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[FAQs](#)  
[Error Codes](#)  
[Request Forms](#)  
[Doc. Library](#)  
[Software Library](#)  
[Fax on Demand](#)  
[Course Listing](#)  
[Contact Information](#)  
[Part Numbers](#)  
[Our Teams](#)

[Search](#)

[Site Map](#)


[Index](#)

[Feedback](#)

[What's New](#)

[PE Home](#)

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**Documentation Library****User Bulletin: ABI 392/4  
Nucleic Acid Synthesizers**

**Subject: Fast, Economical  
Oligonucleotide Synthesis with ABI  
LV40 Columns**

## Description and Advantages

LV40 (low-volume, 40-nmol-scale) columns and accompanying LV40 synthesis cycles enable synthesis at the 40-nmol scale that is comparable in quality to that of the current 40-nmol synthesis, but with a 25% reduction in cycle cost, a 15% reduction in cycle waste, and a 10% reduction in cycle time.

### IMPORTANT

*For optimal performance and maximum economy, all reagent flow rates must be set within specification when using the LV40 cycle. Refer to your User's Manual for flow rate specifications and the auto-dilute calibration procedure.*

Phosphoramidite concentration for LV40 columns is 0.1 M (single dilution), which allows you to switch between scales without changing phosphoramidites. In addition, the G<sup>dmf</sup> LV40 column makes LV40 synthesis compatible with FastPhoramidite reagents (see DNA Synthesis User Bulletin 85, *FastPhoramidite Reagents for DNA Synthesis with Rapid Deprotection at All Scales*, stock no. 239842).

## The LV40 Cycle

The LV40 cycle consumes less reagent per cycle than the 40-nmol CE cycle, a savings of money, time, and the amount of chemical waste generated. Because 40-nmol columns are not compatible with the LV40 cycle, the LV40 column is required to provide optimal synthesis quality. Four print-outs of the LV40 cycle are included at the end of this User Bulletin: ABI 392-5 and ABI 394-8 configurations, both with and without the AutoAnalysis trityl-monitor feature.

LV40 cycles for synthesizers without AutoAnalysis allow for trityl effluent collection through the trityl port. When starting synthesis, select from the trityl collect options of "All," "1st & last 2," or "None." Using the attached cycles as a guide, you can easily create LV40 cycles for the ABI 392-8 and ABI 394-5, without AutoAnalysis. You will find that editing a copy of the 40-nmol CE cycle is the most convenient way to enter the LV40 cycle. An OligoNet floppy disk, containing all the LV40 cycles, is available to OligoNet users.

## LV40 Column Design

A frit has been added to the 40-nmol column to reduce unoccupied volume in the column and to make a packed bed of the polystyrene support beads. This allows

efficient synthesis even with the reduced-consumption LV40 cycle. The LV40 column is compatible with all 40-nmol column applications, but you will realize a cost savings only with the LV40 cycle on the 392/394.

## Instrument Compatibility

LV40 columns are compatible with the 380B, 381, 391, and 392/394 instruments. However, the LV40 cycle takes advantage of the precise control of reagent delivery afforded by the 392/394, whose delivery times can be adjusted in 0.1-second increments. Because the 380B, 381, and 391 allow control only to the nearest second, the economic cycle advantages of the LV40 columns are not obtainable with these instruments.

## 0.02 M Iodine

Successful synthesis depends on the use of 0.02 M iodine (P/N 401732) to reduce frit staining.

## Phosphoramidite Concentration

**392/394:** When LV40 columns are used on these instruments, phosphoramidite concentration should be 0.1 M. This allows you to switch freely between scales without changing phosphoramidites. The 0.1 M phosphoramidites can be used for up to three weeks if LV40 columns are used exclusively during the third week.

**380B, 381, 391:** When LV40 columns are used on these instruments, phosphoramidite concentration should remain at 0.05 M. Phosphoramidite lifetime is three weeks, the same as with current 40-nmol polystyrene columns. See DNA Synthesis User Bulletin 61, *40-nanomole Polystyrene: New Highly Efficient DNA Synthesis Columns*, stock no. 239825.

## Yield

The yield for LV40 syntheses is comparable to that for current 40-nmol columns. A primer-length, crude oligonucleotide yields 5-10 ODU.

## Post-Synthesis Processing

No change in cleavage, deprotection, purification, or analysis protocols are required. We continue to recommend using Teflon-lined caps (Applied Biosystems P/N 400048) for deprotection at the 40-nmol scale (see User Bulletin 61). A primer-length oligonucleotide synthesized with the FastPhoramidite set can be fully deprotected and biologically active in 1 h at 65 °C in 30% concentrated ammonia, or 2 h at 55 °C. To prevent oligonucleotide degradation, deprotection at 65 °C should not exceed 16 h.

## Oligonucleotide Length

When using FastPhoramidites, oligonucleotide length should not exceed 50 bases. Otherwise, there are no restrictions.

## Storage Conditions

Storage conditions are the same for both LV40 and 40-nmol columns. Store unused columns in a dry environment at room temperature.

## Transferring an OligoNet LV40 Cycle to Your

## 392/394

*Note: Select the appropriate LV40 cycle for transfer from an OligoNet 1.0.1 floppy disk to your synthesizer. To select the appropriate cycle, you must determine whether your synthesizer is a 392 (2 columns) or a 394 (4 columns), and if it has 5 base positions or 8.*

1. Insert the New Cycles floppy disk (P/N 603959) into your Macintosh.
2. Open OligoNet, then open your synthesizer.
3. Choose the Edit Cycle function, identify an open cycle location, and scroll to it.
4. Choose Import Cycle from the File menu. You will see the standard directory dialog, which displays a list of files.
5. Click the Desktop button, select New Cycles from the list, and click the Open button.
6. Select the cycle file you wish to import, and click the Open button.
7. Under the File menu, choose Send to Synthesizer, then verify that the cycle is in your synthesizer.

For more information, see Exporting and Importing Cycles and Procedures in the OligoNet User's Manual.

LV40 cycles on OligoNet floppies are for 0.1 M amidites.

## Interrupt Ahead

The LV40 cycle has fewer steps than the 40-nmol CE cycle. This may be important when executing an Interrupt Ahead operation; for example, to change bottles during a synthesis. It is important to examine a print-out of the LV40 cycle and determine an appropriate safe step at which to set the interrupt.

## Cycle Change Required for Specialty Reagents

Cycle changes are required at all scales for some specialty reagents. With appropriate cycle changes made on the 392/394, LV40 columns are compatible with the following specialty phosphoramidites:

- Aminolink 2
- Biotin
- 6-FAM, HEX, and Tet Dye Phosphoramidites
- Inosine
- Phosphalink

## Aminolink 2

(P/N 400808, 250 mg)

**Dilution:** Manually dilute to 0.1 M, using 3.3 mL of anhydrous acetonitrile.

**Bottle Change:** No change. Use ABI 5, 6, 7 or 8 to install Aminolink 2 at positions 5, 6, 7 or 8.

**Begin Procedure:** No change.

**Cycle Change:** Increase to 120 s the coupling wait time of the LV40 cycle at the bottle position where Aminolink 2 is installed. Select "trityl-on" synthesis.

Following is an example of a 392 user cycle in which Aminolink 2 is installed at bottle position 5. Use the base-specifier field to select *only* the base position at

which Aminolink 2 is installed. ABI 394 cycles and other base positions are programmed similarly.

### ABI 392 Cycle Changes for Aminolink 2

Step	Function	Number	Time	Base Specifier
20	B + Tet To Column	33	0.2	AGCT5
22	Tet To Column	34	1.6	AGCT5
23	Flush To Waste	4	0.6	5
24	18 To Waste	64	4.0	
25	Block Flush	1	3.0	AGCT5
26	Column 2 Off	143		
<b>27</b>	<b>Wait</b>	<b>103</b>	<b>120.0</b>	<b>5</b>
28	Flush To Waste	4	0.9	AGCT5
29	Wait	103	3.0	AGCT5
30	Reverse Flush	2	0.4	AGCT5

For more information, see DNA Synthesis User Bulletin 49, *Aminolink 2*, stock no. 239816.

## Biotin Amidite

(85 mg: P/N 401395; 250 mg: P/N 401396)

**Dilution** 0.05 M

**85 mg:** Manually dilute Biotin Amidite with 2 mL of anhydrous acetonitrile.

**250 mg:** Manually dilute Biotin Amidite with 6 mL of anhydrous acetonitrile, or use the 0.5-g auto-dilute procedure.

**Bottle Change:** To conserve reagent, alter the Bottle Change procedure to send the reagent to waste for one second, rather than two. For detailed instructions, see DNA Synthesis User Bulletin 70, *Biotin Labeling of Oligonucleotides on a DNA/RNA Synthesizer*, 1992, stock no. 239832.

**Begin Procedure:** To conserve reagent, alter the Begin procedure to send Biotin Amidite to waste for one second, as described in UB 70.

**Reagent Consumption:** Select "trityl-on" for OPC purification;

**85 mg:** ~12 couplings;

**250 mg:** ~36 couplings

**Cycle Changes:** Increase the B + Tet delivery and coupling wait times of the LV40 cycle at the bottle position where Biotin Amidite is installed. Following is an example of a 392 user cycle in which Biotin Amidite is installed at bottle position 5. Use the base-specifier field to select *only* the base position at which Biotin Amidite is installed. Note that the increased B + Tet delivery time must be specified for every column in the coupling routine. ABI 394 and other base positions are programmed similarly.

### ABI 392 Cycle Changes for Biotin and Dye Amidites

Step	Function	Number	Time	Base Specifier
7	Column 1 On	140		
8	Block Vent	111	2.0	AGCT5
9	Tet To Column	34	0.5	AGCT5
10	B + Tet To Column	33	0.2	AGCT5
<b>11</b>	<b>B + Tet To Column</b>	<b>33</b>	<b>3.3</b>	<b>5</b>
12	Tet To Column	34	1.3	AGCT5
13	Flush To Waste	4	0.6	5
14	18 To Waste	64	4.0	
15	Block Flush	1	3.0	AGCT5
16	Column 1 Off	141		
17	Column 2 On	142		
18	Block Vent	111	2.0	AGCT5
19	Tet To Column	34	0.5	AGCT5
20	B + Tet To Column	33	0.2	AGCT5
<b>21</b>	<b>B + Tet To Column</b>	<b>33</b>	<b>3.3</b>	<b>5</b>
22	Tet To Column	34	1.6	AGCT5
23	Flush To Waste	4	0.6	5
24	18 To Waste	64	4.0	
25	Block Flush	1	3.0	AGCT5
26	Column 2 Off	143		
<b>27</b>	<b>Wait</b>	<b>103</b>	<b>120.0</b>	<b>5</b>
28	Flush To Waste	4	0.9	AGCT5
29	Wait	103	3.0	AGCT5
30	Reverse Flush	2	0.4	AGCT5

## Dye Phosphoramidites

6-FAM Amidite P/N 401527, 85 mg

HEX Amidite P/N 401526, 105 mg

Tet Amidite P/N 401533, 100 mg

**Dilution:** Manually dilute to 0.05 M with 2 mL anhydrous acetonitrile.

**Bottle Change:** Reduce to one second the delivery to waste from the dye phosphoramidite bottle position. For details, see DNA Synthesis User Bulletin 78, *Synthesis and Purification of Fluorescently Labeled Oligonucleotides Using Dye Phosphoramidites*, stock no. 239837-001.

**Begin Procedure:** Reduce to one second the Delivery To Waste from the dye phosphoramidite bottle position. For more details, see UB 78.

**Cycle Change:** Same cycle changes as detailed above for Biotin Amidite.

**Consumption:** Each dye amidite bottle yields ~12 couplings.

**OPC Purification:** Select "trityl-on," using the OPC protocol designed for dye-labeled oligonucleotides and described in UB 78.

## Inosine

(P/N 400402, 0.25 g)

**Dilution:** Dilute to 0.1 M, using the 0.25-g (or 3.4 mL of anhydrous acetonitrile) auto-dilute procedure.

**Begin Procedure:** No change.

**Cycle Change:** No change. Use the LV40 cycle.

## Phosphalink

(P/N 401717, 70 mg)

**Dilution:** Manually dilute to 0.1 M using 1.0-mL anhydrous acetonitrile.

**Bottle Change Procedure:** Reduce to one second the Delivery To Waste from the Phosphalink bottle position.

**Begin Procedure:** No change.

**Cycle Change:** No change. Use the LV40 cycle.

For further information, see DNA Synthesis UB 86, *Phosphalink Amidite for Phosphorylation of Oligonucleotides*, stock no. 239843.

## Ordering Information

P/N	Description
401738	ABI LV40 Column dA <sup>bz</sup>
401742	ABI LV40 Column dG <sup>ibu</sup>
401745	ABI LV40 Column dG <sup>dmf</sup>
401743	ABI LV40 Column dC <sup>bz</sup>
401744	ABI LV40 Column T

Synthesizer - Bufford  
Cycle: 25) LV40 392-5

Step Number	Function		Step Time	Active	Safe Step
	#	Name			
1	106	Begin			Yes
2	64	18 To Waste	3.0		Yes

3	42	18 To Column	10.0	AGCT5	Yes
4	2	Reverse Flush	8.0	AGCT5	Yes
5	1	Block Flush	4.0	AGCT5	Yes
6	101	Phos Prep	3.0		Yes
7	140	Column 1 On			Yes
8	111	Block Vent	2.0	AGCT5	Yes
9	34	Tet To Column	0.5	AGCT5	Yes
10	33	B+Tet To Column	0.2	AGCT5	Yes
11	34	Tet To Column	1.3	AGCT5	Yes
12	4	Flush To Waste	0.6	5	Yes
13	64	18 To Waste	4.0		Yes
14	1	Block Flush	3.0	AGCT5	Yes
15	141	Column 1 Off			Yes
16	142	Column 2 On			Yes
17	111	Block Vent	2.0	AGCT5	Yes
18	34	Tet To Column	0.5	AGCT5	Yes
19	33	B+Tet To Column	0.2	AGCT5	Yes
20	34	Tet To Column	1.6	AGCT5	Yes
21	4	Flush To Waste	0.6	5	Yes
22	64	18 To Waste	4.0		Yes
23	1	Block Flush	3.0	AGCT5	Yes
24	143	Column 2 Off			Yes
25	4	Flush To Waste	0.9	AGCT5	Yes
26	103	Wait	3.0	AGCT5	Yes
27	2	Reverse Flush	0.4	AGCT5	Yes
28	103	Wait	3.0	AGCT5	Yes
29	4	Flush To Waste	0.7	AGCT5	Yes
30	103	Wait	3.0	AGCT5	Yes
31	2	Reverse Flush	0.4	AGCT5	Yes
32	103	Wait	3.0	AGCT5	Yes
33	4	Flush To Waste	0.7	AGCT5	Yes
34	103	Wait	3.0	AGCT5	Yes
35	2	Reverse Flush	0.4	AGCT5	Yes
36	103	Wait	3.0	AGCT5	Yes

37	4	Flush To Waste	0.7	AGCT5	Yes
38	102	Cap Prep	3.0		Yes
39	64	18 To Waste	4.0		Yes
40	2	Reverse Flush	5.0	AGCT5	Yes
41	1	Block Flush	3.0	AGCT5	Yes
42	39	Cap To Column	4.0	AGCT5	Yes
43	103	Wait	5.0	AGCT5	Yes
44	64	18 To Waste	4.0		Yes
45	2	Reverse Flush	5.0	AGCT5	Yes
46	1	Block Flush	3.0	AGCT5	Yes
47	41	15 To Column	4.0	AGCT5	Yes
48	64	18 To Waste	4.0		Yes
49	1	Block Flush	3.0	AGCT5	Yes
50	103	Wait	15.0	AGCT5	Yes
51	42	18 To Column	10.0	AGCT5	Yes
52	4	Flush To Waste	4.0	AGCT5	Yes
53	42	18 To Column	10.0	AGCT5	Yes
54	2	Reverse Flush	5.0	AGCT5	Yes
55	1	Block Flush	3.0	AGCT5	Yes
56	105	Start Detrityl			Yes
57	64	18 To Waste	4.0		Yes
58	42	18 To Column	10.0	AGCT5	Yes
59	2	Reverse Flush	5.0	AGCT5	Yes
60	1	Block Flush	3.0	AGCT5	Yes
61	167	If Monitoring			Yes
62	44	19 To Column	18.0	AGCT5	Yes
63	40	14 To Column	3.0	AGCT5	No
64	135	Monitor trityls			No
65	40	14 To Column	18.0	AGCT5	No
66	136	Monitor noise			No
67	40	14 To Column	10.0	AGCT5	No
68	137	Stop monitor			No
69	42	18 To Column	10.0	AGCT5	No
70	2	Reverse Flush	8.0	AGCT5	No

71	168	If not Monitoring			Yes
72	40	14 To Column	6.0	AGCT5	No
73	103	Wait	5.0	AGCT5	No
74	3	Trityl Flush	5.0	AGCT5	No
75	40	14 To Column	6.0	AGCT5	No
76	103	Wait	5.0	AGCT5	No
77	3	Trityl Flush	5.0	AGCT5	No
78	42	18 To Column	10.0	AGCT5	No
79	3	Trityl Flush	8.0	AGCT5	No
80	169	End Monitoring			Yes
81	42	18 To Column	8.0	AGCT5	Yes
82	2	Reverse Flush	5.0	AGCT5	Yes
83	1	Block Flush	4.0	AGCT5	Yes
84	107	End			Yes

Synthesizer - Bufford  
Cycle: 28) LV40 394-8

Step Number	Function		Step Time	Active	Safe Step
	#	Name			
1	106	Begin			Yes
2	64	18 To Waste	3.0		Yes
3	42	18 To Column	10.0	AGCT5678	Yes
4	2	Reverse Flush	8.0	AGCT5678	Yes
5	1	Block Flush	4.0	AGCT5678	Yes
6	101	Phos Prep	3.0		Yes
7	140	Column 1 On			Yes
8	111	Block Vent	2.0	AGCT5678	Yes
9	34	Tet To Column	0.5	AGCT5678	Yes
10	33	B+Tet To Column	0.2	AGCT5678	Yes
11	34	Tet To Column	1.3	AGCT5678	Yes
12	4	Flush To Waste	0.6	5678	Yes
13	64	18 To Waste	4.0		Yes
14	1	Block Flush	3.0	AGCT5678	Yes
15	141	Column 1 Off			Yes

16	142	Column 2 On			Yes
17	111	Block Vent	2.0	AGCT5678	Yes
18	34	Tet To Column	0.5	AGCT5678	Yes
19	33	B+Tet To Column	0.2	AGCT5678	Yes
20	34	Tet To Column	1.6	AGCT5678	Yes
21	4	Flush To Waste	0.6	5678	Yes
22	64	18 To Waste	4.0		Yes
23	1	Block Flush	3.0	AGCT5678	Yes
24	143	Column 2 Off			Yes
25	144	Column 3 On			Yes
26	111	Block Vent	2.0	AGCT5678	Yes
27	34	Tet To Column	0.5	AGCT5678	Yes
28	33	B+Tet To Column	0.2	AGCT5678	Yes
29	34	Tet To Column	1.7	AGCT5678	Yes
30	4	Flush To Waste	0.6	5678	Yes
31	64	18 To Waste	4.0		Yes
32	1	Block Flush	3.0	AGCT5678	Yes
33	145	Column 3 Off			Yes
34	146	Column 4 On			Yes
35	111	Block Vent	2.0	AGCT5678	Yes
36	34	Tet To Column	0.5	AGCT5678	Yes
37	33	B+Tet To Column	0.2	AGCT5678	Yes
38	34	Tet To Column	2.0	AGCT5678	Yes
39	4	Flush To Waste	0.6	5678	Yes
40	64	18 To Waste	4.0		Yes
41	1	Block Flush	3.0	AGCT5678	Yes
42	147	Column 4 Off			Yes
43	4	Flush To Waste	0.9	AGCT5678	Yes
44	103	Wait	3.0	AGCT5678	Yes
45	2	Reverse Flush	0.4	AGCT5678	Yes
46	103	Wait	3.0	AGCT5678	Yes
47	4	Flush To Waste	0.7	AGCT5678	Yes
48	103	Wait	3.0	AGCT5678	Yes
49	2	Reverse Flush	0.4	AGCT5678	Yes

50	103	Wait	3.0	AGCT5678	Yes
51	4	Flush To Waste	0.7	AGCT5678	Yes
52	103	Wait	3.0	AGCT5678	Yes
53	2	Reverse Flush	0.4	AGCT5678	Yes
54	103	Wait	3.0	AGCT5678	Yes
55	4	Flush To Waste	0.7	AGCT5678	Yes
56	102	Cap Prep	3.0		Yes
57	64	18 To Waste	4.0		Yes
58	2	Reverse Flush	5.0	AGCT5678	Yes
59	1	Block Flush	3.0	AGCT5678	Yes
60	39	Cap To Column	4.0	AGCT5678	Yes
61	103	Wait	5.0	AGCT5678	Yes
62	64	18 To Waste	4.0		Yes
63	2	Reverse Flush	5.0	AGCT5678	Yes
64	1	Block Flush	3.0	AGCT5678	Yes
65	41	15 To Column	4.0	AGCT5678	Yes
66	64	18 To Waste	4.0		Yes
67	1	Block Flush	3.0	AGCT5678	Yes
68	103	Wait	15.0	AGCT5678	Yes
69	42	18 To Column	10.0	AGCT5678	Yes
70	4	Flush To Waste	4.0	AGCT5678	Yes
71	42	18 To Column	10.0	AGCT5678	Yes
72	2	Reverse Flush	5.0	AGCT5678	Yes
73	1	Block Flush	3.0	AGCT5678	Yes
74	105	Start Detrityl			Yes
75	64	18 To Waste	4.0		Yes
76	42	18 To Column	10.0	AGCT5678	Yes
77	2	Reverse Flush	5.0	AGCT5678	Yes
78	1	Block Flush	3.0	AGCT5678	Yes
79	167	If Monitoring			Yes
80	44	19 To Column	18.0	AGCT5678	Yes
81	40	14 To Column	3.0	AGCT5678	No
82	135	Monitor trityls			No
83	40	14 To Column	18.0	AGCT5678	No

84	136	Monitor noise			No
85	40	14 To Column	10.0	AGCT5678	No
86	137	Stop monitor			No
87	42	18 To Column	10.0	AGCT5678	No
88	2	Reverse Flush	8.0	AGCT5678	No
89	168	If not Montring			Yes
90	40	14 To Column	6.0	AGCT5678	No
91	103	Wait	5.0	AGCT5678	No
92	3	Trityl Flush	5.0	AGCT5678	No
93	40	14 To Column	6.0	AGCT5678	No
94	103	Wait	5.0	AGCT5678	No
95	3	Trityl Flush	5.0	AGCT5678	No
96	42	18 To Column	10.0	AGCT5678	No
97	3	Trityl Flush	8.0	AGCT5678	No
98	169	End Monitoring			Yes
99	42	18 To Column	8.0	AGCT5678	Yes
100	2	Reverse Flush	5.0	AGCT5678	Yes
101	1	Block Flush	4.0	AGCT5678	Yes
102	107	End			Yes

Synthesizer - Bufford  
Cycle: 29) LV40 wo/AA 392-5

Step Number	Function		Step Time	Active	Safe Step
	#	Name			
1	106	Begin			Yes
2	64	18 To Waste	3.0		Yes
3	42	18 To Column	10.0	AGCT5	Yes
4	2	Reverse Flush	8.0	AGCT5	Yes
5	1	Block Flush	4.0	AGCT5	Yes
6	101	Phos Prep	3.0		Yes
7	140	Column 1 On			Yes
8	111	Block Vent	2.0	AGCT5	Yes
9	34	Tet To Column	0.5	AGCT5	Yes

10	33	B+Tet To Column	0.2	AGCT5	Yes
11	34	Tet To Column	1.3	AGCT5	Yes
12	4	Flush To Waste	0.6	5	Yes
13	64	18 To Waste	4.0		Yes
14	1	Block Flush	3.0	AGCT5	Yes
15	141	Column 1 Off			Yes
16	142	Column 2 On			Yes
17	111	Block Vent	2.0	AGCT5	Yes
18	34	Tet To Column	0.5	AGCT5	Yes
19	33	B+Tet To Column	0.2	AGCT5	Yes
20	34	Tet To Column	1.6	AGCT5	Yes
21	4	Flush To Waste	0.6	5	Yes
22	64	18 To Waste	4.0		Yes
23	1	Block Flush	3.0	AGCT5	Yes
24	143	Column 2 Off			Yes
25	4	Flush To Waste	0.9	AGCT5	Yes
26	103	Wait	3.0	AGCT5	Yes
27	2	Reverse Flush	0.4	AGCT5	Yes
28	103	Wait	3.0	AGCT5	Yes
29	4	Flush To Waste	0.7	AGCT5	Yes
30	103	Wait	3.0	AGCT5	Yes
31	2	Reverse Flush	0.4	AGCT5	Yes
32	103	Wait	3.0	AGCT5	Yes
33	4	Flush To Waste	0.7	AGCT5	Yes
34	103	Wait	3.0	AGCT5	Yes
35	2	Reverse Flush	0.4	AGCT5	Yes
36	103	Wait	3.0	AGCT5	Yes
37	4	Flush To Waste	0.7	AGCT5	Yes
38	102	Cap Prep	3.0		Yes
39	64	18 To Waste	4.0		Yes
40	2	Reverse Flush	5.0	AGCT5	Yes
41	1	Block Flush	3.0	AGCT5	Yes
42	39	Cap To Column	4.0	AGCT5	Yes
43	103	Wait	5.0	AGCT5	Yes

44	64	18 To Waste	4.0		Yes
45	2	Reverse Flush	5.0	AGCT5	Yes
46	1	Block Flush	3.0	AGCT5	Yes
47	41	15 To Column	4.0	AGCT5	Yes
48	64	18 To Waste	4.0		Yes
49	1	Block Flush	3.0	AGCT5	Yes
50	103	Wait	15.0	AGCT5	Yes
51	42	18 To Column	10.0	AGCT5	Yes
52	4	Flush To Waste	4.0	AGCT5	Yes
53	42	18 To Column	10.0	AGCT5	Yes
54	2	Reverse Flush	5.0	AGCT5	Yes
55	1	Block Flush	3.0	AGCT5	Yes
56	105	Start Detrityl			Yes
57	64	18 To Waste	4.0		Yes
58	42	18 To Column	10.0	AGCT5	Yes
59	2	Reverse Flush	5.0	AGCT5	Yes
60	1	Block Flush	3.0	AGCT5	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	AGCT5	No
69	103	Wait	5.0	AGCT5	No
70	3	Trityl Flush	5.0	AGCT5	No
71	40	14 To Column	6.0	AGCT5	No
72	103	Wait	5.0	AGCT5	No
73	3	Trityl Flush	5.0	AGCT5	No
74	42	18 To Column	10.0	AGCT5	No
75	3	Trityl Flush	8.0	AGCT5	No
76	110	Waste-Bottle			Yes
77	42	18 To Column	8.0	AGCT5	Yes

78	2	Reverse Flush	5.0	AGCT5	Yes
79	1	Block Flush	4.0	AGCT5	Yes
80	107	End			Yes

Synthesizer - Bufford  
Cycle: 32) LV40 wo/AA 394-8

Step Number	Function		Step Time	Active	Safe Step
	#	Name			
1	106	Begin			Yes
2	64	18 To Waste	3.0		Yes
3	42	18 To Column	10.0	AGCT5678	Yes
4	2	Reverse Flush	8.0	AGCT5678	Yes
5	1	Block Flush	4.0	AGCT5678	Yes
6	101	Phos Prep	3.0		Yes
7	140	Column 1 On			Yes
8	111	Block Vent	2.0	AGCT5678	Yes
9	34	Tet To Column	0.5	AGCT5678	Yes
10	33	B+Tet To Column	0.2	AGCT5678	Yes
11	34	Tet To Column	1.3	AGCT5678	Yes
12	4	Flush To Waste	0.6	5678	Yes
13	64	18 To Waste	4.0		Yes
14	1	Block Flush	3.0	AGCT5678	Yes
15	141	Column 1 Off			Yes
16	142	Column 2 On			Yes
17	111	Block Vent	2.0	AGCT5678	Yes
18	34	Tet To Column	0.5	AGCT5678	Yes
19	33	B+Tet To Column	0.2	AGCT5678	Yes
20	34	Tet To Column	1.6	AGCT5678	Yes
21	4	Flush To Waste	0.6	5678	Yes
22	64	18 To Waste	4.0		Yes
23	1	Block Flush	3.0	AGCT5678	Yes
24	143	Column 2 Off			Yes
25	144	Column 3 On			Yes
26	111	Block Vent	2.0	AGCT5678	Yes

27	34	Tet To Column	0.5	AGCT5678	Yes
28	33	B+Tet To Column	0.2	AGCT5678	Yes
29	34	Tet To Column	1.7	AGCT5678	Yes
30	4	Flush To Waste	0.6	5678	Yes
31	64	18 to Flush	4.0		Yes
32	1	Block Flush	3.0	AGCT5678	Yes
33	145	Column 3 Off			Yes
34	146	Column 4 On			Yes
35	111	Block Vent	2.0	AGCT5678	Yes
36	34	Tet To Column	0.5	AGCT5678	Yes
37	33	B+Tet To Column	0.2	AGCT5678	Yes
38	34	Tet To Column	2.0	AGCT5678	Yes
39	4	Flush To Waste	0.6	5678	Yes
40	64	18 To Waste	4.0		Yes
41	1	Block Flush	3.0	AGCT5678	Yes
42	147	Column 4 Off			Yes
43	4	Flush To Waste	0.9	AGCT5678	Yes
44	103	Wait	3.0	AGCT5678	Yes
45	2	Reverse Flush	0.4	AGCT5678	Yes
46	103	Wait	3.0	AGCT5678	Yes
47	4	Flush To Waste	0.7	AGCT5678	Yes
48	103	Wait	3.0	AGCT5678	Yes
49	2	Reverse Flush	0.4	AGCT5678	Yes
50	103	Wait	3.0	AGCT5678	Yes
51	4	Flush To Waste	0.7	AGCT5678	Yes
52	103	Wait	3.0	AGCT5678	Yes
53	2	Reverse Flush	0.4	AGCT5678	Yes
54	103	Wait	3.0	AGCT5678	Yes
55	4	Flush To Waste	0.7	AGCT5678	Yes
56	102	Cap Prep	3.0		Yes
57	64	18 To Waste	4.0		Yes
58	2	Reverse Flush	5.0	AGCT5678	Yes
59	1	Block Flush	3.0	AGCT5678	Yes
60	39	Cap To Column	4.0	AGCT5678	Yes

61	103	Wait	5.0	AGCT5678	Yes
62	64	18 To Waste	4.0		Yes
63	2	Reverse Flush	5.0	AGCT5678	Yes
64	1	Block Flush	3.0	AGCT5678	Yes
65	41	15 To Column	4.0	AGCT5678	Yes
66	64	18 To Waste	4.0		Yes
67	1	Block Flush	3.0	AGCT5678	Yes
68	103	Wait	15.0	AGCT5678	Yes
69	42	18 To Column	10.0	AGCT5678	Yes
70	4	Flush To Waste	4.0	AGCT5678	Yes
71	42	18 To Column	10.0	AGCT5678	Yes
72	2	Reverse Flush	5.0	AGCT5678	Yes
73	1	Block Flush	3.0	AGCT5678	Yes
74	105	Start Detrityl			Yes
75	64	18 To Waste	4.0		Yes
76	42	18 To Column	10.0	AGCT5678	Yes
77	2	Reverse Flush	5.0	AGCT5678	Yes
78	1	Block Flush	3.0	AGCT5678	Yes
79	112	Trityl Advance			Yes
80	109	Waste-Port			Yes
81	120	Advance FC			Yes
82	120	Advance FC			Yes
83	120	Advance FC			Yes
84	120	Advance FC			Yes
85	113	End Advance			Yes
86	40	14 To Column	6.0	AGCT5678	No
87	103	Wait	5.0	AGCT5678	No
88	3	Trityl Flush	5.0	AGCT5678	No
89	40	14 To Column	6.0	AGCT5678	No
90	103	Wait	5.0	AGCT5678	No
91	3	Trityl Flush	5.0	AGCT5678	No
92	42	18 To Column	10.0	AGCT5678	No
93	3	Trityl Flush	8.0	AGCT5678	No
94	110	Waste-Bottle			Yes

95	42	18 To Column	8.0	AGCT5678	Yes
96	2	Reverse Flush	5.0	AGCT5678	Yes
97	1	Block Flush	4.0	AGCT5678	Yes
98	107	End			Yes

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