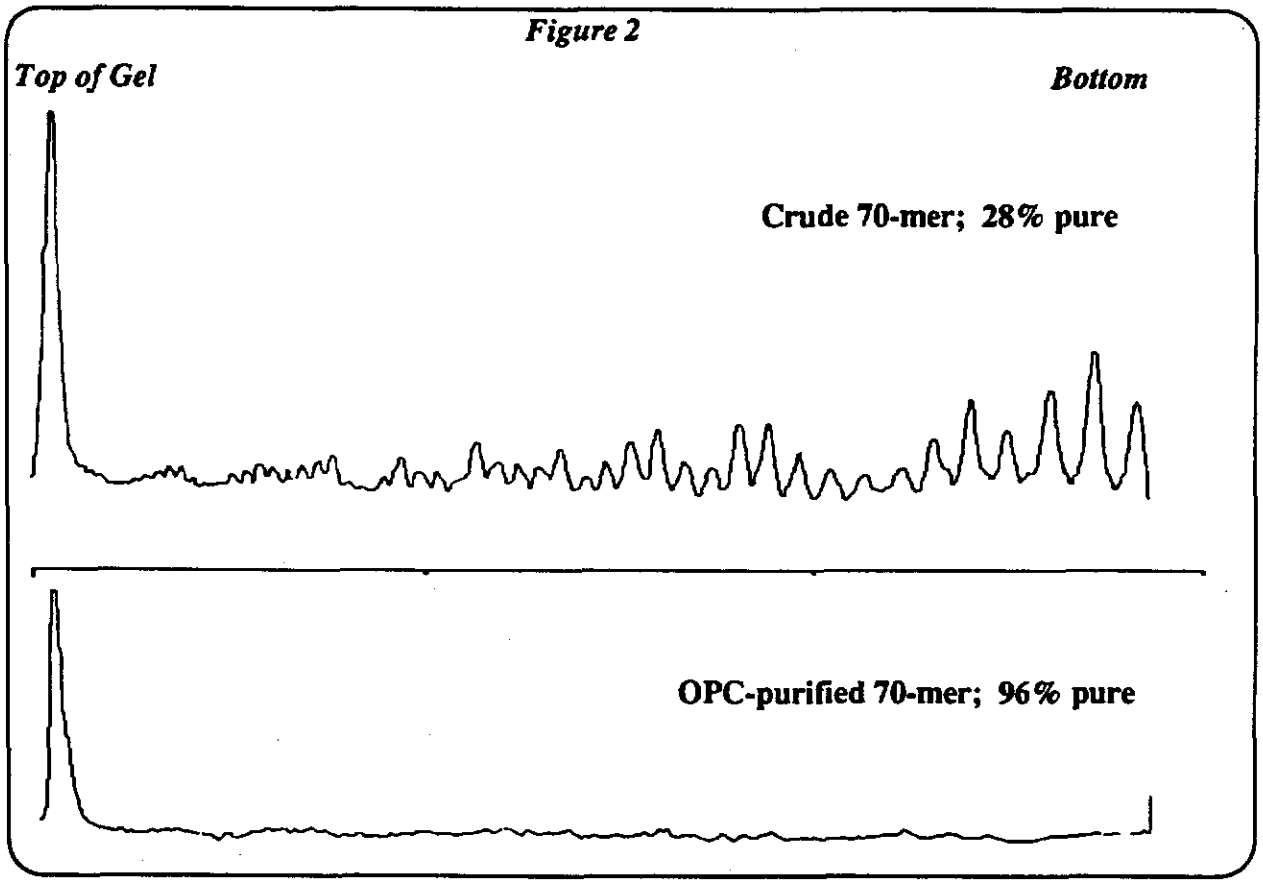

60-MER CRUDE
37%
60-MER BASIC WASH
2%
60-MER "LONGMER" OPC
82%
60-MER OPC
40%
70-MER CRUDE
28%
70-MER BASIC WASH
<1%
70-MER "LONGMER" OPC
96%
70-MER OPC
56%

## Comparison of the Two Methods in Purifying Long DNA

Figure 1 illustrates the differences in purity obtained when purifying long DNAs with the two OPC protocols. The autoradiogram in figure 1 is of crude and purified fractions of two sequences, a 60-mer and a 70-mer. In each sequence series, the first lane depicts the crude oligonucleotide. The second lane is the DNA present in the ammonia wash after detritylation, representing the amount of trityl-bearing (n-minus) mers. The third lane is the OPC-purified product using the modified protocol and the fourth lane is the OPC-purified product using the standard protocol.

The percentage figures at the top of each lane represent the relative amount of each full-length product as determined by scanning densitometry. The modified protocol resulted in product purities of 82% and 96%, for the 60- and 70-mer, respectively. The standard protocols resulted in purity values of 40% and 56%, respectively. Figure 2 shows the densitometry scan of the crude and OPC-purified (modified protocol) 70-mer. Notice the larger amounts of shorter failure products in the crude; these are the trityl-bearing species.

*Figure 2*

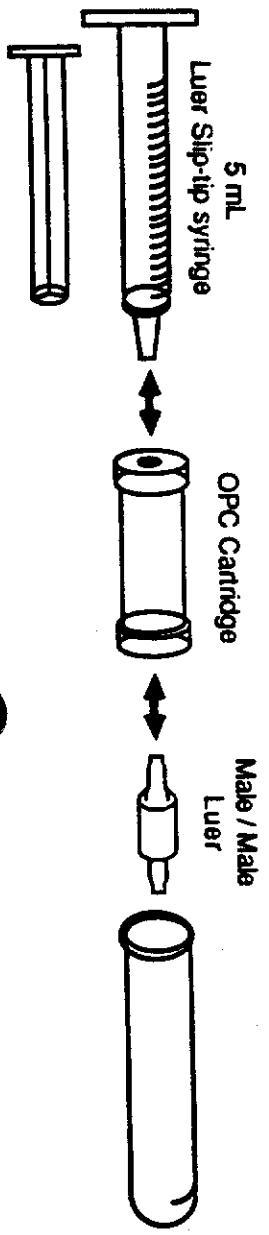



### Conclusion

OPC can now be used for the purification of sequences up to 70-mers. With this increased performance, OPC can be used for virtually any molecular biology application. In addition, OPC has been proven to purify sequences ≤40 bases, even those with major contaminants due to poor coupling or those with significant secondary structures[1]. This kind of performance coupled with its speed and ease of use make OPC the cost effective approach to routine purification of synthetic DNA.

1 McBride, et.al. (1988) A New Reliable Cartridge for the Rapid Purification of Synthetic DNA, *Biotechniques* 6, 362 - 367.

# The Modified OPC Protocol




Solutions Needed:

-HPLC grade acetonitrile, 5 mL
-2.0 M triethylamine acetate (Part No. 400613), 5 mL
-Deionized water, 1 mL
-Dilute ammonium hydroxide, 15 mL
(1:10 dilution of conc. ammonium hydroxide in deionized water)
-2% trifluroacetic acid in deionized water, 10 mL (Neat TFA Part No. 400137)
-20% v/v acetonitrile in deionized water, 1 mL

1. After completion of a "trityl-on" synthesis, cleave the oligonucleotide from the support and deprotect following normal protocols for the synthesis method utilized.

2. Connect an all-polypropylene syringe (Aldrich Z11686-6), OPC cartridge, and male-to-male Luer tip as shown. Make sure all fittings are snug.

3. Flush the cartridge with 5 mL HPLC grade acetonitrile, followed by 5 mL 2.0M triethylamine acetate. Keep the flow rate at 1 to 2 drops per second for all reagent additions.

4. Dilute the ammonium hydroxide solution containing the cleaved, deprotected, "trityl-on" crude oligonucleotide with one-third volume of deionized water.

5. Load the above solution into the syringe and then gently push through the cartridge, saving the eluted fraction. Reload this fraction, and again push through the cartridge.This will load 1 to 5 OD units of the trityl oligonuleotide (depending on length, sequence, and synthesis quality) onto the cartridge. Save this final eluted fraction in ammonia at -20°C, it can be put through another cartridge until exhausted of trityl oligonucleotide.

6. Flush cartridge with 3 x 5 mL dilute ammonium hydroxide.

7. Flush cartridge with 2 x 5 mL deionized water.

8. Detritylate the OPC-bound oligonucleotide with two 1-minute treatments of 5 mL of the 2% trifluoroacetic acid solution.

9. Flush cartridge with 2 x 5 mL deionized water.

**For sequences ≥ 40 bases, add this step:**

9a. Flush cartridge with 1 x 5 mL dilute ammonium hydroxide, then 2 x 5 mL deionized water.

10. Elute the purified, detritylated oligonucleotide by flushing the cartridge with 1 mL of the 20% acetonitrile solution.

11. Evaporate to dryness an aliquot of the eluate (step 10) and dissolve in water to determine the OD units at A-260.

12. Store any unused OPC-purified oligonucleotide as a dry solid at -20°C.

The RNA oligo is then ready for PAGE or HPLC purification/analysis. RNase degradation is not usually a severe problem, except when handling less than 0.1 ODU, although general RNA cautions should be considered. When the oligoribonucleotide is completely deprotected, it is labile to RNase degradation. RNase contamination is ubiquitous, necessitating sterile conditions. Procedures for preventing RNase degradation can be found in several sources[6].

# Conclusion

Oligoribonucleotides are now available from your ABI DNA synthesizer. The Ogilvie chemistry, using 2' silyl protected phosphoramidite monomers, has been demonstrated on all Applied Biosystems DNA synthesizers. The efficiency of RNA synthesis is nearly equivalent to DNA synthesis as judged by trityl yields, PAGE and HPLC. However, it should be realized that subtle chemistry factors influencing the level of biological activity of synthetic oligoribonucleotides, relative to native RNA fragments, have not been exhaustively investigated. Applied Biosystems developed the first fully defined package for RNA synthesis: reagents, monomers, cycles, and procedures. This User Bulletin provides improvements in the methodology to ensure the reliable production of pure oligoribonucleotides.

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# USER BULLETIN

**Models 380A/380B/381A/391 EP DNA Synthesizers**
**Issue No. 53**
**December 1, 1989**

# RNA Synthesis With DMT-Cyanoethyl RNA Phosphoramidites

## Introduction

RNA synthesis chemistry has been recently refined to use DMT protected cyanoethyl RNA phosphoramidites on your Applied Biosystems DNA synthesizer. Oligoribonucleotides of high purity and yield have been obtained on the models 380A, 380B, 381A and 391 EP ABI oligonucleotide synthesizers. All the necessary cycles, end procedures, and cleavage/deprotection protocols, originally described in Applied Biosystems' User Bulletin Number 47 (April 1988), have been revised and are included in this User Bulletin.

The potential utility of defined sequences of oligoribonucleotides in biological experiments has long been recognized. The ability to explore transcription and translation with synthetic constructs of mRNA, tRNA, and rRNA is a powerful prospect. Recently, interest has been heightened by the Nobel prize winning discovery of RNA sequences with catalytic activity[1], RNA with unusual conformations[2], and recombinant RNA technology[3]. Possible chemotherapeutic applications of synthetic RNA are especially exciting and distinct from the use of native double-stranded RNA and interferons as antitumor agents[4]. Historically, these various investigations have been hampered by the unavailability of synthetic RNA. Now, with commercially available RNA monomers and supports, coupled with optimized synthesis cycles on your ABI synthesizer, RNA synthesis is as available as DNA synthesis.

# Reagents

The ancillary reagents for RNA synthesis are the same as for DNA synthesis:

| Reagents | ABI Part No. | Bottle Position |
|---|---|---|
| Tetrazole/acetonitrile | 400233 or 400606 | 9 |
| | (45 ml) (180 ml) | |
| Acetic anhydride/lutidine/THF | 400234 or 400607 | 11 |
| | (45 ml) (180 ml) | |
| NMI/THF | 400777 or 400785 | 12 |
| | (45 ml) (180 ml) | |
| Trichloroacetic acid/DCM | 400236 | 14 |
| Iodine/water/pyridine/THF | 400753 | 15 |
| Acetonitrile | 400262 | 18 (13,16) |

**Important:** **To conduct either RNA or DNA synthesis requires only the changing of the appropriate monomers and support and selecting the appropriate cycle. The above ancillary reagents remain on the instrument and are used at the same bottle positions for RNA and DNA syntheses. Consumption of each reagent per cycle is virtually the same for RNA and DNA.**

# RNA Monomers

As with DNA monomers, the RNA phosphoramidites are diluted with dry acetonitrile to a concentration of 0.1 molar. The acetonitrile solution stability of the RNA monomers is comparable to DNA monomers, which have a useful lifetime on the synthesizer of about 14 days.

# Synthesis Cycles

The RNA synthesis cycles in User Bulletin Number 47 (April 1988) were designed for 5' MMT methylphosphoramidite monomers. Now that 5' DMT cyanoethylphosphoramidite monomers are available, creating an RNA cycle for any scale or ABI synthesizer is extremely simple. *When using DMT cyanoethylphosphoramidite RNA monomers, simply increase the coupling wait time of the appropriate scale DNA cycle to 600 seconds to create your RNA cycle.*

The primary difference from the DNA cycles is the longer wait time for coupling. This is a consequence of the steric hindrance conferred on the reactive 3' phosphoramidite group by the neighboring 2' O-silyl group. RNA cycle times are about 10 minutes longer than DNA cycles. Average yields per cycle have been demonstrated to be nearly as high as DNA synthesis, 98%, as measured by trityl UV quantitation. The high average yield per cycle in the synthesis of long oligoribonucleotides was also demonstrated through the synthesis of a 43-mer[5] and a 77-mer[6], and synthesis at the 10 µmol scale[7] on the Models 381A and 380B DNA synthesizers.

# Cleavage, Deprotection and Purification

The relatively fragile nature of RNA to pH extremes and enzymatic degradation imposes more stringent techniques in deprotection, analysis, purification, and handling[6]. The following protocols describe the conditions and cautions for generating purified synthetic oligoribonucleotides.

After synthesis is complete, the oligoribonucleotide is ready for deprotection. Although RNA syntheses can be conducted either Trityl On or Trityl Off, we recommend that you synthesize all your oligoribonucleotides Trityl Off. Trityl On purifications (HPLC or OPC) require 2'-hydroxyl deprotection *prior* to acidic removal of the trityl group and are not currently recommended. Exposing fully deprotected RNA to acidic conditions (detritylation) enhances the potential for 2' to 3' phosphate migration. RNA syntheses conducted Trityl Off eliminate phosphate migration, since the 2' protecting group is not removed until after detritylation.

The 2' silyl oligoribonucleotides are relatively stable compounds. They are resistant to RNases and may be stored for extended times as a dry pellet or in cold, aqueous, neutral solutions. In the final step, the silyl deprotection reagent (tetrabutylammonium fluoride in tetrahydrofuran) is very selective and mild, preserving the integrity of the RNA[8-10].

The reagent for cleavage from the support and base deprotection (bottle position number 10) is a 3:1 mixture of concentrated ammonia:ethanol. The inclusion of ethanol in the ammonia is necessary to adequately solubilize the more lipophilic 2' O-silyl oligoribonucleotide and to minimize desilylation. Cleavage from the support with the ammonia:ethanol (3:1) is slower than cleavage with ammonia only. Therefore, the cleavage time has been doubled from previously described procedures. The post-RNA synthesis procedures for cleavage from the support and deprotection are described below.

The post-synthesis protocol for cleavage/deprotection of RNA oligos, originally described in Applied Biosystems' User Bulletin Number 47, has been improved and simplified. As with DNA, deprotecting the *cyanoethyl*phosphoramidite derived RNA oligonucleotide does not require thiophenol.

## For 380A and 380B users:

Support cleavage may be conducted on the 380B and the 380A with minor alterations to the standard DNA synthesis end procedures. To achieve complete cleavage of the RNA oligo, simply double the four ammonia/ethanol wait times to 1800 seconds in the standard DNA end procedure. Store this new end procedure for future RNA syntheses. Upon completion of the end procedure, remove and cap the collection vial, then heat at 55° C for 8-16 hours for complete base deprotection.

## For 391 and 381A users:

For deprotection off the synthesizer, ammonia treatment may be implemented through the double-syringe method.

1. At completion of the synthesis, dry the synthesis column with 60 seconds of reverse flush (function 2), then remove the column from the instrument.

2. Load a fresh syringe (with luer tip not a locking tip) with a 2 mL solution of 3:1 concentrated ammonia (Baker Part No. 9721-01): ethanol (Aldrich Part No. 27,764-9)
3. Mount another, empty syringe, with plunger fully inserted, into one end of the synthesis column. Mount the ammonia/ethanol loaded syringe into the other end of the column.
4. Holding a syringe in each hand, *gently* inject the reagent through the column to the empty syringe and return several times.
5. Let stand for 2 hours at ambient temperature.
6. Withdraw the ammonia/ethanol solution into one syringe and eject the solution into a screw-cap vial.
7. Heat at 55° C for 8-16 hours for complete base deprotection.

## 2'-Hydroxyl deprotection:

1. Dry the ammonia/ethanol solution of crude RNA oligo (DMT OFF), after heating 8-16 hours at 55° C.
2. Add 10 microliters (per ODU of crude RNA oligo) of 1 molar tetrabutylammonium fluoride/THF (Aldrich Part No. 21,614-3). Vortex thoroughly for at least one minute, then let stand for 6-24 hours at room temperature.
3. Add an equivalent volume of 0.1M TEAA, mix, and evaporate. The pellet is substantial and appears as a viscous, semi-solid.
4. Add 1 ml of 0.1M TEAA , mix and apply to an OPC cartridge which has been prewashed with 5 ml acetonitrile, then 5 ml 2M TEAA.
5. Load the RNA oligo, recycling it through the cartridge twice at 1-2 drops per second.
6. Wash the OPC cartridge with 5 ml 0.1M TEAA, then 10 ml deionized water.
7. Elute the desalted RNA oligo (up to 50 ODU) with 1 ml 50% acetonitrile.

**Important:** **This OPC procedure replaces a long and troublesome Sephadex column for removal of the tetrabutylammonium salts, which complicate further analysis and purification.**

# USER BULLETIN

Model 380A, 380B, 381A, 391, 392, 394
DNA Synthesizers

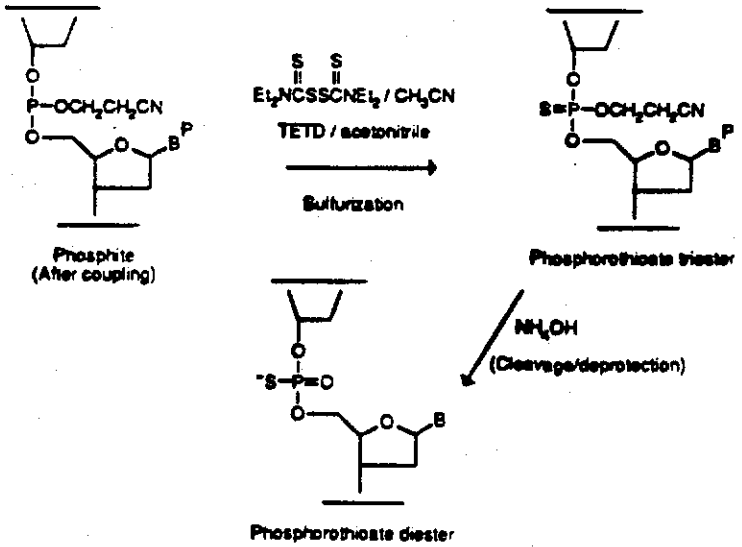
Number 58
February 1991

# Sulfurization With TETD: Phosphorothioate Oligonucleotide Synthesis Via Phosphoramidite Chemistry

A new sulfurizing reagent is now available that significantly improves the ease, cost, and efficiency of synthesizing internucleotide analog, phosphorothioate oligonucleotides. Tetraethylthiuram disulfide (TETD) in acetonitrile (ABI P/N 401147) converts internucleotide cyanoethyl phosphite to the phosphorothioate triester within 15 minutes at room temperature.[1] It replaces only the iodine reagent, with all other reagents used for standard phosphoramidite chemistry remaining unchanged. It is available in the standard 180 mL (8 oz.) bottle and is used in the same position as the iodine reagent.

The TETD reagent, which has been specially formulated and prepared, can be used with all Applied Biosystems DNA synthesizers that use current software versions except the pre-124 non-programmable 380A, and the non-programmable (non-EP) 391 PCR-MATE™. The cycles specified for the 380B are designed to be used with Version 2.00; those specified for the 381A are to be used with Version 1.50.

With the attached, optimized cycles, the synthesis efficiency and purity of phosphorothioate oligonucleotides is comparable to that of phosphodiester oligonucleotides. Also, mixed phosphorothioate/phosphodiester DNA can be easily prepared. Even after long exposure to the TETD reagent, analysis of oligonucleotides by enzymatic digestion shows no detectable base modifications.[2]




Applied Biosystems

# Antisense Investigations

The study of phosphorothioate oligonucleotides in the antisense experiment has flourished recently. Replacement of one of the non-bridging, equivalent internucleotide oxygens with sulfur results in a chiral phosphorothioate diester, resistant to nuclease metabolism *in vivo*. Specific sequences nullify transcription of mRNA in the expression of specific genes, termed the antisense effect.[3] Potential therapeutic candidates—against HIV, for example—have progressed to the preclinical, pharmacokinetic stage.[4]

The hydrogen-phosphonate chemistry was developed to furnish phosphorothioates by automated, solid-phase DNA synthesis.[5] A single sulfurization of all the hydrogen-phosphonate internucleoside linkages is conducted post-synthesis, with elemental sulfur dissolved in carbon disulfide.[6] This method was adequate to meet the initial demand until recently when the issues of synthesis efficiency, economy, and large-scale production intensified. The new reagent, TETD, greatly simplifies and improves the synthesis of this class of DNA analog oligonucleotide.[7]

# Installation

Using the standard #15 bottle change procedure, replace the iodine reagent with a bottle of acetonitrile. Conduct the same procedure a second time, removing the acetonitrile bottle and installing the TETD reagent. This double-bottle change procedure minimizes cross contamination of the sulfurizing and oxidizing reagents.

Note: **The TETD reagent may be installed on the 380B at Position #17, keeping the iodine reagent on Position #15. Two function changes in the synthesis cycles are necessary to ensure delivery of TETD from #17. To make an oligonucleotide that has both phosphodiester and phosphorothioate linkages, use the Cycle Editor to switch the cycles during a single synthesis at programmed base numbers (Model 380B only).**

| 380B Cycle | Steps | TETD #15 Function | TETD #17 Function |
|---|---|---|---|
| 1 µM SULF | 40 | 81 | 30 |
| | 41 | 13 | 11 |
| 10 µM SULF | 26 | 81 | 30 |
| | 27 | 13 | 11 |

- The TETD reagent is stable at room temperature.
- Keep it tightly closed when not in use.
- The TETD reagent may be installed and removed from the synthesizer several times. It will retain peak performance for six weeks after initial use.
- Either the Fast Oligonucleotide Deprotection (FOD) reagents or standard phosphoramidites and supports may be used.[8]
- Phosphorothioate oligonucleotides, like phosphodiester oligonucleotides, are cleaved and deprotected at the completion of synthesis with concentrated ammonia.
- No additional post-synthesis procedures are necessary.

# Cycles

- Use the Cycle Editor to enter the cycles provided here.

Note: **Cycles for Models 392 and 394 will be encoded on the software update, Version 1.01. Contact ABI (DNA technical support) if you require preliminary copies in printed form.**

- Check the cycles carefully after entering them. Cycle times are approximately 15 minutes longer than the corresponding, normal phosphodiester cycles.
- Because most phosphorothioate applications require higher quantities of oligonucleotide, only 1- and 10-µmole scale cycles are provided. For smaller scale syntheses, the 1-µmole cycles will perform well.
- Model 380A users should contact ABI (DNA technical support).
- Cycles for the 391 PCR-MATE EP™ and the 381A, Version 1.50, are identical.

# Analysis/Purification

Phosphorothioate oligonucleotides may be analyzed and purified by the same techniques of HPLC, PAGE, and gel capillary electrophoresis techniques as normal, phosphodiester DNA.[9]

- Because the sulfur-containing phosphorothioates are more hydrophobic, retention times on reverse phase HPLC are longer, or require a higher organic content in the mobile phase. Product peaks may be broad, relative to phosphodiester DNA.

- The sulfur analog has virtually the same electrophoretic mobility on **PAGE**, relative to normal DNA (Figure 1).

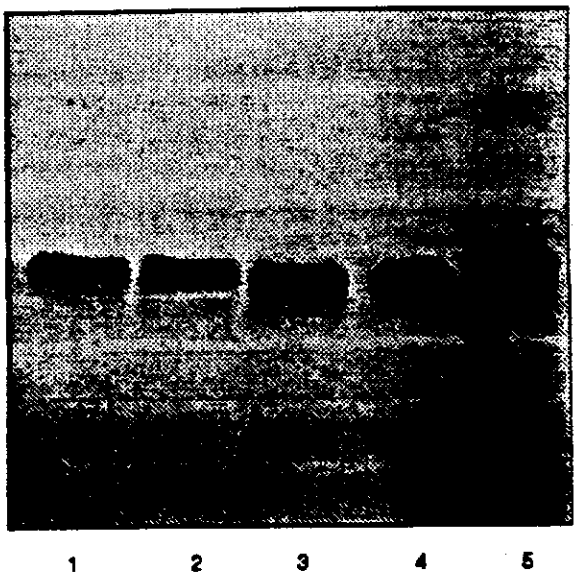



**Figure 1.** PAGE, UV shadow analysis, of a 21-mer sequence. Lane 1: all P-S, OPC purified, 380B; lane 2: all P-S, OPC purified, 392; lane 3: 19 P-S, 1 P-O, OPC purified, 380B; lane 4: 1 P-S, 19 P-O, OPC purified, 380B; lane 5: all P-S, crude, 380B.

- For oligonucleotide purification cartridge (OPC) purification, phosphorothioate oligonucleotides are eluted from the OPC with a 35% acetonitrile solution.
- **MICRO-GEL™ 100 Capillary Electrophoresis** is a new, high-resolution method of oligonucleotide analysis that is useful for both phosphodiester and phosphorothioate DNA[10] (Figure 2).

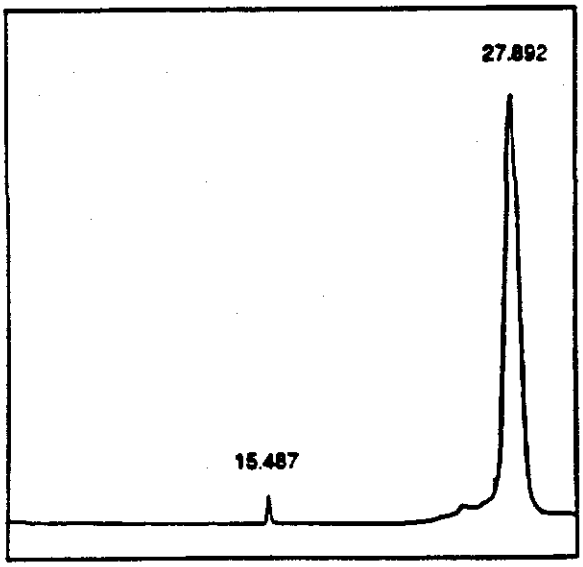



**Figure 2.** MICRO-GEL™ 100 Capillary Electrophoresis of lane 1, Figure 1, phosphorothioate 21-mer.

- Phosphorothioates are *not* good substrates for polynucleotide kinase and thus do not 5' label efficiently with $^{32}P$ ATP. Mixed oligonucleotides should have at least three phosphodiester linkages at the 5' end for efficient labeling.
- The relative phosphodiester-to-phosphorothioate content of oligonucleotides can be conveniently and quantitatively measured by $^{31}P$-NMR. Using high field NMR spectrometers, a single 1-µmole synthesis provides sufficient sample to conduct analysis (Figures 3 and 4).

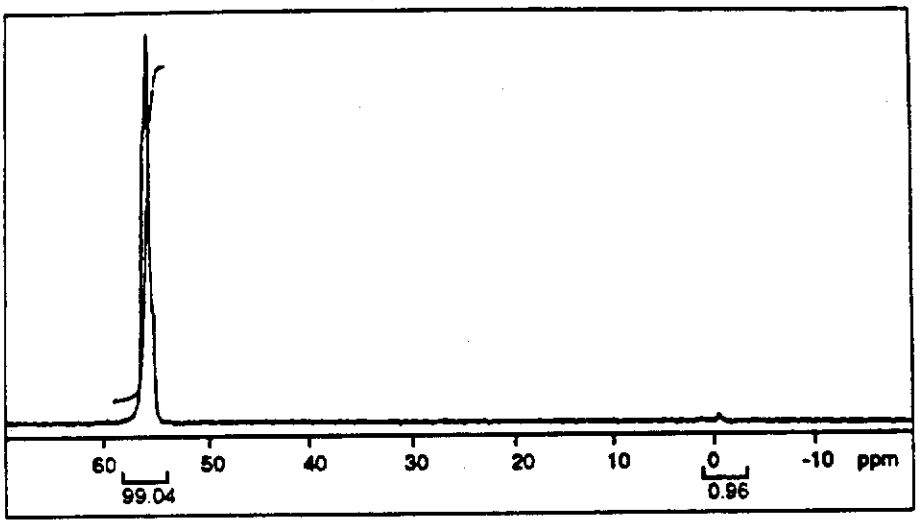



Figure 3. Analysis by $^{31}P$-NMR, showing that sulfurization with TETD typically results in less than 1% phosphodiester contamination, based on the integrated area of the phosphorothioate diastereomer resonances at approximately 55 ppm, and the phosphodiester region at about 0 ppm (relative to phosphoric acid).

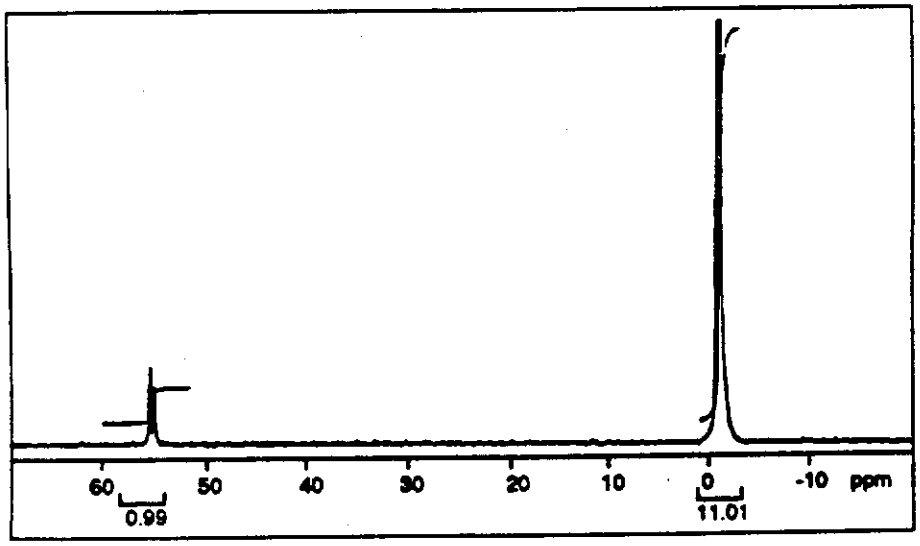



Figure 4. Analysis by $^{31}P$-NMR, showing that the correct, integrated areas are obtained in a mixed sequence. In this example, the 13-mer containing a single phosphorothioate linkage near the 3' terminus yielded the expected 11:1 ratio of phosphodiester:phosphorothioate. No loss of sulfur was incurred upon subsequent iodine oxidations during synthesis.

# Reagent Consumption

The flow rate of TETD is 10-20% higher than the iodine reagent. Since the delivery times in the cycles are the same, the consumption rate of TETD is 10-20% higher than iodine, depending on the DNA synthesizer model.

# Conclusion

The TETD reagent simplifies phosphorothioate oligonucleotide synthesis and significantly reduces costs. It replaces the hydrogen-phosphonate reagents, facilitating investigations of the antisense effect via phosphate analogs of DNA.

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SYNTHESIS CYCLE
VERSION 2.00



CYCLE NAME: 1UMSULF
NUMBER OF STEPS: 94
DATE: Oct 12, 199
TIME: 7:36

380B 1umole TETD Sulfurizing Reagent
3 column version 2.00 large bottle update

| STEP NUMBER | FUNCTION # | NAME | STEP TIME | STEP ACTIVE FOR BASES A | G | C | T | 5 | 6 | 7 | SAFE STEP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 10 | #18 To Waste | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 2 | 9 | #18 To Column | 20 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 3 | 2 | Reverse Flush | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 1 | Block Flush | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 28 | Phos Prep | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | +45 | Group 1 On | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 7 | 90 | TET To Column | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 8 | 19 | B+TET To Col 1 | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | 90 | TET To Column | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 10 | 19 | B+TET To Col 1 | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 11 | 90 | TET To Column | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 12 | 19 | B+TET To Col 1 | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 13 | 9 | #18 To Column | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 14 | -46 | Group 1 Off | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 15 | +47 | Group 2 On | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 16 | 10 | #18 To Waste | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 17 | 1 | Block Flush | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 18 | 90 | TET To Column | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 19 | 20 | B+TET To Col 2 | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 20 | 90 | TET To Column | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | 20 | B+TET To Col 2 | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 22 | 90 | TET To Column | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 23 | 20 | B+TET To Col 2 | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 24 | 9 | #18 To Column | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 25 | -48 | Group 2 Off | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 26 | +49 | Group 3 On | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 27 | 10 | #18 To Waste | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 28 | 1 | Block Flush | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 29 | 90 | TET To Column | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 30 | 21 | B+TET To Col 3 | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 31 | 90 | TET To Column | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 21 | B+TET To Col 3 | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 33 | 90 | TET To Column | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 34 | 21 | B+TET To Col 3 | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 35 | 9 | #18 To Column | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 36 | -50 | Group 3 Off | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 37 | 4 | Wait | 15 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 38 | 2 | Reverse Flush | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 1 | Block Flush | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 40 | 81 | #15 To Waste | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 41 | 13 | #15 To Column | 12 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 10 | #18 To Waste | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 4 | Wait | 900 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

(Continued next page.)

CYCLE NAME: 1UMSULF
NUMBER OF STEPS: 94

| STEP NUMBER | FUNCTION # | NAME | STEP TIME | STEP ACTIVE FOR BASES A | G | C | T | 5 | 6 | 7 | SAFE STEP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 44 | 2 | Reverse Flush | 6 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 45 | 1 | Block Flush | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 9 | #18 To Column | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 47 | 34 | Flush to Waste | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 48 | 9 | #18 To Column | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 2 | Reverse Flush | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 50 | 9 | #18 To Column | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 51 | 2 | Reverse Flush | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 9 | #18 To Column | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 53 | 2 | Reverse Flush | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 54 | 1 | Block Flush | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 55 | 16 | Cap Prep | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 56 | 91 | Cap To Column | 12 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 57 | 10 | #18 To Waste | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 58 | 4 | Wait | 8 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 59 | 2 | Reverse Flush | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 60 | 9 | #18 To Column | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 61 | 2 | Reverse Flush | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 62 | 9 | #18 To Column | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 63 | 2 | Reverse Flush | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 64 | 9 | #18 To Column | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 65 | 2 | Reverse Flush | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 66 | 1 | Block Flush | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 67 | 33 | Cycle Entry | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 68 | 10 | #18 To Waste | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 69 | 9 | #18 To Column | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 70 | 2 | Reverse Flush | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 71 | 1 | Block Flush | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 72 | 6 | Waste-Port | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 73 | 5 | Advance FC | 2 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 74 | 5 | Advance FC | 2 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 75 | 5 | Advance FC | 2 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 76 | 5 | Advance FC | 2 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 77 | 82 | #14 To Waste | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 78 | 14 | #14 To Column | 10 | | | Yes | Yes | Yes | Yes | Yes | No |
| 79 | 34 | Flush to Waste | 1 | | | Yes | Yes | Yes | Yes | Yes | No |
| 80 | 14 | #14 To Column | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 81 | 34 | Flush to Waste | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 82 | 14 | #14 To Column | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 83 | 34 | Flush to Waste | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 84 | 14 | #14 To Column | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 85 | 34 | Flush to Waste | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 86 | 14 | #14 To Column | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 87 | 34 | Flush to Waste | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 88 | 14 | #14 To Column | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |

(Continued next page.)

CYCLE NAME: 1UMSULF
NUMBER OF STEPS: 94

| STEP NUMBER | FUNCTION # | NAME | STEP TIME | STEP ACTIVE FOR BASES A | G | C | T | 5 | 6 | 7 | SAFE STEP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 89 | 34 | Flush to Waste | 8 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 90 | 9 | #18 To Column | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 91 | 34 | Flush to Waste | 8 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 92 | 7 | Waste-Bottle | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 93 | 9 | #18 To Column | 10 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 94 | 2 | Reverse Flush | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

SYNTHESIS CYCLE
VERSION 2.00



CYCLE NAME: 10UMSULF
NUMBER OF STEPS: 80
DATE: Sep 7, 1990
TIME: 10:12

380B 10umole Sulfurizing Reagent TETD

3 column version 2.00 large bottle update

| STEP NUMBER | FUNCTION # | NAME | STEP TIME | STEP ACTIVE FOR BASES A | G | C | T | 5 | 6 | 7 | SAFE STEP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 10 | #18 To Waste | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 2 | 9 | #18 To Column | 60 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 3 | 2 | Reverse Flush | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 4 | 1 | Block Flush | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 5 | 28 | Phos Prep | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 6 | +45 | Group 1 On | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 7 | 90 | TET To Column | 2 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 8 | 19 | B+TET To Col 1 | 60 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 9 | -46 | Group 1 Off | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 10 | +47 | Group 2 On | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 11 | 10 | #18 To Waste | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 12 | 1 | Block Flush | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 13 | 90 | TET To Column | 2 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 14 | 20 | B+TET To Col 2 | 60 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 15 | -48 | Group 2 Off | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 16 | +49 | Group 3 On | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 17 | 10 | #18 To Waste | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 18 | 1 | Block Flush | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 19 | 90 | TET To Column | 2 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 20 | 21 | B+TET To Col 3 | 60 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 21 | -50 | Group 3 Off | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 22 | 4 | Wait | 15 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 23 | 10 | #18 To Waste | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 24 | 2 | Reverse Flush | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 25 | 1 | Block Flush | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 26 | 81 | #15 To Waste | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 27 | 13 | #15 To Column | 70 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 28 | 10 | #18 To Waste | 5 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 29 | 4 | Wait | 900 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 30 | 2 | Reverse Flush | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 31 | 10 | #18 To Waste | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 32 | 1 | Block Flush | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 33 | 9 | #18 To Column | 60 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 34 | 2 | Reverse Flush | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 35 | 9 | #18 To Column | 60 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 36 | 2 | Reverse Flush | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 37 | 9 | #18 To Column | 60 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 38 | 2 | Reverse Flush | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 39 | 1 | Block Flush | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 40 | 16 | Cap Prep | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 41 | 91 | Cap To Column | 60 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 42 | 4 | Wait | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 43 | 2 | Reverse Flush | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

(Continued next page.)

SYNTHESIS CYCLE
VERSION 2.00



CYCLE NAME: 10UMSULF
NUMBER OF STEPS: 80

| STEP NUMBER | FUNCTION # | NAME | STEP TIME | STEP ACTIVE FOR BASES A | G | C | T | 5 | 6 | 7 | SAFE STEP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 44 | 10 | #18 To Waste | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 45 | 1 | Block Flush | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 46 | 9 | #18 To Column | 60 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 47 | 2 | Reverse Flush | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 48 | 9 | #18 To Column | 60 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 49 | 2 | Reverse Flush | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 50 | 9 | #18 To Column | 60 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 51 | 2 | Reverse Flush | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 52 | 1 | Block Flush | 4 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 53 | 33 | Cycle Entry | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 54 | 10 | #18 To Waste | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 55 | 9 | #18 To Column | 60 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 56 | 2 | Reverse Flush | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 57 | 1 | Block Flush | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 58 | 6 | Waste-Port | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 59 | 5 | Advance FC | 2 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 60 | 5 | Advance FC | 2 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 61 | 5 | Advance FC | 2 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 62 | 5 | Advance FC | 2 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 63 | 82 | #14 To Waste | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 64 | 14 | #14 To Column | 30 | | | Yes | Yes | Yes | Yes | Yes | No |
| 65 | 34 | Flush to Waste | 3 | | | Yes | Yes | Yes | Yes | Yes | No |
| 66 | 14 | #14 To Column | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 67 | 34 | Flush to Waste | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 68 | 14 | #14 To Column | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 69 | 34 | Flush to Waste | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 70 | 14 | #14 To Column | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 71 | 34 | Flush to Waste | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 72 | 14 | #14 To Column | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 73 | 34 | Flush to Waste | 3 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 74 | 14 | #14 To Column | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 75 | 2 | Reverse Flush | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 76 | 9 | #18 To Column | 60 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 77 | 2 | Reverse Flush | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | No |
| 78 | 7 | Waste-Bottle | 1 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 79 | 9 | #18 To Column | 60 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| 80 | 2 | Reverse Flush | 30 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

NAME: CYCLE-4
NUMBER OF STEPS: 74
TIME: 11:28
DATE: 01/11/91

TETD Sulfurizing Reagent
391 PCR-MATE EP 1 umole
381A version 1.50

| STEP NUMBER | FUNCTION # | NAME | STEP TIME | ACTIVE FOR BASES A | G | C | T | X |
|---|---|---|---|---|---|---|---|---|
| 1 | 10 | $18 TO WASTE | 2 | Yes | Yes | Yes | Yes | Yes |
| 2 | 9 | $18 TO COLM | 15 | Yes | Yes | Yes | Yes | Yes |
| 3 | 2 | REVERSE FLUSH | 5 | Yes | Yes | Yes | Yes | Yes |
| 4 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 5 | 28 | PHOS PREP | 3 | Yes | Yes | Yes | Yes | Yes |
| 6 | 90 | TET TO COLUMN | 3 | Yes | Yes | Yes | Yes | Yes |
| 7 | 19 | B+TET TO COLM | 3 | Yes | Yes | Yes | Yes | Yes |
| 8 | 90 | TET TO COLUMN | 2 | Yes | Yes | Yes | Yes | Yes |
| 9 | 19 | B+TET TO COLM | 3 | Yes | Yes | Yes | Yes | Yes |
| 10 | 90 | TET TO COLUMN | 2 | Yes | Yes | Yes | Yes | Yes |
| 11 | 19 | B+TET TO COLM | 3 | Yes | Yes | Yes | Yes | Yes |
| 12 | 90 | TET TO COLUMN | 2 | Yes | Yes | Yes | Yes | Yes |
| 13 | 4 | WAIT | 15 | Yes | Yes | Yes | Yes | Yes |
| 14 | 2 | REVERSE FLUSH | 5 | Yes | Yes | Yes | Yes | Yes |
| 15 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 16 | 81 | $15 TO WASTE | 3 | Yes | Yes | Yes | Yes | Yes |
| 17 | 13 | $15 TO COLUMN | 10 | Yes | Yes | Yes | Yes | Yes |
| 18 | 10 | $18 TO WASTE | 5 | Yes | Yes | Yes | Yes | Yes |
| 19 | 4 | WAIT | 900 | Yes | Yes | Yes | Yes | Yes |
| 20 | 2 | REVERSE FLUSH | 5 | Yes | Yes | Yes | Yes | Yes |
| 21 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 22 | 10 | $18 TO WASTE | 5 | Yes | Yes | Yes | Yes | Yes |
| 23 | 9 | $18 TO COLM | 10 | Yes | Yes | Yes | Yes | Yes |
| 24 | 2 | REVERSE FLUSH | 5 | Yes | Yes | Yes | Yes | Yes |
| 25 | 9 | $18 TO COLM | 10 | Yes | Yes | Yes | Yes | Yes |
| 26 | 2 | REVERSE FLUSH | 5 | Yes | Yes | Yes | Yes | Yes |
| 27 | 9 | $18 TO COLM | 10 | Yes | Yes | Yes | Yes | Yes |
| 28 | 2 | REVERSE FLUSH | 5 | Yes | Yes | Yes | Yes | Yes |
| 29 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 30 | 16 | CAP PREP | 3 | Yes | Yes | Yes | Yes | Yes |
| 31 | 22 | CAP TO COLUMN | 12 | Yes | Yes | Yes | Yes | Yes |
| 32 | 4 | WAIT | 8 | Yes | Yes | Yes | Yes | Yes |
| 33 | 10 | $18 TO WASTE | 3 | Yes | Yes | Yes | Yes | Yes |
| 34 | 2 | REVERSE FLUSH | 5 | Yes | Yes | Yes | Yes | Yes |
| 35 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 36 | 10 | $18 TO WASTE | 3 | Yes | Yes | Yes | Yes | Yes |
| 37 | 9 | $18 TO COLM | 10 | Yes | Yes | Yes | Yes | Yes |
| 38 | 2 | REVERSE FLUSH | 5 | Yes | Yes | Yes | Yes | Yes |
| 39 | 9 | $18 TO COLM | 10 | Yes | Yes | Yes | Yes | Yes |
| 40 | 2 | REVERSE FLUSH | 5 | Yes | Yes | Yes | Yes | Yes |
| 41 | 9 | $18 TO COLM | 10 | Yes | Yes | Yes | Yes | Yes |
| 42 | 2 | REVERSE FLUSH | 5 | Yes | Yes | Yes | Yes | Yes |
| 43 | 9 | $18 TO COLM | 10 | Yes | Yes | Yes | Yes | Yes |
| 44 | 2 | REVERSE FLUSH | 5 | Yes | Yes | Yes | Yes | Yes |
| 45 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 46 | 33 | CYC ENTRY | 1 | Yes | Yes | Yes | Yes | Yes |

( Continued next page )

NAME: CYCLE-4
NUMBER OF STEPS: 74
TIME: 11:28
DATE: 01/11/91

| STEP NUMBER | FUNCTION # | NAME | STEP TIME | ACTIVE FOR BASES A | G | C | T | X |
|---|---|---|---|---|---|---|---|---|
| 47 | 10 | #18 TO WASTE | 3 | Yes | Yes | Yes | Yes | Yes |
| 48 | 9 | #18 TO COLM | 10 | Yes | Yes | Yes | Yes | Yes |
| 49 | 2 | REVERSE FLUSH | 5 | Yes | Yes | Yes | Yes | Yes |
| 50 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 51 | 5 | ADVANCE FC | 1 | Yes | Yes | Yes | Yes | Yes |
| 52 | 6 | WASTE PORT | 1 | Yes | Yes | Yes | Yes | Yes |
| 53 | 82 | #14 TO WASTE | 3 | Yes | Yes | Yes | Yes | Yes |
| 54 | 14 | #14 TO COLUMN | 10 | Yes | Yes | Yes | Yes | Yes |
| 55 | 108 | FLUSH TO TRIT | 1 | Yes | Yes | Yes | Yes | Yes |
| 56 | 14 | #14 TO COLUMN | 10 | Yes | Yes | Yes | Yes | Yes |
| 57 | 108 | FLUSH TO TRIT | 1 | Yes | Yes | Yes | Yes | Yes |
| 58 | 14 | #14 TO COLUMN | 10 | Yes | Yes | Yes | Yes | Yes |
| 59 | 108 | FLUSH TO TRIT | 1 | Yes | Yes | Yes | Yes | Yes |
| 60 | 14 | #14 TO COLUMN | 10 | Yes | Yes | Yes | Yes | Yes |
| 61 | 108 | FLUSH TO TRIT | 1 | Yes | Yes | Yes | Yes | Yes |
| 62 | 14 | #14 TO COLUMN | 10 | Yes | Yes | Yes | Yes | Yes |
| 63 | 108 | FLUSH TO TRIT | 1 | Yes | Yes | Yes | Yes | Yes |
| 64 | 14 | #14 TO COLUMN | 10 | Yes | Yes | Yes | Yes | Yes |
| 65 | 108 | FLUSH TO TRIT | 1 | Yes | Yes | Yes | Yes | Yes |
| 66 | 9 | #18 TO COLM | 10 | Yes | Yes | Yes | Yes | Yes |
| 67 | 108 | FLUSH TO TRIT | 8 | Yes | Yes | Yes | Yes | Yes |
| 68 | 7 | WASTE BOTTLE | 1 | Yes | Yes | Yes | Yes | Yes |
| 69 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 70 | 2 | REVERSE FLUSH | 1 | Yes | Yes | Yes | Yes | Yes |
| 71 | 9 | #18 TO COLM | 20 | Yes | Yes | Yes | Yes | Yes |
| 72 | 2 | REVERSE FLUSH | 5 | Yes | Yes | Yes | Yes | Yes |
| 73 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 74 | 34 | CYC END | 1 | Yes | Yes | Yes | Yes | Yes |

391 CYCLE/PROCEDURE
381

NAME: CYCLE-4
NUMBER OF STEPS: 66
TIME: 08:29
DATE: 10/15/90

391 PCR-MATE 10umole TETD Sulfurizing Reagent
381A version 1.50

| STEP NUMBER | FUNCTION # | NAME | STEP TIME | ACTIVE FOR BASES A | G | C | T | X |
|---|---|---|---|---|---|---|---|---|
| 1 | 10 | #18 TO WASTE | 3 | Yes | Yes | Yes | Yes | Yes |
| 2 | 9 | #18 TO COLM | 50 | Yes | Yes | Yes | Yes | Yes |
| 3 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 4 | 9 | #18 TO COLM | 50 | Yes | Yes | Yes | Yes | Yes |
| 5 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 6 | 9 | #18 TO COLM | 50 | Yes | Yes | Yes | Yes | Yes |
| 7 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 8 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 9 | 28 | PHOS PREP | 3 | Yes | Yes | Yes | Yes | Yes |
| 10 | 61 | TET TO WASTE | 6 | Yes | Yes | Yes | Yes | Yes |
| 11 | 19 | B+TET TO COLM | 55 | Yes | Yes | Yes | Yes | Yes |
| 12 | 4 | WAIT | 15 | Yes | Yes | Yes | Yes | Yes |
| 13 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 14 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 15 | 81 | #15 TO WASTE | 3 | Yes | Yes | Yes | Yes | Yes |
| 16 | 13 | #15 TO COLUMN | 50 | Yes | Yes | Yes | Yes | Yes |
| 17 | 10 | #18 TO WASTE | 5 | Yes | Yes | Yes | Yes | Yes |
| 18 | 4 | WAIT | 900 | Yes | Yes | Yes | Yes | Yes |
| 19 | 10 | #18 TO WASTE | 3 | Yes | Yes | Yes | Yes | Yes |
| 20 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 21 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 22 | 10 | #18 TO WASTE | 3 | Yes | Yes | Yes | Yes | Yes |
| 23 | 9 | #18 TO COLM | 50 | Yes | Yes | Yes | Yes | Yes |
| 24 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 25 | 9 | #18 TO COLM | 50 | Yes | Yes | Yes | Yes | Yes |
| 26 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 27 | 9 | #18 TO COLM | 50 | Yes | Yes | Yes | Yes | Yes |
| 28 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 29 | 9 | #18 TO COLM | 50 | Yes | Yes | Yes | Yes | Yes |
| 30 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 31 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 32 | 16 | CAP PREP | 3 | Yes | Yes | Yes | Yes | Yes |
| 33 | 22 | CAP TO COLUMN | 40 | Yes | Yes | Yes | Yes | Yes |
| 34 | 4 | WAIT | 5 | Yes | Yes | Yes | Yes | Yes |
| 35 | 10 | #18 TO WASTE | 3 | Yes | Yes | Yes | Yes | Yes |
| 36 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 37 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 38 | 10 | #18 TO WASTE | 3 | Yes | Yes | Yes | Yes | Yes |
| 39 | 9 | #18 TO COLM | 50 | Yes | Yes | Yes | Yes | Yes |
| 40 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 41 | 9 | #18 TO COLM | 50 | Yes | Yes | Yes | Yes | Yes |
| 42 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 43 | 9 | #18 TO COLM | 50 | Yes | Yes | Yes | Yes | Yes |
| 44 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 45 | 9 | #18 TO COLM | 50 | Yes | Yes | Yes | Yes | Yes |
| 46 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |

( Continued next page )

391 CYCLE/PROCEDURE
381



NAME: CYCLE-4
NUMBER OF STEPS: 66
TIME: 08:29
DATE: 10/15/90

| STEP NUMBER | FUNCTION # | NAME | STEP TIME | ACTIVE FOR BASES A | G | C | T | X |
|---|---|---|---|---|---|---|---|---|
| 47 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 48 | 33 | CYC ENTRY | 1 | Yes | Yes | Yes | Yes | Yes |
| 49 | 10 | #18 TO WASTE | 3 | Yes | Yes | Yes | Yes | Yes |
| 50 | 9 | #18 TO COLM | 80 | Yes | Yes | Yes | Yes | Yes |
| 51 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 52 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 53 | 5 | ADVANCE FC | 1 | Yes | Yes | Yes | Yes | Yes |
| 54 | 6 | WASTE PORT | 1 | Yes | Yes | Yes | Yes | Yes |
| 55 | 82 | #14 TO WASTE | 3 | Yes | Yes | Yes | Yes | Yes |
| 56 | 14 | #14 TO COLUMN | 160 | Yes | Yes | Yes | Yes | Yes |
| 57 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 58 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 59 | 10 | #18 TO WASTE | 3 | Yes | Yes | Yes | Yes | Yes |
| 60 | 9 | #18 TO COLM | 80 | Yes | Yes | Yes | Yes | Yes |
| 61 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 62 | 9 | #18 TO COLM | 80 | Yes | Yes | Yes | Yes | Yes |
| 63 | 2 | REVERSE FLUSH | 45 | Yes | Yes | Yes | Yes | Yes |
| 64 | 1 | BLOCK FLUSH | 3 | Yes | Yes | Yes | Yes | Yes |
| 65 | 7 | WASTE BOTTLE | 1 | Yes | Yes | Yes | Yes | Yes |
| 66 | 34 | CYC END | 1 | Yes | Yes | Yes | Yes | Yes |

# USER BULLETIN

Model 380A, 380B, 381A, 391, 392, 394
DNA Synthesizers

Number 59
March 1991

# New Applications for the Oligonucleotide Purification Cartridge

Since its introduction in January of 1988, the Oligonucleotide Purification Cartridge (OPC™, P/N 400771) has provided the level of purity required for such synthetic oligonucleotide applications as primers for the GeneAmp™ PCR process, sequencing primers, and hybridization probes. New applications for the OPC, however, have recently been developed. This User Bulletin details these applications and provides answers to common questions regarding the use of OPC.

## Is there a faster, easier protocol for the OPC?

Yes. Several improvements designed to make OPC purification simpler and more convenient are now available. They include increased capacity, faster operation, multiplexed operation, purification of "bigmers" (long oligonucleotides), and purification of RNA and DNA analogs (e.g., phosphorothioates).

## Can the capacity of the OPC be increased?

Yes. The presence of ammonia in the loading solution of the oligonucleotide provides the desired non-denaturing media but limits the binding capacity of dimethoxytritanol (DMT) oligonucleotides. The present protocol specifies adding one-third volume of water to the crude oligonucleotide/ammonia solution to yield 1-5 ODU (optical density units, $A_{260}$nm/mL) of purified, detritylated oligonucleotide. If more water is used, or if ammonia is partially removed after several minutes in a vacuum centrifuge, the binding capacity of DMT oligonucleotides will increase to yield about 10 ODU of purified, detritylated oligonucleotide.

## Can "bigmers" be purified with the OPC?

Yes. As detailed in User Bulletin No. 51 (November 1988), purification with the OPC is useful in the 40- to 70-mer (and even longer) range with two additional washes (steps 8 and 9).

## The OPC Purification Protocol:

1. Pass 5 mL of acetonitrile through the OPC to waste.
2. Pass 5 mL of 2M Triethylammonium Acetate (TEAA, ABI P/N 400613) through the OPC to waste.
3. Dilute the oligonucleotide ammonia deprotection solution with an equal volume of water, e.g., 1 mL of each. Pass the diluted solution through the OPC at a rate of about 1 drop per second. Collect the eluate and pass it through a second time.
4. Pass 5 mL of dilute ammonia through the OPC to waste.
5. Pass 10 mL of water through the OPC to waste.
6. Fill the syringe barrel with 5 ml 2% trifluoroacetic acid (TFA) in water and pass a portion through the OPC to waste. Let stand 3-5 minutes before passing the remainder through to waste.
7. Pass 10 mL of water through the OPC to waste.
8. **Pass 5 mL of dilute ammonia through the OPC to waste.***
9. **Pass 5 mL of water through the OPC to waste.***
10. Elute drop-by-drop with 1 mL of 20% Acetonitrile (P/N 400314) in water and collect the purified oligonucleotide in a 1.5-mL Eppendorf vial.

*Additional steps for >40-mers
Dilute ammonia: 1.5M = 9:1 ($H_2$0:concentrated ammonia)

## Can I purify synthetic RNA with the OPC?

No. To minimize phosphate migration, which gives rise to 2'-5' phosphate linkages, the final step in RNA deprotection should be the 2' desilylation (see User Bulletin No. 53, December 1989). Therefore, RNA syntheses should be conducted trityl-OFF. After desilylation, the crude, fully deprotected RNA can be efficiently separated from the tetrabutylammonium salts with the OPC by the *desalting* protocol.

## Can I purify phosphorothioate oligonucleotides with the OPC?

Yes. Purification of the antisense, phosphate DNA analog, phosphorothioate oligonucleotide is very efficient with the OPC. Since phosphorothioates are more hydrophobic, use 1 mL of 35% acetonitrile in water to elute them (step 10).

## What happened to the desalting protocol?

When the OPC format changed to the new column design in a tray format, the desalting protocol was omitted due to lack of space. The desalting protocol is useful for:

- trityl-off oligonucleotides;
- RNA oligonucleotides, after desilylation;
- polyacrylamide gel purification extracts;
- anion-exchange HPLC fractions.

## The OPC Desalting Protocol:

1. Pass 5 mL of acetonitrile through the OPC to waste.
2. Pass 5 mL of 2M TEAA through the OPC to waste.
3. Dissolve the oligonucleotide in 1-3 mL of aqueous solution (e.g., 0.1M TEAA). The loading solution should not contain organic solvents. Pass the diluted solution through the OPC at a rate of about 1 drop per second. Collect the eluate and pass it through a second time.
4. Pass 15 mL of 0.1M TEAA through the OPC to waste.
5. Elute drop-by-drop with 1 mL of 50% acetonitrile in water and collect the purified oligonucleotide in a 1.5-mL Eppendorf vial.

## Does detritylation with TFA cause depurination?

No. By base composition analysis, no degradation can be detected in a pure oligonucleotide subjected to normal OPC conditions nor in one subjected to extreme conditions (1 hour 2% TFA in water).

## How long should TFA be in the OPC?

A 2% solution of TFA should be in the OPC for 3 to 5 minutes to effect complete detritylation. The TFA solution should be prepared carefully to ensure that the concentration is 2%.

## Why did the OPC body change?

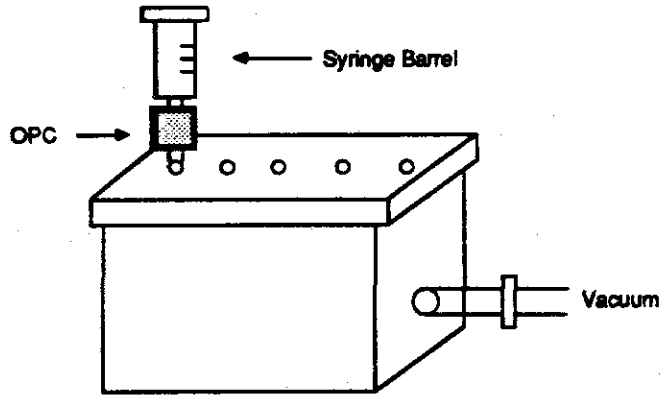
In 1989 we implemented a new automated system for assembling, packaging, and testing both the OPC and DNA synthesis columns. The new "snap" OPCs are individually pressure tested. Another improvement is the heavier frits that withstand higher pressure and greater force than did the thinner, "crimp" styled filters.

## Why was the OPC designed to be disposable?

Reuse of any purification matrix can lead to problems with carryover and sample contamination. Avoiding these problems is especially important in synthetic DNA applications because of the potential for errant priming and hybridization. Analytical HPLC studies demonstrate that approximately 5% of an oligonucleotide product is carried over as a contaminant into a second, different oligonucleotide sequence upon reuse of an OPC. Also, the DMT that is released upon TFA treatment is very strongly bound to the OPC matrix. This results in diminished capacity and selectivity for DMT oligonucleotides. The safest, most reliable solution was to adopt a disposable format for the OPC.

## Can I process more than one OPC purification at a time?

Yes. Dr. Bruce Levison of Case Western Reserve University developed the following protocol with an inexpensive solid-phase extraction device to conduct many OPC purifications simultaneously. In addition to increased throughput, this protocol entails fewer manual operations.




OPC Vacuum Manifold

Dr. Levison finds that a vacuum manifold such as the Analytichem (a division of Varian) Vac Elut SPS 24, or the Vac Elut 10-place manifold with a 12 x 75-mm test tube rack is ideally suited for multiplexed OPC processing. Both units have been tested at Applied Biosystems laboratories and gave excellent results, with no cross-contamination of samples. Other commercially available manifolds may work equally well. In selecting a manifold, you will want to consider such factors as the inertness of the device to ammonia and TFA, the available collection vial sizes, convenience of collection or removal of the washes, and the desired throughput.

## Recommended Protocol for the Analytichem/ Varian Manifolds:

Equip the vacuum manifold with an in-line trap for waste and a water aspirator pump or a small vacuum pump. The optimal vacuum is about 10-15 inches Hg. For each oligonucleotide to be purified, connect a luer-luer adapter to one end of an OPC and a 5-mL plastic syringe barrel to the other. Insert the free end of the luer-luer adapter into a luer fitting on top of the manifold. Plug all remaining sites.

- Vac Elut 10: Remove the test tube rack.
- Vac Elut SPS 24: Place the clean test tubes into the test tube rack in anticipation of the loading step. Rotate the top to the waste position.
- Turn on the vacuum.

1. Wash each OPC with 5 mL of acetonitrile.

   A wash step consists of filling the syringe barrel, which serves as a solvent reservoir, with 5 mL of the wash solvent and letting the vacuum pull the wash and some air through the OPC. Unless otherwise stated, the vacuum does not need to be regulated to reduce the flow rate.

2. Wash with 5 mL of 2M TEAA.
3. Turn off the vacuum. Remember to vent as necessary.
   - Vac Elut 10: Place the rack, containing clean 12 X 75-mm test tubes, into the box.
   - Vac Elut SPS 24: Rotate to the collect position. Make sure that the test tubes are in place.
4. Load each oligonucleotide, dissolved in 1 mL of concentrated ammonia and 1 mL of water, into the syringe barrels. Turn on the vacuum but regulate it to about 2 inches Hg, using the bleed or the quick release valve. *A low flow rate is essential to avoid cross-contamination of the samples.*
   - Return the oligomer solution to the barrel and reload it onto the cartridge with the vacuum set at about 2 inches Hg.
   - Vac Elut 10 : Remove the rack.
   - Vac Elut SPS 24 place: Rotate to the waste position.
5. Wash the OPC with 5 mL of 1.5M (dilute) ammonia.
6. Wash twice with 10 mL of water.
7. Detritylate by pulling 1-2 mL of a 5-mL, 2% TFA solution through the OPC. Then vent the system and turn off the vacuum. Allow the remaining 3 mL of 2% TFA to fill the OPC. Let it stand for about 5 minutes to detritylate completely. Pull any remaining TFA solution through the OPC.
8. Wash twice with 5 mL of water.

   **For sequences ≥40 bases:** Wash with 5 mL of 1.5M ammonia, followed by two 5-mL water washes.
   - Vac Elut 10: Place the rack into the box again with clean 12 X 75-mm test tubes.
   - Vac Elut SPS 24: Rotate to the collection position. Make sure that clean test tubes are in place.
9. Elute with 1 mL of 20% Acetonitrile in water (35% acetonitrile for phosphorothioate DNA) with the vacuum set at about 2 inches Hg.

   Purification with the OPC, using the bigmer protocol described above, works well even for 120-mers. Figure 1 shows a MICRO-GEL™100 capillary electrophoresis comparison (ABI Model 270A) of a crude, and an OPC-purified, 120-mer (synthesized on the Model 380B, Version 2.00).

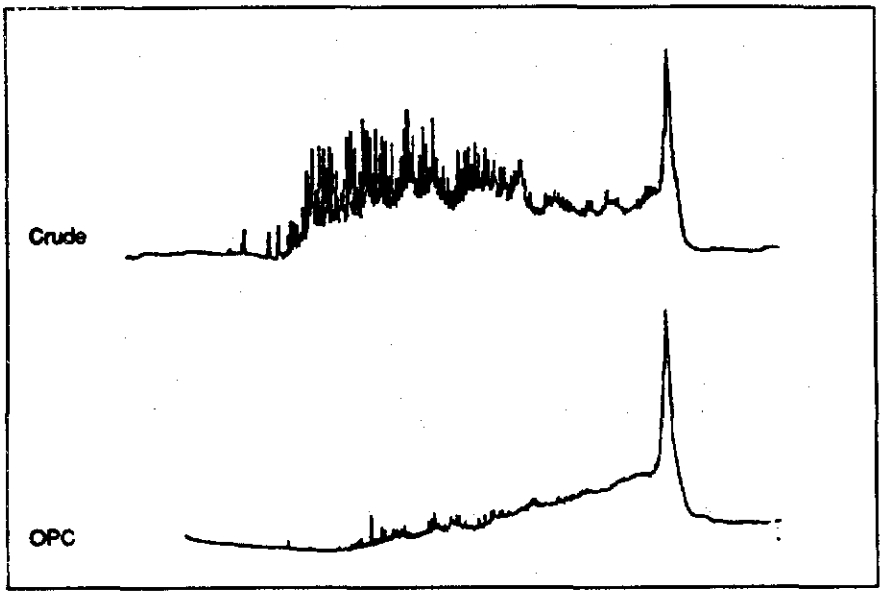



**Figure 1.** MICRO-GEL™100 capillary electrophoresis comparison of a crude, and an OPC-purified, 120-mer.

**References**

McBride, L.J., McCollum, C., Davidson, S., Efcavitch, J.W., Andrus, A. and Lombardi, S. 1988. "A New, Reliable Cartridge for the Rapid Purification of Synthetic DNA," *BioTechniques* 6:362-367.

Moring, S.E., Colburn, J.C., Grossman, P.D. and Lauer, H.H. 1990. "Analytical Aspects of an Automated Capillary Electrophoresis System," *LC-GC* 8:34.

Dubrow, B., DeDionisio, L. and Andrus, A. "Gel Capillary Electrophoresis for the Analysis of Synthetic Oligonucleotides," International Meeting of the Electrophoresis Society, Washington, DC, March 1991.

The Polymerase Chain Reaction (PCR) process is covered by U.S. patents issued to Cetus Corporation and licensed from Perkin-Elmer Cetus Instruments by Applied Biosystems. GeneAmp™ is a trademark of Cetus Corporation.

USER BULLETIN
Model 380A, 380B, 381A, 391, 392, 394 DNA Synthesizers
Number 61
May 1991

# 40-nanomole Polystyrene: New Highly Efficient DNA Synthesis Columns

New, smaller scale DNA synthesis columns are now available from Applied Biosystems. The 40-nanomole columns feature a novel polystyrene support that provides efficient and economical synthesis for oligonucleotides of all lengths. Phosphoramidites can be used with these columns at a double dilution of 0.05 M, saving about 25% of the cycle cost. In addition, the LOW cycle of the Model 391 and the primer cycle of the 392 and 394 can be used with the 40-nanomole columns while maintaining excellent synthesis performance. Finally, these columns extend the life of phosphoramidites in acetonitrile on the instrument to three weeks from the previous standard of two weeks.

The 40-nanomole columns can be used on all Applied Biosystems' DNA synthesizers, and no new cycles are needed. Oligonucleotides of any length can be synthesized on the new support. Currently, 40-nanomole columns are available only with standard protecting groups, and therefore are not compatible with FOD™ (fast oligonucleotide deprotection) reagents, except for those sequences that have a 3' T.

## A New Support Material

A form of polystyrene has been developed as the solid support for these columns. Manufactured as rigid beads that do not swell, this solid support has the attractive features of mechanical stability, rapid reaction kinetics, and the ability to be washed quickly and efficiently with organic solvents. Parameters such as pore size, particle size, and both polymerization formula and conditions have been optimized.[1]

Phosphoramidite DNA synthesis using controlled pore glass (CPG) supports can attain 98-99% repetitive trityl yields. However, as oligonucleotide synthesis scale and phosphoramidite excess are both reduced to achieve more economical cycles, the deficiencies of CPG become more apparent. Although the silanol matrix of CPG is exhaustively capped prior to synthesis, a small number of trimethylsilyl capping groups are removed during detritylation with trichloroacetic acid, allowing the phosphoramidite to react with the nucleophilic silanols.[2] Extraneous oligonucleotides can be initiated and propagated during each synthesis cycle, resulting in 3' phosphate terminus failure sequences. These failures become more problematic at smaller scales.[3] With an inert polystyrene support, side reactions during the preparation of the support and during oligonucleotide synthesis are minimized by the lack of reactive functionality.

A 21-mer oligonucleotide primer was synthesized on a Model 380B DNA Synthesizer equipped with both 40-nanomole polystyrene and 0.2-μmole CPG columns. Four milligrams of phosphoramidite were delivered each cycle to test the supports stringently, using the fast cycle and 0.05 M phosphoramidites. Both the crude oligonucleotides and those purified by the oligonucleotide purification cartridge (OPC™)[4] were analyzed on the Model 270A by MICRO-GEL™100 Capillary Electrophoresis (CE)[5] using a new gel-filled matrix optimized for oligonucleotide analysis. The trityl yield for polystyrene was excellent (99.5%), but the CPG trityl yield was only fair (97.1%). The trityl data was corroborated by the quantitation of product on CE. Examination of the CE electropherograms suggests that undesired growth off the support may contribute to the comparatively high (n-1 mer) contamination seen in the CPG samples. Trityl-selective OPC purification and subsequent CE analysis demonstrate that these contaminants are trityl bearing.

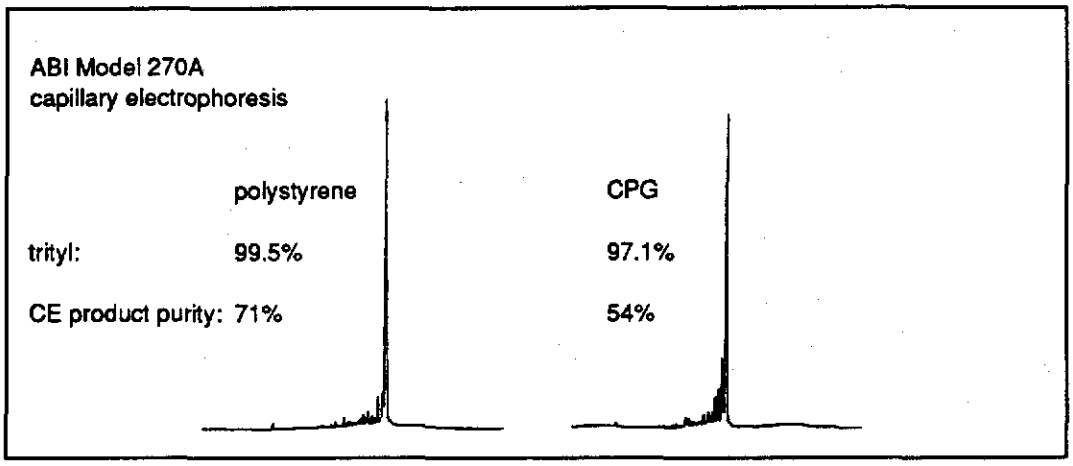



**Figure 1.** CEs of crude 21-mers.

Electropherograms of crude and OPC-purified samples also demonstrate the superior quality of oligonucleotides synthesized on polystyrene supports. Syntheses conducted with aged, partially degraded reagents also give better results when polystyrene supports are used. In addition to decreased extraneous propagation off the support, polystyrene supports offer coupling efficiency superior to that attainable with CPG. The advantage is especially evident under impaired or more stringent synthesis conditions, such as low phosphoramidite excess. The difference may reflect a more anhydrous environment of the hydrophobic polystyrene.

# Recommendations for Use

## Dilution of Phosphoramidites

The 40-nanomole polystyrene is especially intended for use with 0.05 M

phosphoramidites to reduce cycle costs and still provide high-quality oligonucleotides. Both the 250- and 500-mg phosphoramidite bottles can be diluted with twice the normal volume of acetonitrile to achieve a concentration of 0.05 M, as shown below:

**Table 1.** 0.05 M Phosphoramidite Dilution Volumes

| Phosphoramidite | Bottle Size | Acetonitrile Volume |
|---|---|---|
| $A^{bz}$ | 500 mg | 11.2 mL |
| $A^{bz}$ | 250 | 5.6 |
| $G^{ib}$ | 500 | 11.6 |
| $G^{ib}$ | 250 | 5.8 |
| $C^{bz}$ | 500 | 11.8 |
| $C^{bz}$ | 250 | 5.9 |
| T | 500 | 13.2 |
| T | 250 | 6.6 |

The auto dilute feature of the Model 392 and the 394 can provide 0.05 M phosphoramidites by using 500-mg bottles with the 1.0-g dilution option, or 250-mg bottles with the 0.5-g dilution option.

## Increased Phosphoramidite Lifetime

The 40-nanomole polystyrene columns contribute to efficient synthesis even with 0.05 M phosphoramidites that have been on the instrument for 21 days. A parallel synthesis using a 0.2-µmole (small-scale) CPG column demonstrated inferior quality after 14 days. The 40-nanomole polystyrene columns require fewer equivalents of phosphoramidite for efficient synthesis.

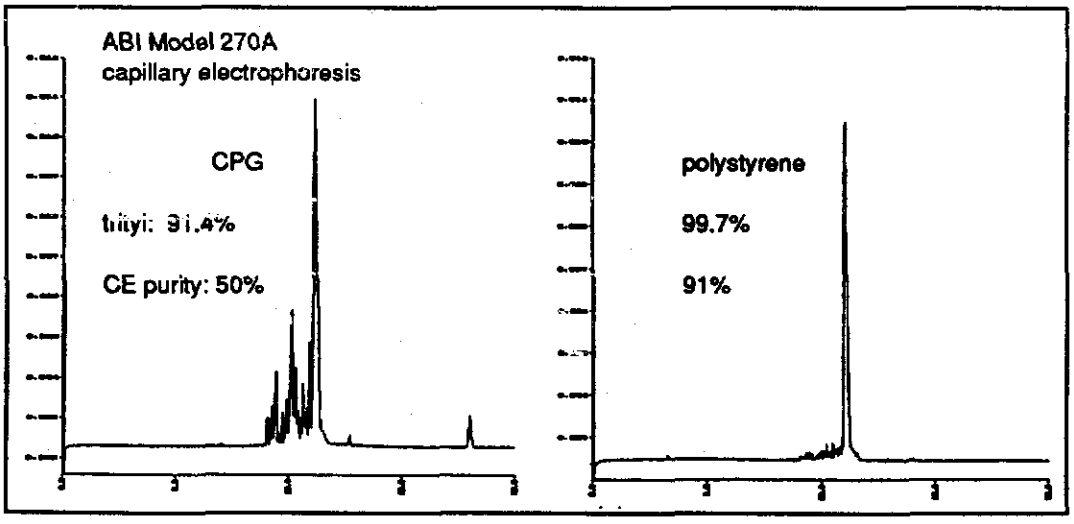



**Figure 2.** 21-day-old, 0.05 M phosphoramidites, concurrent syntheses of an 18-mer.

## Cycle Recommendations

These new columns may be used on all Applied Biosystems' DNA synthesizers. No new cycles are necessary.

**Table 2.** Cycle Recommendations for 40-nanomole Synthesis

| Synthesizer | Cycle |
|---|---|
| 380A, 380B | small-scale cycle (e.g., ssceaf 3, Version 2.01, 380B) |
| 381 | .2 UM cycle |
| 391 | LOW or .2 UM cycle |
| 392-394 | PRIMER or .2 UM CE |

- The 40-nanomole columns will support synthesis of oligonucleotides to 150 bases on the above cycles with 0.05 M phosphoramidites.
- The LOW cycle on the Model 391 is not recommended for synthesis of oligonucleotides greater than 50 bases with 0.05 M phosphoramidites and the 40-nanomole columns.

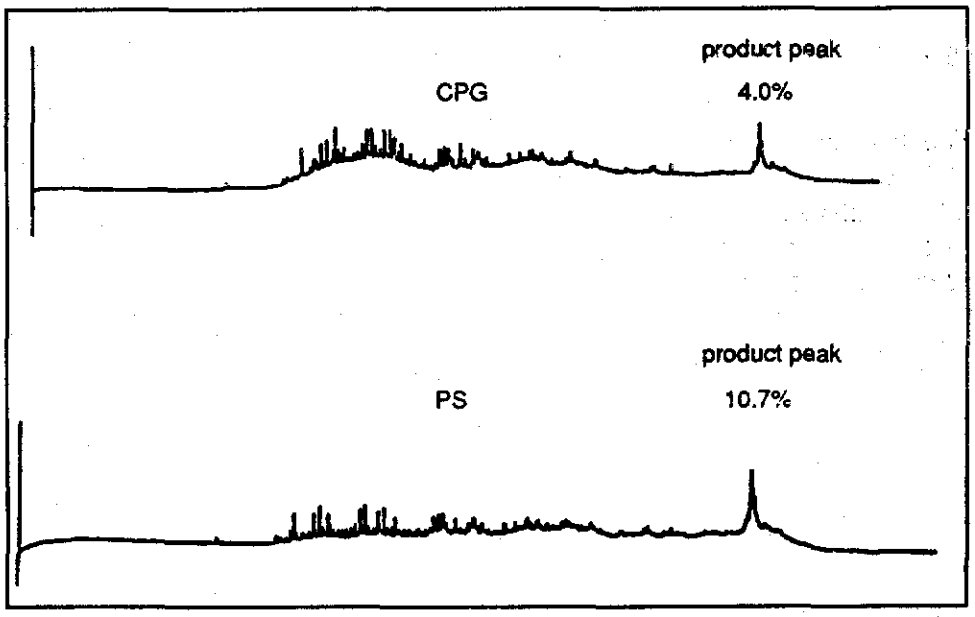



**Figure 3.** Comparison of crude 120-mers on CE. Syntheses conducted on a 391, with .2 UM cycle, and 0.05 M phosphoramidites.

## Trityl Yields

Repetitive trityl yields are consistently at least 98-99% when used as described above. Because of the small amount of trityl released each cycle, an accurate reading of the trityls produced is difficult. Users who wish to read trityls may find it more convenient to dilute trityl solutions to 5.0 mL rather than 10.0 mL. The trityl concentrations will be more appropriate, and less 0.1 M p-toluenesulfonic acid in acetonitrile will be consumed.

Monitoring the trityl cation can be important, but the results must be interpreted with caution. The trityl assay is only an indirect measure of synthesis efficiency. Certainly, trityl cation yields of 98-99% must be present for a good synthesis. However, high trityl yields can be present even when a poor synthesis occurs because this chemistry, although highly refined, is not perfect. Unwanted side reactions do occur, some of which contribute to the trityl cation released each cycle. Sites other than the 5' hydroxyl group can participate in coupling (e.g., branched sites on the nucleic bases).

Low trityl yields (≤ 97%) always predict a less than optimal synthesis. In practical terms, trityl yields of less than 95% do not allow successful synthesis of even short oligonucleotides. For the above reasons, the trityl assay must be viewed only as a preliminary yet convenient monitor of the synthesizer's performance. The assay is useful for the early detection and diagnosis of instrument-related problems. Many laboratories monitor their trityl fractions only by visual inspection. With experience, a failed synthesis is detected this way. Evaluation of the oligonucleotide by PAGE, HPLC or CE is much more informative than the trityl assay. Synthesizer or reagent problems can be adequately diagnosed only by these methods, which are *direct* analyses of the product.

## Yield

Most users will find that the 40-nanomole scale provides sufficient quantities of oligonucleotide for their experiments. This scale is compatible with a single OPC purification. With 40-nanomole polystyrene, a typical primer gives 5-10 crude ODU (150-300 µg) and 1-2 OPC-purified ODU (30-60 µg).

## Protecting Groups

The current 40-nanomole columns (PN 401072-401075) have the standard protecting groups $A^{bz}$, $G^{ib}$, and $C^{bz}$. Because T has no protecting group, T columns are also compatible with FOD phosphoramidites.

## Cleavage and Deprotection

Covering the ammonia collection vials that are used in 40-nanomole syntheses with Teflon-lined caps is especially important. Ammonia will leach a contaminant from Wheaton rubber-lined caps (catalogue no. 225002, for 4-mL vials) into the DNA solution. This contaminant absorbs at 260 nm and 310 nm and will be seen as a fast moving band in a UV shadow gel of the product. The ratio of contaminant to DNA is such that it might not be seen in larger scale syntheses but is readily apparent at the 40-nanomole scale. Teflon-lined caps may be obtained from Baxter (catalogue no. B7508-1). For more details, see User Bulletin No. 32 (July 1986).

A very small amount of pyridine can be retained in the column from the oxidation step, only to be washed into the collection vial along with the DNA during cleavage. This is not noticeable at larger synthesis scales, but at the 40-nanomole scale it may be detected in a UV spectrum of the diluted ammonium hydroxide by the spikes of the pyridine absorbance superimposed on the normal DNA profile. The pyridine should be of no concern because it is easily removable either by evaporation or by OPC. Users who are still concerned may incorporate a 60-second acetonitrile wash at the beginning of the end procedure (before the 60-second reverse flush) to remove the pyridine before cleavage starts. The deprotection time in ammonia for oligonucleotides made on 40-nanomole polystyrene columns with standard protecting groups remains at 8-24 hours at 55° C.

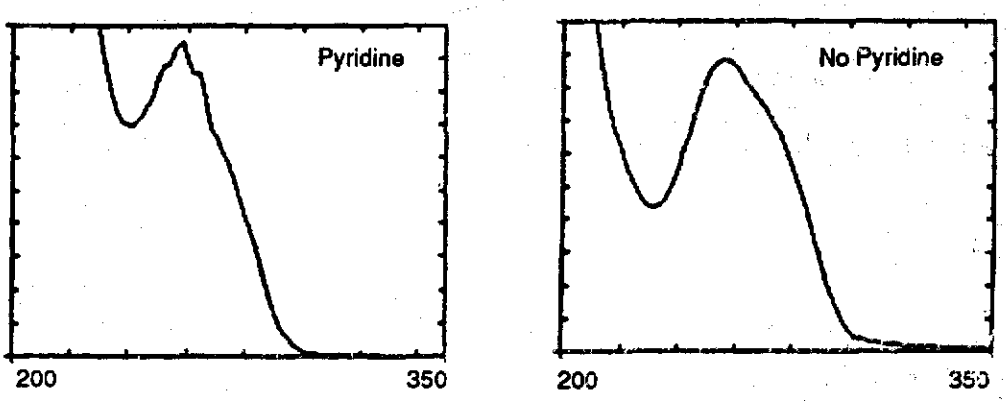



**Figure 4.** DNA spectra with and without pyridine

## Manual Cleavage

Manual cleavage should be performed in the column with a syringe, rather than by emptying the support into a vial containing concentrated ammonium hydroxide. A quick acetonitrile, methanol or ethanol wash and subsequent 60-second reverse flush is recommended before manual or auto cleavage of columns that have dried out since synthesis was performed.

# References


1. Andrus, A. and McCollum, C. "A New Support for Automated Oligonucleotide Synthesis," presented at the Symposium on Solid Phase Synthesis, Oxford, U.K., August 1989.
2. Pon, R.T., Usman, N. and Ogilvie, K.K. (1988) "Derivatization of Controlled Pore Glass Beads for Solid Phase Oligonucleotide Synthesis," *Biotechniques* 6:768-775.
3. Andrus, A. and McCollum, C. "A New Support for Automated Oligonucleotide Synthesis," presented at the Ninth International Round Table: Nucleosides, Nucleotides and Their Biological Applications, Uppsala, Sweden, July 1990.
4. McBride, L.J., McCollum, C., Davidson, S., Efcavitch, J.W., Andrus, A. and Lombardi, S. (1988) "Oligonucleotide Purification Cartridge," *Biotechniques* 6:362-367.
5. Moring, S.E., Colburn, J.C., Grossman, P.D. and Lauer, H.H. (1990) "Analytical Aspects of an Automated Capillary Electrophoresis System," *LC•GC* 8:34.



These colums contain microporous particles and are required to be identified by the following legend:

MADE IN THE UK

This product contains media manufactured under one or more of US patents 4,224,415; 4,256,840; 4,382,124; and 4,501,826 and the purchaser is entitled to utilize this product under US Patent 4,297,220 in analytical, preparative and process chromatography (excluding treatment of fruit juice), medical diagnostics and catalyst and chemical synthesis supports and for no other use.