TaqMan® Genetically Modified Organism (GMO) Detection Kits

User Guide

Applied Biosystems

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1 Introduction

Overview

About This User Guide

The TaqMan® Genetically Modified Organism (GMO) Detection Kits User Guide contains general information that pertains to TaqMan Genetically Modified Organism Assays.

IMPORTANT Please refer to the product insert for kit-specific procedures and for other information that is specific for an individual GMO assay.

Topic	See
Sample Preparation	Chapter 2
Setup on the ABI Prism 7700 SDS	Chapter 3
Preparing the Reactions	Chapter 4
Thermal Cycling	Chapter 5
Data Analysis and Interpretation	Chapter 6
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About TaqMan GMO Assays

PCR-Based Assay

TaqMan GMO Detection Kits provide a simple, reliable procedure for detecting the presence of genetically modified maize or soy. The assay utilizes the polymerase chain reaction (PCR) to amplify an endogenous reference target that is unique to either maize or soy, plus a target that indicates the presence of genetically modified maize or soy.

The TaqMan GMO Detection Kits utilize the fluorogenic 5´ nuclease assay. For more information on the fluorogenic 5´ nuclease assay, refer to “5´ Nuclease Assay” on page A-1. Fluorescence readings are taken on the ABI PRISM® 7900 Sequence Detection System (SDS), the ABI PRISM® 7000 SDS or the ABI PRISM® 7700 SDS and analyzed using the instrument’s SDS software.

Two Dye Layers

The TaqMan GMO Detection Kits detect PCR products by monitoring the increase in fluorescence from two dye-labeled DNA probes. Each reaction contains two independent PCR systems, each with its own DNA primers and dye-labeled probe.

One PCR system detects a GMO-specific target DNA sequence. The other is an endogenous reference (ER) system designed to serve as a quantitative reference that detects genetically modified or non-genetically modified maize and soy.

Separate dye layers are defined in the SDS software sample setup window to determine the proper analysis of results from the GMO-specific and ER PCR systems.

- The GMO system’s probe is labeled with the FAM™ dye.
- The ER system’s probe is labeled with the VIC™ dye.

The FAM (GMO-specific) dye layer results are compared to their respective VIC layer results to calculate a %GMO figure for each sample. In the VIC ER dye layer, samples are defined as IPC+. The analysis method uses these sample designations and dye layers to determine the final assay results for each reaction.

Endogenous Reference System

Primers and probe to detect the ER are contained in the TaqMan GMO Mix that is used in every reaction. It detects a gene specific to either maize (TaqMan GMO Maize Detection Kit) or soy (TaqMan GMO Soy Detection Kit). The ER systems are crucial for assay quantitation, and also indicate whether DNA from the plant being tested for (maize or soy) is present in the sample.

GMO Concentration Reference Standards

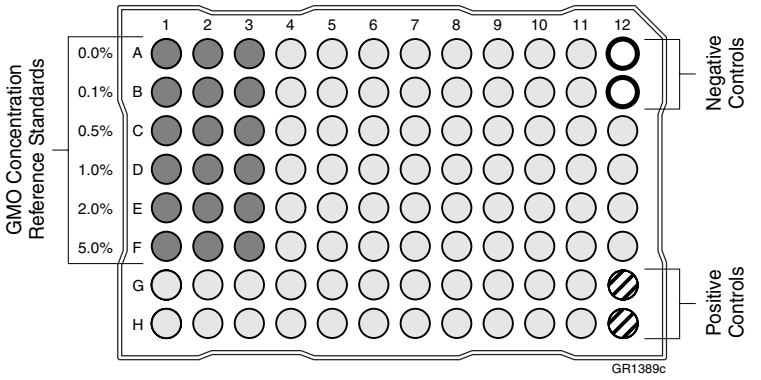
For quantitative results with the TaqMan GMO Detection Kits, it is necessary to prepare samples of a set of GMO concentration reference standards such as those produced by the Institute for Reference Materials and Methods (IRMM), a branch of the European Commission. There are separate standards sets available for genetically modified soy and genetically modified maize. These are sold through outlets such as Fluka Chemical Company and Resource Technology Corporation (Laramie, WY). DNA extracts of the GMO standards should be prepared by the same method as the food samples to be analyzed. The GMO Concentration reference standards must be analyzed on each reaction plate of the TaqMan GMO Detection Kit assays.

Other Controls

Two positive control reactions and two negative control reactions are recommended for good laboratory practice, but are not required for data analysis.

Plate Configuration

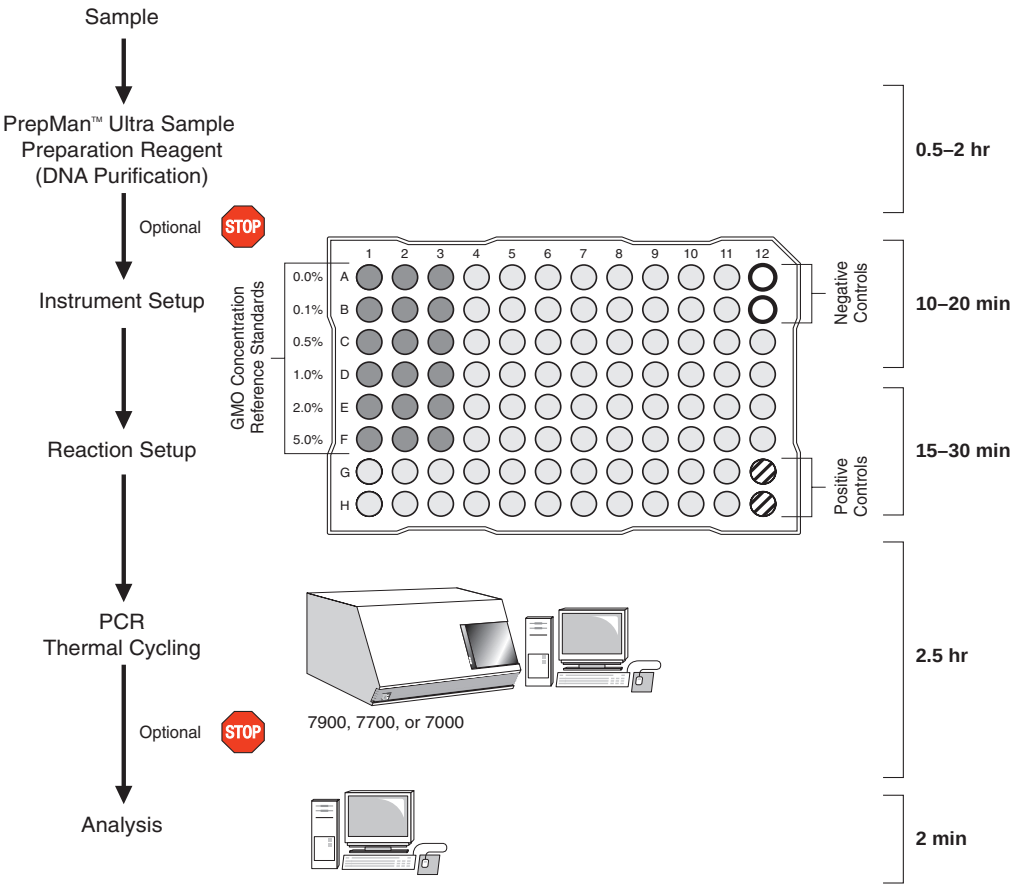
The following is an example of one possible setup used for the TaqMan GMO assays:



Procedure Flowchart

The diagram below shows an overview of how to use the TaqMan GMO Detection Kits.

Note The Stop sign indicates places in the procedure where optional breaks can occur.



About the Kits

Two Kits

There are TaqMan GMO Detection Kits for two different crops (soy and maize):

Kit	Kit Contents	Part Number
TaqMan GMO Soy 35S Detection Kit	Kit with Quick Reference Card and User Guide	4327692
TaqMan GMO Soy 35S Detection Kit	Kit without Quick Reference Card and User Guide	4327691
TaqMan GMO Maize 35S Detection Kit	Kit with Quick Reference Card and User Guide	4327693
TaqMan GMO Maize 35S Detection Kit	Kit without Quick Reference Card and User Guide	4327690

Kit Contents

The TaqMan GMO Detection Kits contain the following items:

- TaqMan GMO Mix
- AmpliTaq Gold® DNA Polymerase
- Negative Control
- Positive Control

Kit Storage

Store the TaqMan GMO Detection Kit at –15 °C to –25 °C in a constant-temperature freezer.

Kit Performance

The TaqMan GMO Detection Kit will detect the GMO only if all of the recommended components are used and the recommended protocols are followed.

Materials Required but Not Supplied

The following items are required in addition to the reagents supplied with the TaqMan GMO Detection Kit.

Reagents and Equipment

Reagent / Equipment Item	Source
ABI PRISM 7900 Sequence Detection System	Contact your local Applied Biosystems representative for the instrument and software best suited to meet your needs.
ABI PRISM 7700 Sequence Detection System	
ABI PRISM 7000 Sequence Detection System	
MicroAmp® Optical 96-Well Reaction Plates and MicroAmp Optical Caps	Applied Biosystems (P/N 403012)
MicroAmp 9600 Base	Applied Biosystems (P/N N801-0531)
MicroAmp® Cap Installing Tool	Applied Biosystems (P/N N801-0438)
PrepMan Ultra Sample Preparation Reagent	Applied Biosystems (P/N 4322547)
Printer, Color, Hewlett Packard Model No. 1600CM or equivalent	Applied Biosystems (P/N 100892)
GMO Concentration Reference Standards	Fluka Chemical Company OR Resource Technology Corporation
Microcentrifuge	Major laboratory suppliers (MLS)
Microsoft® Excel Spreadsheet	Microsoft
Boiling water bath	MLS
Pipettors, positive-displacement or air-displacement	MLS
Pipette tips, with filter plugs	MLS
Polypropylene tubes, 1.5-, 2.0-, or 5.0-mL	MLS
Vortexer	MLS

Safety

Documentation User Attention Words

Five user attention words appear in the text of all Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below.

Note Calls attention to useful information.

IMPORTANT Indicates information that is necessary for proper instrument operation.

⚠ CAUTION Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

⚠ WARNING Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

⚠ DANGER Indicates an imminently hazardous situation which, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Chemical Hazard Warning

CHEMICAL HAZARD. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Do not leave chemical containers open. Use only with adequate ventilation.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Hazard Warning

⚠ WARNING CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems kits are potentially hazardous and can cause injury or illness.

- Read and understand the material safety data sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Handle chemical wastes in a fume hood.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.,* safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (*e.g.*, fume hood). For additional safety guidelines, consult the MSDS.

Site Preparation and Safety Guide

A site preparation and safety guide is a separate document sent to all customers who have purchased an Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, chemical safety, and waste profiles.

Ordering MSDSs

You can order free additional copies of MSDSs for chemicals manufactured or distributed by Applied Biosystems using the contact information below.

<table>
<tr><th>To order MSDSs...</th><th>Then...</th></tr>
<tr><td>Over the Internet</td><td>a. Go to our Web site at www.appliedbiosystems.com/techsupp
b. Click MSDSs

<table><tr><th>If you have...</th><th>Then...</th></tr><tr><td>The MSDS document number or the Document on Demand index number</td><td>Enter one of these numbers in the appropriate field on this page.</td></tr><tr><td>The product part number</td><td rowspan="2">Select Click Here, then enter the part number or keyword(s) in the field on this page.</td></tr><tr><td>Keyword(s)</td></tr></table>
c. You can open and download a PDF (using Adobe® Acrobat® Reader™) of the document by selecting it, or you can choose to have the document sent to you by fax or email.</td></tr>
<tr><td>By automated telephone service</td><td>Use “To Obtain Documents on Demand” under “Technical Support.”</td></tr>
<tr><td>By telephone in the United States</td><td>Dial 1-800-327-3002, then press 1.</td></tr>
<tr><td>By telephone from Canada</td><td><table><tr><th>To order in...</th><th>Dial 1-800-668-6913 and...</th></tr><tr><td>English</td><td>Press 1, then 2, then 1 again</td></tr><tr><td>French</td><td>Press 2, then 2, then 1</td></tr></table></td></tr>
<tr><td>By telephone from any other country</td><td>See the specific region under “To Contact Technical Support by Telephone or Fax” under “Technical Support.”</td></tr>
</table>

For chemicals not manufactured or distributed by Applied Biosystems, call the chemical manufacturer.

Recommended PCR Amplification Practices

General PCR Practices

The DNA amplification capability of the PCR process makes special laboratory practices necessary. The following precautions will minimize sample cross-contamination and PCR product carryover (Dieffenbach and Dveksler, 1993).

IMPORTANT Maintain separate areas with dedicated equipment and supplies including lab coats, gloves, and pipettors. Three laboratory areas may be designated as shown in the table below. This is also shown in the "Diagram of Designated Areas" on page 1-11.

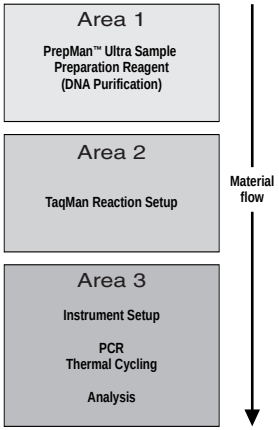
Area	Procedures
1	DNA purification
2	TaqMan reaction setup
3	Instrument setup, pre-read, PCR thermal cycling, post-read, and analysis of PCR products

- Wear a clean lab coat and clean gloves when preparing samples for PCR amplification.
- Change gloves whenever you suspect that they are contaminated.
- Never bring amplified PCR products into the DNA purification area.
- Open and close all sample tubes carefully. Try not to splash or spray samples.
- Use positive-displacement or air-displacement pipettors with filter-plugged tips. Change tips after each use.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution.

Note If you have questions concerning PCR amplification practices, please contact Technical Support.

Diagram of Designated Areas

Below is a diagram showing which steps should be performed in each of the three separate areas of the laboratory.



Reference

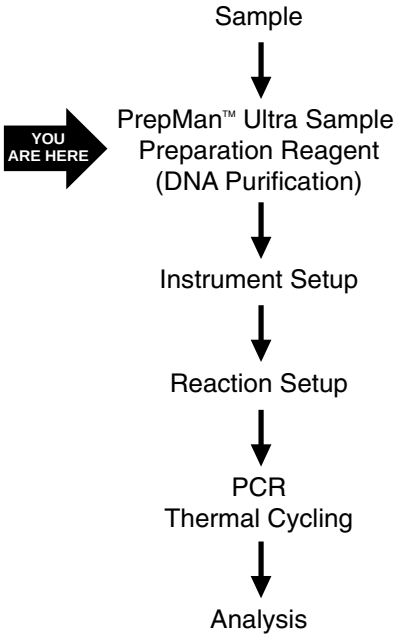
Dieffenbach, C. and Dveksler. 1993. Setting up a PCR Laboratory. *PCR Methods and Applications* 3:S2–S7.

2 Sample Preparation

Overview

Where You Are in the Procedure

This chapter describes sample preparation.



Sample Preparation

Introduction

Samples of processed food or their ingredients to be tested must first be prepared to extract their DNA and inactivate or remove impurities that would otherwise inhibit PCR. Different DNA preparation procedures may be used, which accomplish these ends in different ways.

Preventing Cross-Contamination Between Samples

Whatever sample-prep method is used, it is important to completely contain and isolate individual samples to prevent their cross-contamination, so that the sample volumes added to the TaqMan® GMO Detection assays are pure. The detection method is so sensitive that tiny amounts of cross-contamination can result in apparent false-positive assay results.

It is crucial to follow the general guidelines in Chapter 1 in "Recommended PCR Amplification Practices". Additionally, it bears mentioning that all steps leading up to sample preparation: acquiring the samples, handling the samples during pre-prep processing (grinding, transferring, weighing, etc.), must be done in so that the chance of cross-contamination between samples is minimized. To aid in this, use disposable, single use equipment when possible.

Processing Samples

If the food samples are not already in a powdered or granular form, they should be processed before DNA extraction. Samples such as flour or meal are already in an ideal form for DNA extraction, but coarser samples must first be ground or smashed into relatively fine particles to increase the efficiency of DNA extraction.

Many DNA extraction methods can use as little as 20 to 50 mg of starting material, so large-scale processing with blenders or homogenizers is usually not necessary. A simple method that works well is to place the sample between two clean disposable plastic weigh boats and smash/grind the sample with a hard, heavy object such as a hammer. Even dried corn kernels can be smashed this way with good results. As mentioned above, care must be taken to ensure that dust or particles are contained, and work surfaces are protected to eliminate cross-contamination.

DNA Extraction

Once the sample has been pulverized, it can be weighed and the appropriate amount added according to whichever DNA extraction method being used, and processed by the method's protocol. DNA extraction methods which have been used successfully are:

- PrepMan™ Ultra Sample Preparation Reagent
  Applied Biosystems (P/N 4322547)
- DNeasy™ Plant Mini Kit
  Qiagen, Inc. (P/N 69104)
  Valencia, CA 91355
- FastDNA® Kit
  BIO 101, Inc. (P/N 6540-400)
  Vista, CA 92083

Note The FastDNA kit requires the use of a special FastPrep® homogenizer.

PrepMan Ultra Sample Prep Method

The following method works for TaqMan GMO analysis of soy or maize samples.

To use PrepMan Ultra for sample preparation:

Step	Action
1	Weigh 20 mg of each sample and standard into a screw-cap microcentrifuge tube.
2	Add 400 µL of PrepMan Ultra to each 20 mg sample. Vortex to suspend the food sample completely. **⚠ WARNING CHEMICAL HAZARD**. **PrepMan Ultra** contains a material that may cause eye, skin, and respiratory tract irritation, and adverse effects on the kidneys and blood and central nervous system. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3	Boil the samples in a water bath for 10 min.
4	Centrifuge the samples for 2 min at high speed in a microcentrifuge.
5	Remove the cleared supernatant from each preparation and place it in a fresh tube. **Note** Samples may have some slight color, and there may be slight turbidity which forms immediately or with storage. This is normal, and no additional steps need to be taken.
6	Store samples at –20 °C indefinitely.

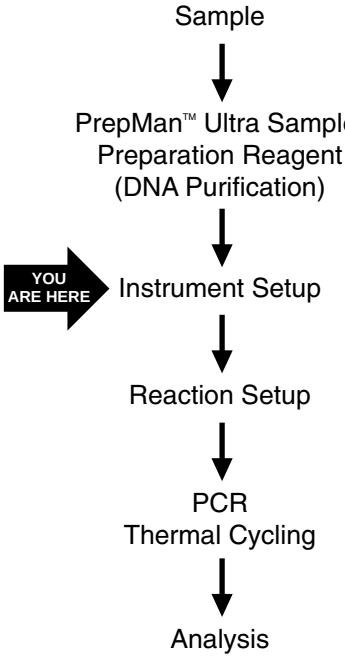
Setup on the ABI PRISM 7700 SDS

3

Overview

Where You Are in the Procedure

This section describes how to configure the ABI PRISM® 7700 Sequence Detection System (SDS).



Stationery File

Definition

A stationery file is a user-defined template that is generated within the SDS software. The template makes it easier to set up the ABI PRISM® 7700 Sequence Detection System for experiments that are repeated. The stationery file contains the thermal cycling conditions.

For additional information about stationery files refer to the *ABI PRISM 7700 Sequence Detection System User Guide* (P/N 904989).

First-Time Users Generate Stationery Files

It is recommended that first-time users generate a stationery file before beginning the procedure. For information on how to generate the stationery file refer to Appendix B, “The Thermal-Cycling Stationery File.”

Configuring the SDS Software

Introduction

This procedure describes how to set up the plate configuration in the SDS software. The procedure consists of two parts:

- Opening a stationery file
- Setting up the plate configuration

Order of the Procedure

Because setting up the software is a potentially time-consuming step, especially for the first-time user, it should be completed first. By setting up the software before pipeting your reagents, your samples are not left at room temperature for an extended period of time.

Opening the Thermal Cycling Stationery File

To open the thermal cycling stationery file:

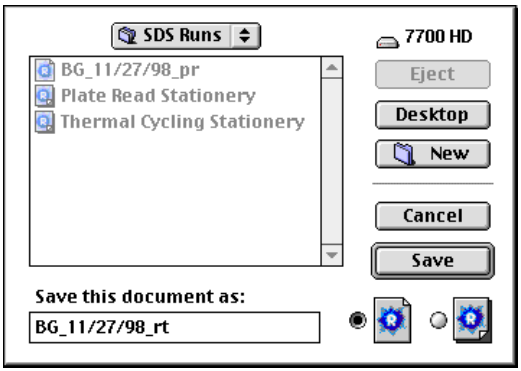
Step	Action
1	Open the **Thermal Cycling Thermal** file.
2	If you have created a pre-configured stationery file for TaqMan GMO analysis, launch the SDS software.
3	Open the **Template** file. **Note** If you do not have a stationery file, follow the steps in Appendix B to create one. Then, follow the remaining steps in this section.

Defining Sample Types, Names, and Replicates

To define sample types, names, and replicates:

Step	Action
1	a. In the Plate Setup window, select **FAM** from the Dye Layer pull-down menu. b. Using the mouse, click and drag to select all wells to be used for all controls, and assay samples. c. Designate all reaction wells as **UNKN** from the Sample Type pull-down menu.
2	a. Select **VIC** on the Dye Layer pull-down menu. b. Using the mouse, select all the reaction wells of every type. c. Designate all reaction wells as **IPC+** from the Sample Type pull-down menu.
3	a. Once sample types have been defined, reactions can be labeled in the Sample Name field. b. Use the mouse to click and highlight any reaction well. c. Click in the Sample Name field, and type in the sample name. d. Repeat for all samples.
4	**Note** If replicate reactions are to be run (*i.e.*, more than one reaction per sample), they should be designated using the Replicate field in the Plate Setup window. This should be done even when only one reaction per sample is being run. a. Using the mouse, select all reactions of the same replicate set together at the same time. b. With replicate wells highlighted, click the mouse in the Replicate field and give the replicate set a name by typing, *e.g.* `1.0% GMO Standard`, in the Replicate field. c. Repeat for all replicate groups. **Note** This will ensure that the results from replicate reactions will be grouped together for post-PCR data analysis.

To define sample types, names, and replicates: *(continued)*

Step	Action
5	a. Once all sample types, sample names and replicates have been defined, select **Save** from the File menu. The file will contain all data from the run.  b. Give the file a name and designate a storage location on the computer. c. Click **Save**.

Labeling the Positive Control Wells

The positive control wells are labeled UNKN. For clarity, use the Sample Name field to label the positive control. See step 3 in the table "Defining Sample Types, Names, and Replicates" on page 3-4 for how to do this.

Adding a Comment for the Experiment Report

Comments can be typed in the comment field of the plate setup window. This is a convenient place to provide details about the experiment such as:

- The date of the experiment
- The type of assay

Any information that is typed in the comment field of the plate setup window will appear on the Experiment Report. The Experiment Report is a summary of the results from the experiment.

Step	Action
1	a. Click in the Comment field of the plate setup window. b. Type a description for the experiment that you would like to appear on the Experiment Report.
2	Choose **Save** from the File menu.

Once this is saved, the software setup is completed and the instrument is ready to run the assay.

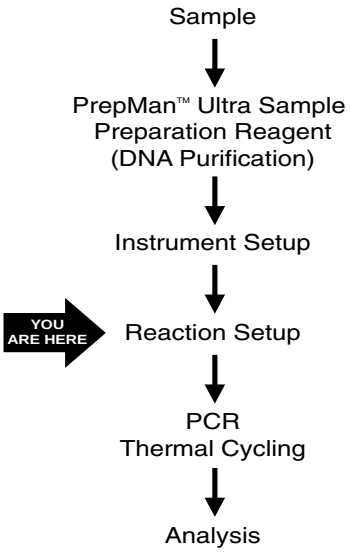
Preparing the Reactions

4

Overview

Where You Are in the Procedure

This section describes how to set up control and sample reactions.



Required Reactions and Their Components

Introduction

For proper data analysis of each run it is recommended that you prepare:

- 1 to 2 negative control samples
- 1 to 2 positive control samples
- Your unknown samples (including GMO concentration reference standards and food samples)

TaqMan GMO Mix

The TaqMan® GMO Mix is an assay-specific component of each TaqMan GMO Detection Kit. It contains buffer, primers, TaqMan assay probes, and other reaction components.

GMO PCR Cocktail

The GMO PCR Cocktail is made by combining TaqMan GMO Mix and AmpliTaq® Gold DNA Polymerase.

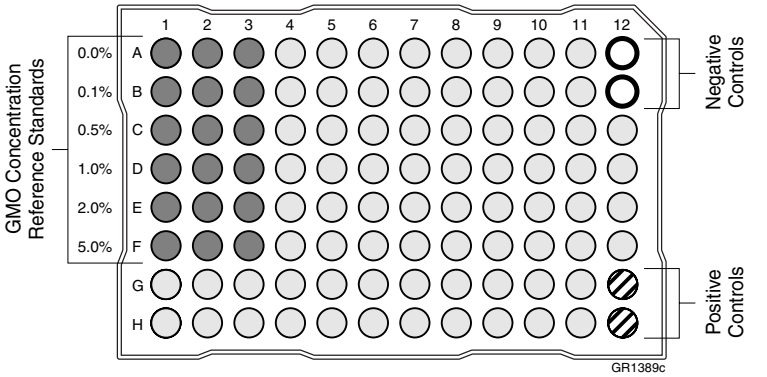
Preparing the Reactions

Preparing Control and Sample Reactions

To prepare the reactions:

Step	Action
1	a. Calculate the amount of GMO PCR Cocktail needed for all of the samples. Each sample requires 44 µL of TaqMan GMO Mix and 1 µL of AmpliTaq Gold DNA Polymerase. **Note** Refer to the table of commonly used reaction volumes on the TaqMan® GMO Detection Kit Quick Reference Card (P/N 4327689A). **⚠ CAUTION CHEMICAL HAZARD. AmpliTaq Gold DNA Polymerase** may cause eye and skin irritation. It may cause discomfort if swallowed or inhaled. Always use adequate ventilation such as that provided by a fume hood. Please read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Note** The GMO PCR Cocktail must be prepared at the time of use. Take the kit out of the freezer and allow it to thaw at room temperature for several minutes. Mix the tubes for 5 sec using a vortexer. Make the cocktail within 1 hr of use. b. Label a sterile tube with the name of the TaqMan GMO assay being performed.
2	In the labeled tube make the GMO PCR Cocktail. Vortex mixture for 10 sec. **Note** Use sterile pipette tips with aerosol barriers for all pipetting. **IMPORTANT** Because of the high glycerol content in the AmpliTaq Gold DNA Polymerase, ensure complete addition of the volume into the GMO PCR Cocktail. Rinse out the pipette tip by repetitive uptake and expulsion of the GMO PCR Cocktail.
3	Place a MicroAmp® Optical 96-Well Reaction Plate (P/N 403012) in a base (P/N N801-0531). Set up a MicroAmp Optical 96-Well Reaction Plate as shown below. **Note** There is a 96-well optical plate template for copying on page C-2. Do not write on the 96-well optical plate.

To prepare the reactions: *(continued)*

Step	Action
	
4	Pipet 45 µL of the prepared GMO PCR Cocktail in all of the wells to be used.
5	Pipet 5 µL of your sample DNA preparations into each of the sample wells. **IMPORTANT** Change pipet tips between samples.
6	After adding sample DNA to one vertical row of wells, cover the wells with optical caps. Use the MicroAmp® Cap Installing Tool (P/N N801-0438). Repeat this until all of the sample wells are capped. **Note** For a detailed description of how to use the Cap Installing Tool, refer to the *ABI PRISM 7700 Sequence Detection System User's Manual* (P/N 904989).
7	Pipet 5 µL of the Negative Control into each of the two negative control wells and cap the wells with optical caps.
8	Pipet 5 µL of the Positive Control into each of the two positive control wells and cap the wells with optical caps.
9	**Note** Though not strictly required, it is recommended to briefly centrifuge the loaded and capped 96-well reaction plate in a swinging bucket centrifuge designed specifically for such plates. This ensures that all GMO PCR Cocktail and sample volumes loaded in reaction wells are mixed together in the bottom of the wells, with no splashes or bubbles on the sides or caps. a. Spin the 96-well reaction plate at 1000 rpm for 20 sec. b. Spin the 96-well reaction plate prior to loading the 96-well reaction plate in the ABI PRISM® 7700 Sequence Detection System.

Loading the 96-Well Plate into the Instrument

Loading the Plate

To load the 96-well reaction plate on to the 7700 Sequence Detector:

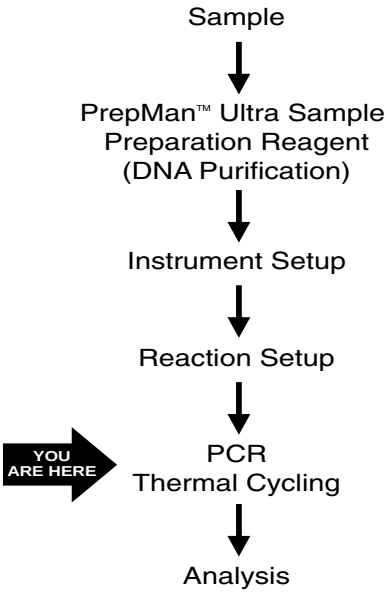
Step	Action
1	Loosen the knob on the sample block cover of the 7700 Sequence Detector and slide the cover back.
2	Place the 96-well reaction plate on the sequence detector sample block. Be sure to orient the plate such that: ♦ The notched corner is placed in the right-back corner of the sample block. ♦ Well A1 is located in the left-back corner of the sample block. **Note** For a detailed description of how to place the 96-well sample tray on the sequence detector, refer to the *ABI PRISM 7700 Sequence Detection System User's Manual*.
3	Slide the sample block cover back over the sample block.
4	Securely tighten the knob on the sample block cover. **Note** Do not over-tighten the knob.

5 Thermal Cycling

Overview

Where You Are in the Procedure

This section describes how to begin thermal cycling.



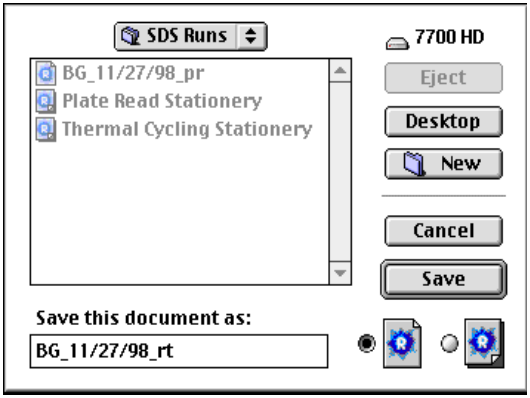
PCR Amplification

Timing

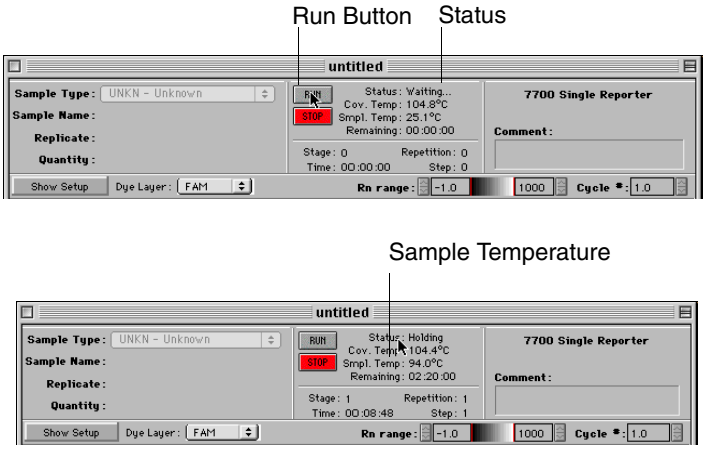
Thermal cycling takes approximately 2.5 hours to complete.

How to Begin Thermal Cycling

To begin thermal cycling:

Step	Action
1	Start the ABI PRISM® 7700 Sequence Detection System.
2	Open the **Thermal Cycling Stationery File** that you created in Appendix B, "The Thermal-Cycling Stationery File." The Plate setup window appears.
3	Define the sample wells, sample names and replicate groups for this particular run.
4	Save the file by clicking **Save**, and give it a name and storage location on the computer. 
5	Click **Show Analysis** on the Plate setup window. The Analysis plate window appears.

To begin thermal cycling: *(continued)*

Step	Action
6	Click the **Run** button on the Analysis plate window. **Note** The run will not start until the temperature of the lid has reached 104 °C. ♦ The Status will change from No Response to Waiting. ♦ After 25 sec you will hear a beep. ♦ The Smpl. Temp (sample temperature) will begin increasing. 
7	Thermal cycling is complete when the Remaining (time remaining) is 00:00:00.

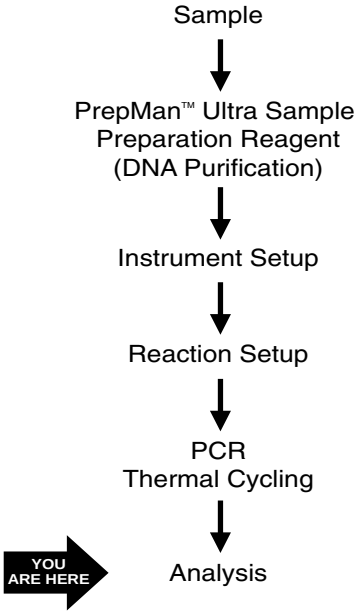
Data Analysis and Interpretation

6

Overview

Where You Are in the Procedure

This section describes how to analyze and interpret the data.



Analyzing the Data

Overview

After running the samples on the instrument, the data is analyzed using the ABI PRISM® 7700 Sequence Detection System software to produce C_T values of each reporter dye for each sample run. These values are then used to quantify the amount of GMO material in each sample.

The amount of GMO material (FAM™ dye) is normalized to the amount of plant material (VIC™ dye) detected in each sample. This produces a ΔC_T value which is averaged for replicate samples. These values are compared to a calibration curve produced from the ΔC_T values of the known GMO concentration standards. This enables a %GMO result for each unknown sample to be produced.

Use of Reference Standards

To do the quantitative GMO analysis correctly, you should prepare DNA extracts of a set of certified GMO concentration standards using the same method you used for the food samples to be tested. The certified concentration standards are prepared by the Institute for Reference Materials and Methods (IRMM), a subsidiary of the European Commission, and sold through vendors such as Fluka Chemical Company (now part of Sigma Chemical Company), or Resource Technology Corporation (Laramie, WY).

The standard set consists of four to six individual standards, containing a range of %GMO concentrations, usually from 0% to 5%, and are specific to the plant being tested for: soy or maize. TaqMan assays should be run for the set of concentration standards on each assay plate, using the standards specific to the food being tested (genetically modified maize or genetically modified soy). If both soy and maize assays are run together on the same plate, then both soy and maize concentration standards must be run.

Plate Setup Window

It is important to properly name analysis samples and standards in the SDS software Plate Setup window. Each sample should be given a unique name in the “Replicate” field in the Plate Setup window; replicate samples should be given exactly the same name (even if the sample is unique, it should be given a “Replicate” name).

Note If replicate names were not assigned initially, they can be named even after thermal cycling by following the steps in Chapter 3, “Setup on the ABI PRISM 7700 SDS.” This will aid subsequent data analysis.

Analyzing the Run

To analyze the run:

Step	Action
1	After thermal cycling, select **Save** from the File menu. Analyze the run by selecting **Analyze** from the Analysis menu. **IMPORTANT** If you are you are using SDS software v1.7, the following settings are recommended: a. Select the **Analysis** menu under Options. In the dialog box, uncheck the box **Automatically compute PCR stage**. Select the radio button **PCR Stage** and enter 2 for **PCR Stage** and 3 for **Extension Step**. b. Select the **Analysis** menu under Options. Uncheck **Show Threshold Dialog After Analyze**.
2	Export a text file of the real-time data by going to the File menu, and selecting **Export, Results.** Click **Save.** **Note** It helps to select in the Analysis Plate window only the wells that contain reactions by click-dragging with the mouse. In the Export results file dialog window, select the radio button labeled **Export Selected Wells** to eliminate empty wells from the export file.

Using Microsoft Excel

To use Microsoft Excel:

Step	Action
1	Open the exported results file in Microsoft® Excel. A table containing two blocks of data corresponding to the FAM dye layer and VIC dye layer appears.
2	Select all of the cells in the FAM dye data block, including the column headers. The selected cells are highlighted.
3	Select **Sort** from the Data menu.
4	a. Enter **`Replicate`** as the primary sort parameter. b. Enter **`Well`** as the secondary sort parameter. c. Click **Sort** or press the Return key. The FAM dye data is sorted.
5	Repeat steps 4a through 4c for the block of VIC dye data. The FAM dye and VIC dye blocks are sorted in exactly the same order, so that a given sample is in the same relative position within each block of data.
6	a. To the right of the right-most column in the table, add a new column header called "ΔC_T." b. Starting with the first row in the FAM dye table, click under the ΔC_T column heading and type `=`.
7	a. To the left in the table in the same row, find the corresponding FAM dye C_T for the sample, and **Click**. b. Enter a minus (`-`) sign.
8	a. Navigate to the block of VIC dye layer data, identify the same sample (it should be in the same sorted order as the FAM dye layer data). b. Select the sample's VIC dye C_T well.

To use Microsoft Excel: *(continued)*

Step	Action
9	Press Enter. **Note** You are simply preparing to subtract the VIC dye C_T from the FAM dye C_T for this sample. This is called ΔC_T. a. Once this has been done for the first sample, assuming that all other samples are in the same order between the FAM dye and VIC dye data blocks, you can simply click-drag from the first ΔC_T calculation well, down to the end of the FAM dye layer data block in the ΔC_T column to automatically calculate ΔC_T values for the remaining samples. **Note** Samples which have a FAM dye layer C_T = 40 do not require that their ΔC_T be calculated. Since there was no amplification of the GMO target. These samples are GMO negative. No further analysis is required for these samples. **Note** Likewise, samples which have a VIC dye C_T = 40 cannot be further analyzed since they contained none of the plant DNA being tested for.
10	a. If there are replicate reactions of samples, the initial ΔC_T values should be calculated individually for each sample, then the resulting ΔC_T values should be averaged for each group of replicates. b. If average ΔC_T values are calculated, add these in a column next to the ΔC_T values. c. Use the AVERAGE function in Excel to calculate the ΔC_T means for each group of replicates.

To use Microsoft Excel: *(continued)*

Step	Action
11	If replicate reactions were run, calculate the ΔC_T standard deviation for the replicates. In a new column labeled ΔC_T StDev, calculate individual standard deviations using the “STDEV” function in Excel. The new ΔC_T, ΔC_T Mean, and ΔC_T St Dev (Standard Deviation) columns appear with calculated data. **Note** This example has two replicate reactions per sample.
12	a. Locate the concentration standards in the data table. b. Transcribe the concentration standards’ basic data (%GMO and average ΔC_T) into a new table on the same worksheet by selecting those cells and pasting them to a new section of the spreadsheet.

K	L	M	N
IPC	ΔCt	ΔCt Mean	ΔCt St Dev
No Amp			
-			
+	9.81		
+	10.59	10.20	0.55
+	8.11		
+	7.87	7.99	0.17
+	7.16		
+	7.21	7.19	0.04
+	6.17		
+	5.89	6.03	0.20

To use Microsoft Excel: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>13</td><td>
a. Paste the %GMO and ΔC_T data into the spreadsheet leaving one blank column between them.
b. Label the blank column Log (%GMO) as shown below.
c. Calculate the logarithm of each concentration standard's known %GMO. (Omit the 0.00 %GMO standard from this and subsequent steps since its logarithm is undefined.)

To calculate the Log (%GMO) values:
a. Click to select the cell in the Log (%GMO) column immediately to the right of the corresponding %GMO cell.
b. Enter the formula = LOG ( ).
c. Place the cell number of the %GMO value to be converted between the parentheses.
d. Press ENTER. The logarithm of the %GMO value will appear in the cell.

The transcribed GMO standards table should appear similar to the following data:

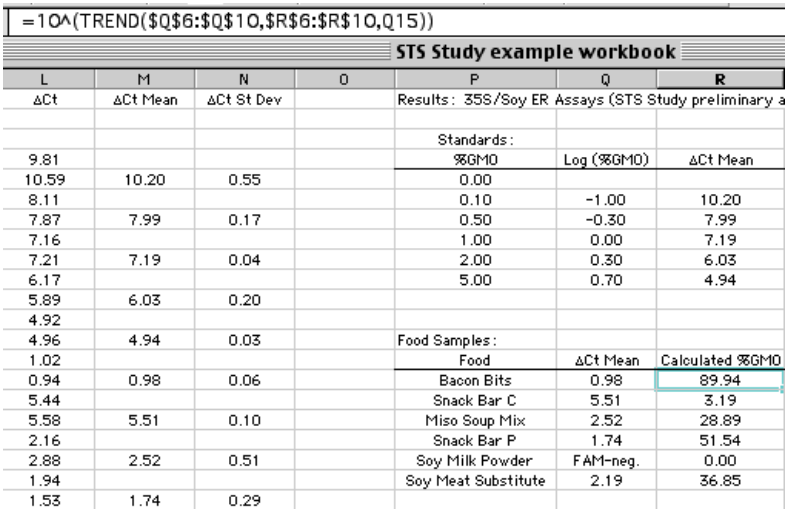
<table>
<tr><td>Standards:</td><td></td><td></td></tr>
<tr><td>%GMO</td><td>Log (%GMO)</td><td>ΔCt Mean</td></tr>
<tr><td>0.00</td><td></td><td></td></tr>
<tr><td>0.10</td><td>-1.00</td><td>10.20</td></tr>
<tr><td>0.50</td><td>-0.30</td><td>7.99</td></tr>
<tr><td>1.00</td><td>0.00</td><td>7.19</td></tr>
<tr><td>2.00</td><td>0.30</td><td>6.03</td></tr>
<tr><td>5.00</td><td>0.70</td><td>4.94</td></tr>
</table>
</td></tr>
<tr><td>14</td><td>Make another table on the same worksheet to transcribe the sample names and ΔC_T values (or average ΔC_T values) for the food analysis samples by clicking and dragging on these values, then pasting these values into the spreadsheet.</td></tr>
</table>

Calculating %GMO

For each food sample, %GMO is calculated by using an Excel function to analyze the sample's ΔC_T, comparing it to the set of log (%GMO) and ΔC_T values for the concentration standards set. The Excel function is called TREND and is used in the following formula:

%GMO(food sample) = 10^(TREND(Standards Log%GMO values cells, Standards ΔC_T values cells, food sample ΔC_T value)).

The calculated food sample analysis table (with the %GMO calculation shown as entered in the upper window) should appear similar to the following example:



=10^(TREND($Q$6:$Q$10,$R$6:$R$10,Q15))

STS Study example workbook

L	M	N	O	P	Q	R
ΔCt	ΔCt Mean	ΔCt St Dev		Results: 35S/Soy ER Assays (STS Study preliminary a		
				Standards:		
9.81				%GMO	Log (%GMO)	ΔCt Mean
10.59	10.20	0.55		0.00		
8.11				0.10	-1.00	10.20
7.87	7.99	0.17		0.50	-0.30	7.99
7.16				1.00	0.00	7.19
7.21	7.19	0.04		2.00	0.30	6.03
6.17				5.00	0.70	4.94
5.89	6.03	0.20				
4.92						
4.96	4.94	0.03		Food Samples:		
1.02				Food	ΔCt Mean	Calculated %GMO
0.94	0.98	0.06		Bacon Bits	0.98	89.94
5.44				Snack Bar C	5.51	3.19
5.58	5.51	0.10		Miso Soup Mix	2.52	28.89
2.16				Snack Bar P	1.74	51.54
2.88	2.52	0.51		Soy Milk Powder	FAM-neg.	0.00
1.94				Soy Meat Substitute	2.19	36.85
1.53	1.74	0.29				

7 Troubleshooting

Overview

Introduction

This section contains information about troubleshooting for the TaqMan® GMO Detection Assay.

For a detailed description of issues related to the computer and software, refer to the *ABI PRISM 7700 Sequence Detection System User' Manual (*P/N 904989).

Troubleshooting Table

Troubleshooting

Observation	Possible Cause	Recommended Action
Cannot find VIC™ dye in the Plate Setup window Dye Layer pull-down menu.	An older dye set was installed on the ABI PRISM® 7700 Sequence Detection System that does not contain VIC dye, or the dye set was improperly installed.	Install a current dye set on the 7700 SDS that contains VIC dye along with the other reporter dyes.
Initial post-PCR data analysis indicates that reactions amplified, but ΔR_n data is far outside of the usual range.	Incorrect settings were used in the SDS software.	If the SDS software settings are incorrect, then correct them and re-analyze the data. ♦ Check that Sample Type Setup (Plate Setup window) shows the correct assignment of reporter dyes, quencher and passive reference. ♦ Check in the Plate Setup window that the FAM dye and VIC dye layers, and their respective sample type designations, were correctly assigned. ♦ Check under Advanced Options (Select **Diagnostics** from the Instrument menu) that the option **Use Spectral Compensation for Real-Time** is checked.

Troubleshooting *(continued)*

Observation	Possible Cause	Recommended Action
Initial post-PCR data analysis shows no amplification, or amplification plots look grossly abnormal.	Inadequate seating of optical caps caused sample evaporation.	Redo the assay beginning with Reaction Setup. Use the MicroAmp® Cap Installing Tool (Applied Biosystems P/N N801-0438) to secure the optical caps.
	Did not use the proper consumables.	Restart the assay. Use only the optical grade 96-well plates and caps (Applied Biosystems P/N 403012).
	Samples were not properly prepared.	Remake the sample DNA preps. Ensure that the DNA extraction method is properly performed.
	GMO PCR Cocktail was not properly formulated.	Restart the assay. Ensure that the PCR Mix and AmpliTaq Gold® enzyme were combined in the appropriate amounts during assay setup.
	The sample block cover (heated lid) of the 7700 Sequence Detection System was not properly aligned and secured.	Restart the assay. Make sure that the cover is slid all the way towards the front, and that the knob is securely tightened.

Troubleshooting *(continued)*

Observation	Possible Cause	Recommended Action
After the completion of thermal cycling, the computer is unable to save the SDS real-time file, the file is accidentally discarded, or the application is quit without saving the file.	The computer has a shortage of disk space, or operator error.	The file can be recovered from the automatic backup Run file in the SDS Runs folder. a. If disk space is limited, first discard unneeded files from the disk to create more room. b. Relaunch the SDS software. c. Choose **New Plate** from the File menu (the New Plate dialog box appears). d. In the New Plate dialog box, choose **Real Time** in the Run box. e. Choose **Import** from the File menu and **Import LabView Format Raw Data** from the sub-menu. f. Locate and open the SDS Runs folder on the computer hard-drive. g. Open the real-time thermal cycling file corresponding to the lost run data (it will be designated only by the date and time of the run, not its original file name). h. Click **Open**. The data is imported to the new plate. i. Assign the correct sample types to each of the wells to correspond to the original plate setup. j. Save the file by choosing **Save** from the File menu. k. Continue with data analysis.

Troubleshooting *(continued)*

Observation	Possible Cause	Recommended Action
Upon returning to the 7700 Sequence Detection System after beginning a thermal cycling run, the computer is off, but the thermal cycler is on.	Power outage during or after thermal cycling.	Follow steps b through k listed on page 7-4 to determine if the thermal cycling was completed. If the thermal cycling was complete, proceed with data analysis after recovering the Run file (see previous directions on recovering a Run file). If thermal cycling was interrupted before finishing, the assay will need to be repeated entirely.
Positive Control reactions failed to amplify, but other reactions appear OK.	Positive Control DNA was not added to the reaction wells.	If other reactions look normal, there may be no need to repeat the run.
Negative Control reactions are positive on both the FAM dye and VIC dye layers.	Contamination of the Negative Control solution or the GMO PCR Cocktail with GMO-positive DNA.	Use more care to prevent contamination while handling kit reagents and setting up assays.

Troubleshooting *(continued)*

Observation	Possible Cause	Recommended Action
After initial post-PCR data analysis, all samples appear negative. The Amplification Plot window appears to show only one thermal cycle (instead of the usual 40) on the horizontal axis, and the amplification plot is blank.	Incorrect settings in the SDS software.	Select **Options** from the Analysis menu in the Analysis window. Check that the **Automatically Compute PCR Stage** box is checked. Re-analyze data with the correct settings.
Amplification plots for very high-concentration samples show a steep drop in ΔR_n after initial amplification.	The sample C_T was within the cycle range for Baseline calculation.	In the Amplification Plot window, change the default Baseline Stop box value from 15 to a lower cycle number such as 8 (the Start box value can be left at 3). After re-analyzing the data, the amplification plot(s) should appear normal.

5´ Nuclease Assay

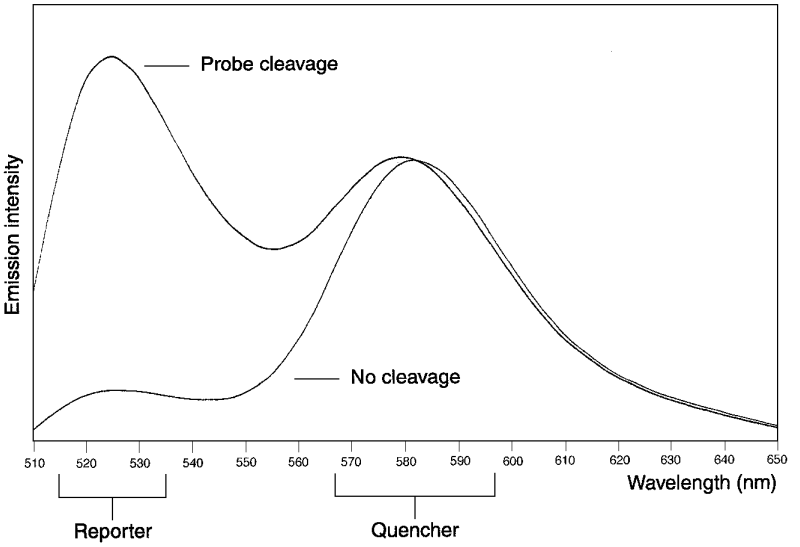
A

Overview

TaqMan Probe

The TaqMan® probe consists of an oligonucleotide with a 5´-reporter dye and a 3´-quencher dye. The reporter dye, FAM™ or VIC™ is covalently linked to the 5´ end of the oligonucleotide. The fluorescence of the reporter is quenched by TAMRA™, located at the 3´ end.

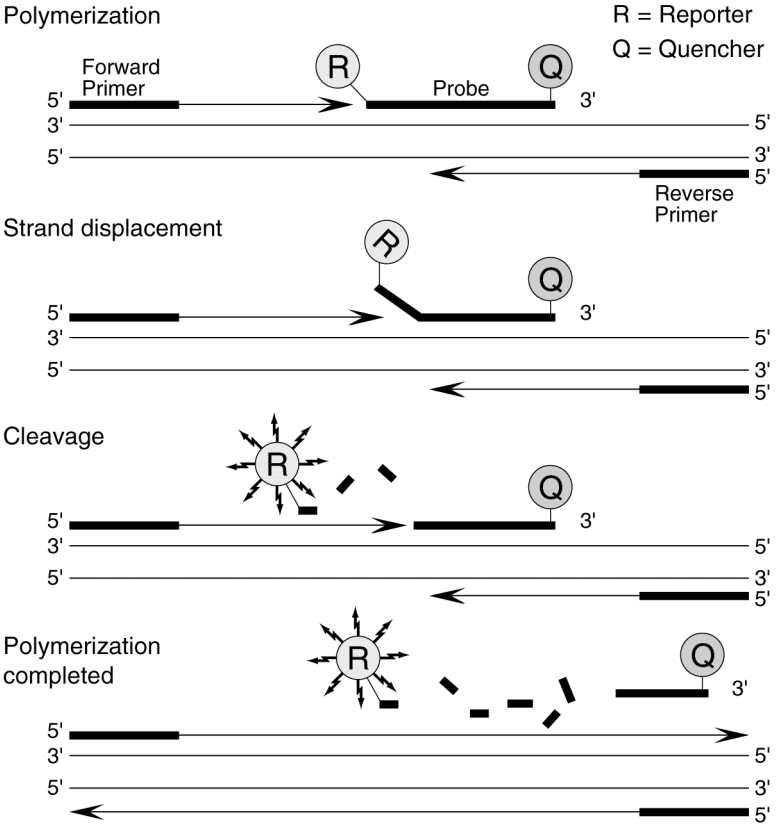
When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. If the probe is cleaved, the reporter and quencher dyes are separated, causing the reporter dye fluorescence to increase (see figure below).



TaqMan Chemistry

The TaqMan method exploits the 5´–3´ nuclease activity of AmpliTaq Gold® DNA Polymerase to allow direct detection of the PCR product by the release of the fluorescent reporter during PCR amplification.

In PCR, forward and reverse primers hybridize to a specific sequence of the target DNA. The target sequence is then amplified. The TaqMan probe hybridizes to a target sequence within the PCR product. When the PCR product is amplified in subsequent cycles, AmpliTaq Gold DNA Polymerase cleaves the TaqMan probe (5´–3´ nuclease activity) so that the enzyme can continue to copy its target sequence. The reporter dye and quencher dye are separated, resulting in increased fluorescence of the reporter (see figure below).



The Thermal-Cycling Stationery File

B

Overview

When to Generate the Stationery File

The first time that you run a TaqMan® GMO Detection assay it is recommended that you create a thermal-cycling stationery file to facilitate software setup for future runs.

Overview of Thermal Cycling Conditions

The TaqMan GMO Detection Kits are amplified using the following PCR thermal cycling conditions:

Thermal Cycler	Times and Temperatures			
	Initial Step	Each of 40 Cycles		
		Melt	Anneal	Extend
ABI PRISM® 7700 Sequence Detector	HOLD	CYCLE		
	9 min 94 °C	20 sec 95 °C	1 min 60 °C	30 sec 72 °C

Steps for Generating a Thermal-Cycling Stationery File

To generate a thermal-cycling stationary file:

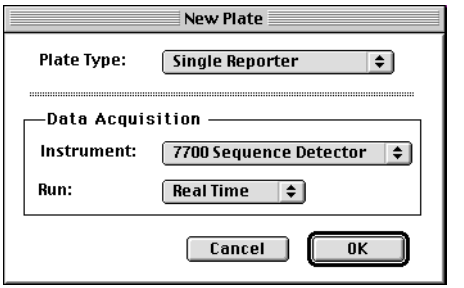
Step	See Page
New Blank Plate Setup	B-2
Programming and Saving the Thermal Cycling Stationery File	B-8

New Blank Plate Setup

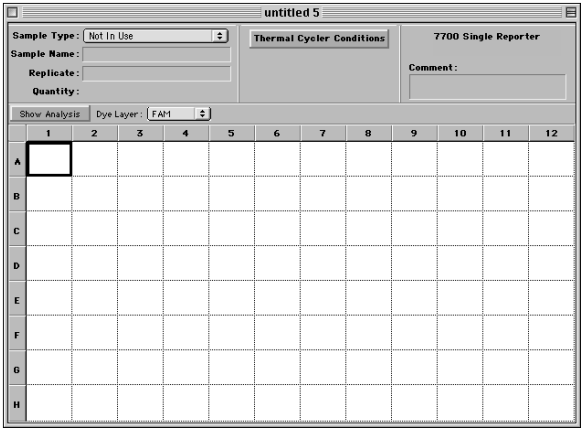
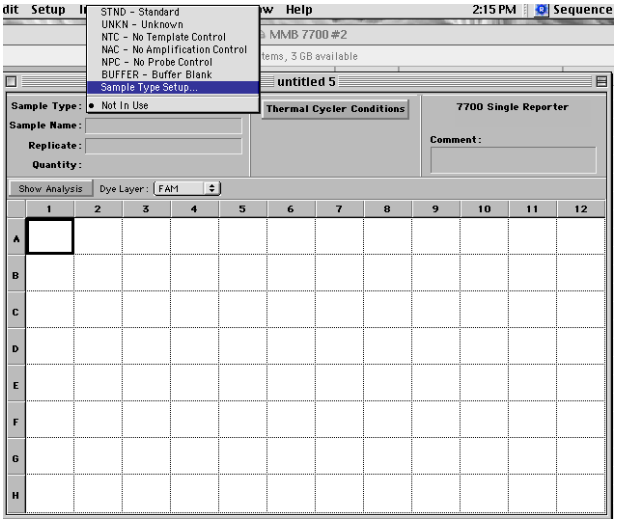
Opening a New Blank Plate Setup Window

A new plate setup window will be opened, configured for TaqMan GMO analysis, then saved as a stationery file.

To open a new plate setup window:

Step	Action
1	Select **New Plate** from the File menu. The New Plate dialog box appears. 
2	In the New Plate dialog box: ♦ Select the **Single Reporter** from the Plate Type pop-up menu. ♦ Select **7700 Sequence Detector** from the Instrument pop-up menu. **IMPORTANT** Select **Real Time** from the Run pop-up menu.

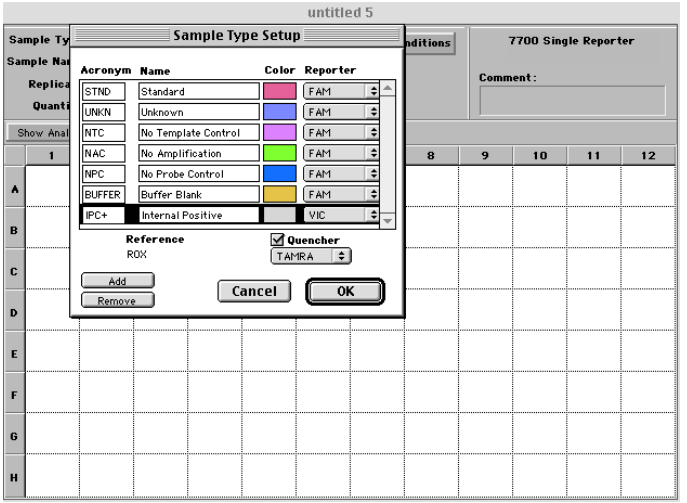
To open a new plate setup window: *(continued)*

Step	Action
3	Click **OK**. A Plate Setup window appears. 
4	Select **Sample Type Setup** from the Sample Type menu. The Sample Type Setup dialog box appears. 

Configuring Sample Types, Reporter Dyes, and Quenchers

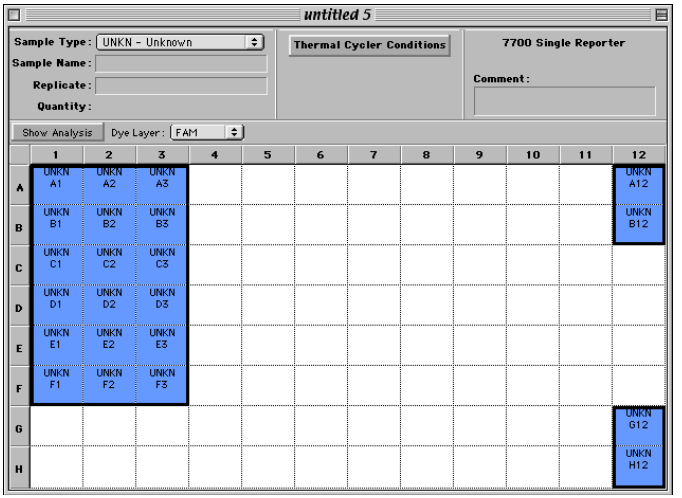
To configure the IPC sample type that is associated with the VIC™ dye, follow these instructions:

To configure the IPC sample:

Step	Action
1	Open the **Sample Type Setup** dialog box: a. Click the row labeled **IPC+**. b. Verify that the box marked **Quencher** is checked. c. Verify that **TAMRA** is chosen as the **Quencher** from the pull-down menu. d. Verify that **ROX** is listed as the reference dye. e. Click **OK**. The VIC dye is selected as the reporter for the IPC+ sample type. 

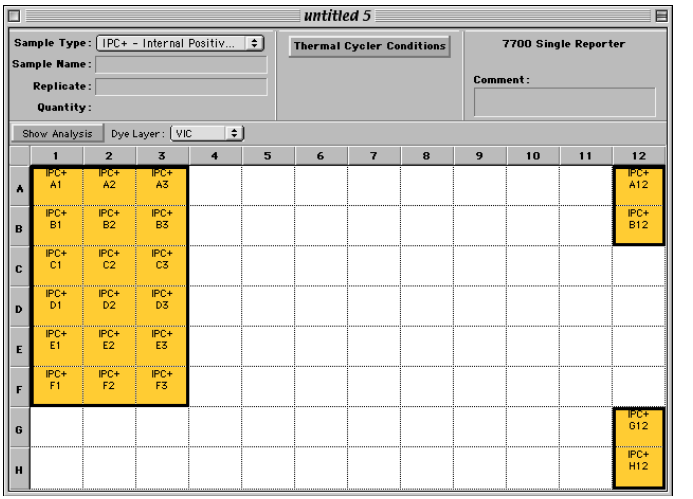
Selecting the FAM Dye Layer

To select the FAM dye layer:

Step	Action
1	a. Select the wells containing the samples by clicking and dragging the mouse over the wells. The selected wells are highlighted. **Note** All samples, concentration standards and control reactions are designated with the **UNKN** sample type on the FAM dye layer. In this example, the template file is configured with predefined reactions for concentration standards and controls. b. Select **UNKN** from the Sample Type pull-down menu. The new UNKN sample type designation will appear in the wells. 

Selecting the VIC Dye Layer

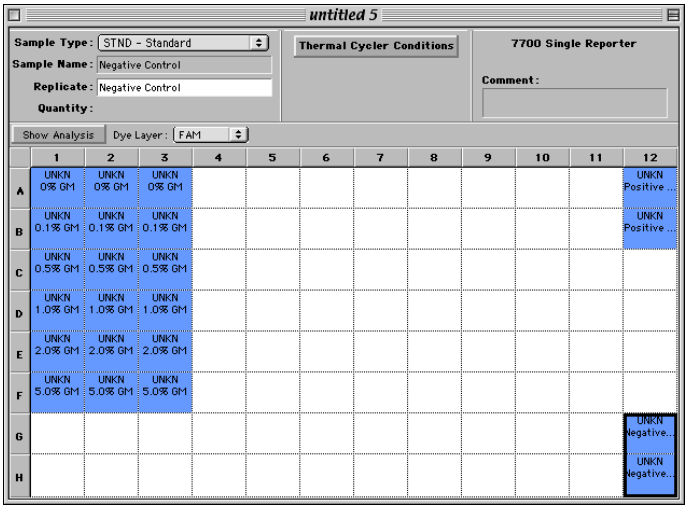
To select the VIC dye layer:

Step	Action
1	a. Choose **VIC** from the Dye Layer pull-down menu. The sample type designations associated with the VIC dye layer will appear in the plate window. b. Select the wells that were previously defined as UNKN in the FAM layer. The selected wells will be highlighted. c. Define all the VIC layer samples by selecting the **IPC+** sample type from the from the Sample Type menu. 

Applying Replicate Sample Names

Reactions may be given Sample Names and Replicate designations by clicking the reaction wells, then typing in the Sample Name and Replicate fields on the Plate Setup window. In the example below, three replicate reactions of GMO concentration standards, and two replicate reactions each of Positive and Negative Controls are defined. As long as this arrangement is always used in future runs, it saves the effort of defining and labeling wells.

To apply replicate sample names:

Step	Action
1	a. Click on the reaction wells. The selected wells will be highlighted. **Note** Multiple wells may be grouped and given the same sample name and replicate name for statistical analysis. b. Click to set the cursor in the **Sample Name** field. c. Enter the sample name. d. Click to set the cursor in the **Replicate** field. e. Enter the replicate name. 

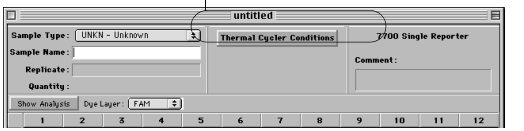
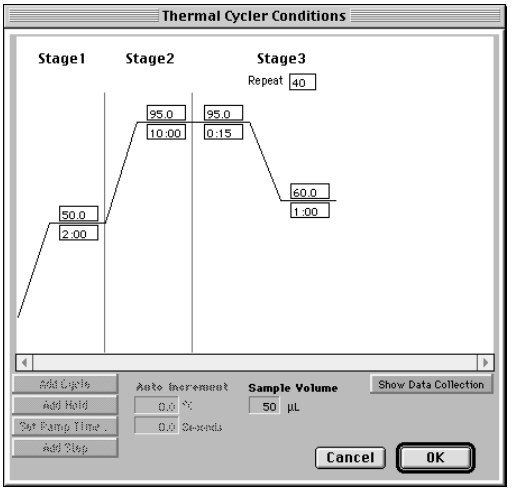
Programming and Saving the Thermal Cycling Stationery File

Introduction

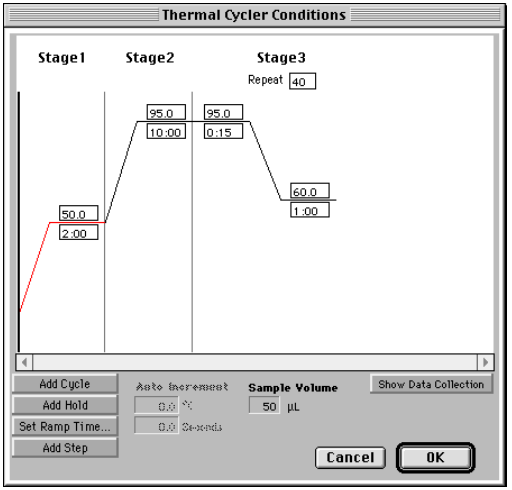
By generating and saving a Thermal-Cycling Stationery file, the thermal cycling conditions will only have to be configured once.

How to Program the Thermal Cycling Conditions

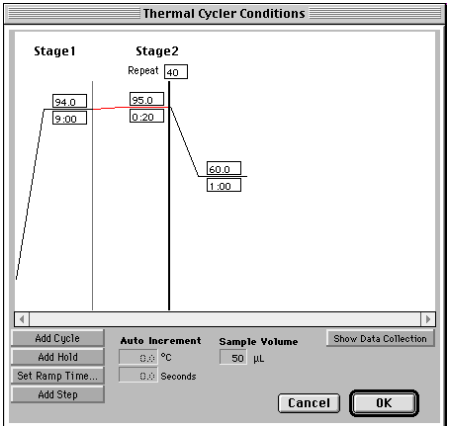
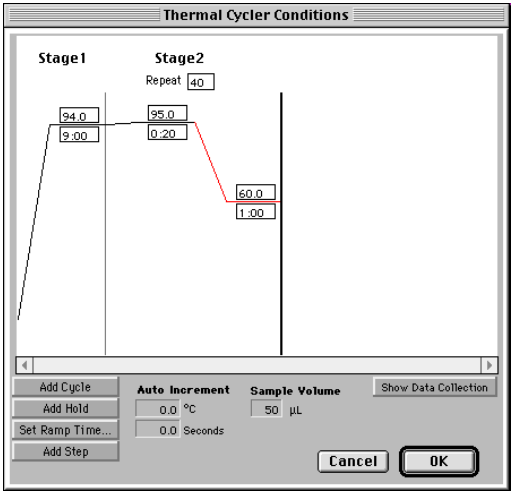
To set the thermal cycling conditions:

Step	Action
1	Click the **Thermal Cycler Conditions** button on the Plate Setup window. Thermal Cycler Conditions Button  The Thermal Cycler Conditions dialog box appears. 

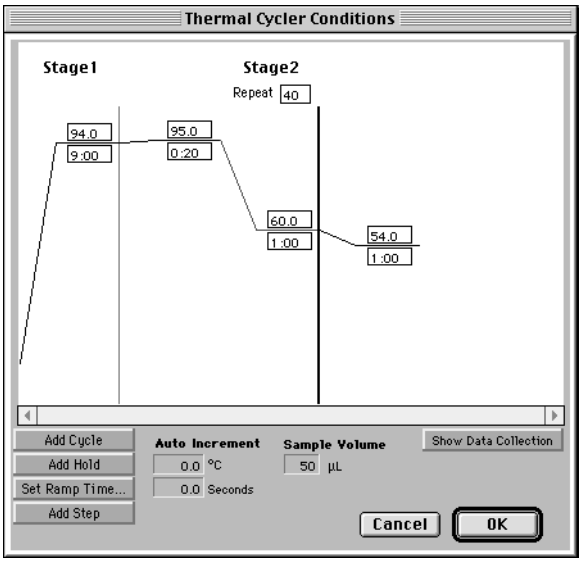
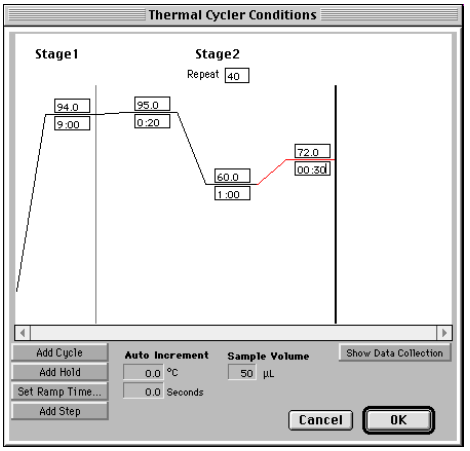
To set the thermal cycling conditions: *(continued)*

Step	Action
2	Select the **Stage 1** box by clicking in the Stage 1 portion of the Thermal Cycler Conditions dialog box. A black line (insert bar) appears in the left-hand margin of the Stage 1 box and the sloped line is changed from black to red. 
3	Drag the cursor from the insert bar to the right across the Stage 1 box. The Stage 1 box is highlighted.
4	Select **Clear** from the Edit menu. The Stage 1 box is deleted. The box that was Stage 2, now becomes Stage 1.
5	In the Stage 1 box, click in the temperature field containing **95.0** and drag the cursor across the number to select it. Change the number to **94.0**.
6	In the Stage 1 box, select the time field containing **10:00** and change it to **9:00**.

To set the thermal cycling conditions: *(continued)*

Step	Action
7	In the box labeled Stage 2 select the time field containing 0:15 and change it to **0:20**. 
8	Click at the end of the line located between the temperature field containing 60.0 and the time field containing 1:00. The insert bar appears to the right of the 60.0 and 1:00 temperature and time fields. 

To set the thermal cycling conditions: *(continued)*

Step	Action
9	Click the **Add Step** button. Additional temperature (54.0) and time (1:00) fields are added to the thermal cycling program. 
10	Select the temperature field containing **54.0** and change it to **72.0**.
11	Select the time field containing **1:00** and change it to **00:30**. 

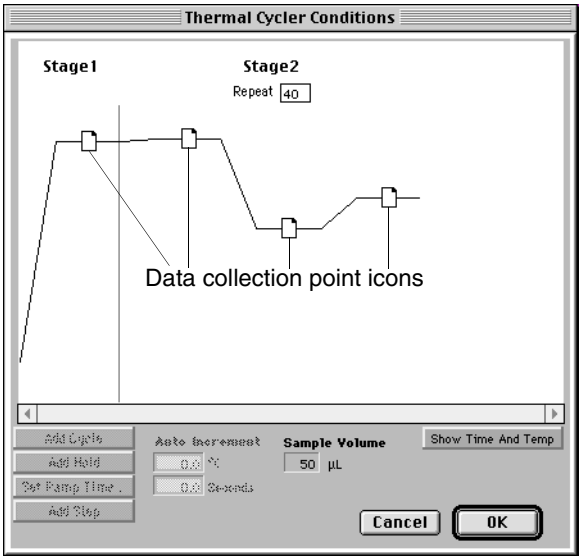
To set the thermal cycling conditions: *(continued)*

Step	Action
12	Check that the sample volume is **50 µL**.
13	Check that the number **40** is in the Repeat field, indicating 40 cycles. **Note** This completes the steps for defining the thermal cycling conditions.

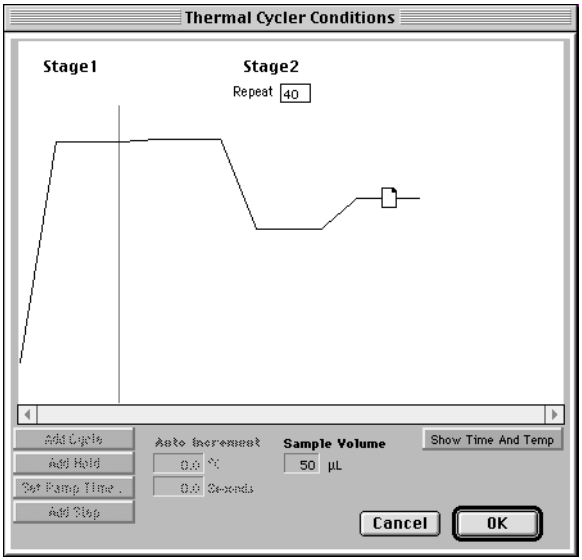
Setting Up for Data Collection

This section describes how to set up the data collection points during thermal cycling.

To set the thermal cycling conditions:

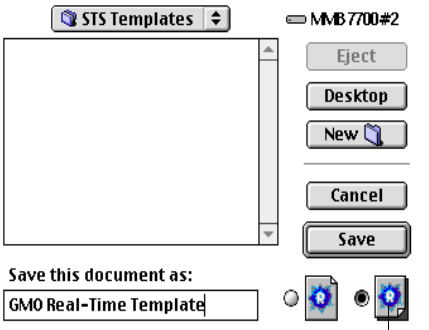
Step	Action
1	Click **Show Data Collection**. The following dialog box appears. 

To set the thermal cycling conditions: *(continued)*

Step	Action
2	Click on the first, second, and third data collection point icons. The first, second, and third data collection point icons disappear. 
3	Click **OK**.

Naming and Saving the Thermal-Cycling Stationery Template

To name the stationery template:

Step	Action
1	Choose **Save** from the File menu. The following dialog box appears: 
2	Type the file name.
3	Click the **Stationery Format** icon. The file is saved as a Stationery Template.
4	Save the Stationery file in the SDS Runs folder.

96-Well Optical Plate Template

C

Overview

How to Use This Template

The template for the MicroAmp® 96-well optical plate is available on the next page. Copies of this template can be used to write down the location of samples in the plate, without writing on the plate itself.

IMPORTANT Writing on the optical plate interferes with data analysis.

Template

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Technical Support

D

Contacting Technical Support

You can contact Applied Biosystems for technical support:

- By e-mail
- By telephone or fax
- Through the Applied Biosystems web site

You can order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents 24 hours a day. In addition, you can download documents in PDF format from the Applied Biosystems web site. (Please see the section "To Obtain Technical Documents" following the telephone information below.)

To Contact Technical Support by E-Mail

You can contact Applied Biosystems Technical Support by e-mail for help in the following product areas:

Product/Product Area	E-mail address
Genetic Analysis (DNA Sequencing)	galab@appliedbiosystems.com
Sequence Detection Systems (Real-Time PCR) and PCR	pcrlab@appliedbiosystems.com
Protein Sequencing, Peptide, and DNA Synthesis	corelab@appliedbiosystems.com

Product/Product Area	E-mail address
♦ Biochromatography (BioCAD®, SPRINT™, VISION™, and INTEGRAL® Workstations and POROS® Perfusion Chromatography Products) ♦ Expedite™ 8900 Nucleic Acid Synthesis Systems ♦ MassGenotyping Solution 1™ (MGS1) Systems ♦ PNA Custom and Synthesis ♦ Pioneer™ Peptide Synthesizers ♦ Proteomics Solution 1™ (PS1) Systems ♦ ICAT™ Reagent ♦ FMAT™ 8100 HTS Systems ♦ Mariner™ ESI-TOF Mass Spectrometry Workstations ♦ Voyager™ MALDI-TOF Biospectrometry Workstations ♦ CytoFluor® 4000 Fluorescence Plate Reader	tsupport@appliedbiosystems.com
LC/MS (Applied Biosystems/MDS Sciex)	support@sciex.com
Chemiluminescence (Tropix)	tropix@appliedbiosystems.com

To Contact Technical Support by Telephone or Fax (North America)

To contact Applied Biosystems Technical Support in North America, use the telephone or fax numbers in the table below.

Note To schedule a service call for other support needs, or in case of an emergency, dial **1.800.831.6844**, then press **1**.

Product/Product Area	Telephone	Fax
ABI PRISM® 3700 DNA Analyzer	**1**.**800**.**831**.**6844**, then press **8**[a]	1.650.638.5981
DNA Synthesis	**1**.**800**.**831**.**6844**, press **2**, then press **1**[a]	1.650.638.5981

Product/Product Area	Telephone	Fax
Fluorescent DNA Sequencing	**1**.**800**.**831**.**6844**, press **2**, then press **2**[a]	1.650.638.5981
Fluorescent Fragment Analysis (including GeneScan® applications)	**1**.**800**.**831**.**6844**, press **2**, then press **3**[a]	1.650.638.5981
Integrated Thermal Cyclers (ABI PRISM® 877 and Catalyst 800 instruments)	**1**.**800**.**831**.**6844**, press **2**, then press **4**[a]	1.650.638.5981
ABI PRISM® 3100 Genetic Analyzer	**1**.**800**.**831**.**6844**, press **2**, then press **6**[a]	1.650.638.5981
Peptide Synthesis (433 and 43x Systems)	**1**.**800**.**831**.**6844**, press **3**, then press **1**[a]	1.650.638.5981
Protein Sequencing (Procise® Protein Sequencing Systems)	**1**.**800**.**831**.**6844**, press **3**, then press **2**[a]	1.650.638.5981
Sequence Detection Systems (Real-Time PCR) and PCR	**1**.**800**.**762**.**4001**, then press: **1** for PCR[a] **2** for TaqMan® applications and Sequence Detection Systems including ABI Prism, 7700, 7900, and 5700[a] **6** for the 6700 Automated Sample Prep System[a] or **1**.**800**.**831**.**6844**, then press **5**[a]	1.240.453.4613

Product/Product Area	Telephone	Fax
♦ Mariner™ ESI-TOF Mass Spectrometry Workstations ♦ Voyager™ MALDI-TOF Biospectrometry Workstations ♦ MassGenotyping Solution 1™ (MGS1) Systems ♦ Proteomics Solution 1™ (PS1) Systems ♦ ICAT™ Reagent	**1.800.899.5858**, press **1**, then press **3**[b]	1.508.383.7855
Biochromatography (BioCAD®, SPRINT™, VISION™, and INTEGRAL® Workstations and POROS® Perfusion Chromatography Products)	**1.800.899.5858**, press **1**, then press **4**[b]	1.508.383.7855
Expedite™ 8900 Nucleic Acid Synthesis Systems	**1.800.899.5858**, press **1**, then press **5**[b]	1.508.383.7855
Pioneer™ Peptide Synthesizers	**1.800.899.5858**, press **1**, then press **5**[b]	1.508.383.7855
PNA Custom and Synthesis	**1.800.899.5858**, press **1**, then press **5**[b]	1.508.383.7855
♦ FMAT™ 8100 HTS Systems ♦ CytoFluor® 4000 Fluorescence Plate Reader	**1.800.899.5858**, press **1**, then press **6**[b]	1.508.383.7855
Chemiluminescence (Tropix)	**1.800.542.2369** (U.S. only), or **1.781.271.0045**[c]	1.781.275.8581
LC/MS (Applied Biosystems/MDS Sciex)	**1.800.952.4716**	1.508.383.7899

a. 5:30 AM to 5:00 PM Pacific time.

b. 8:00 AM to 6:00 PM Eastern time.

c. 9:00 AM to 5:00 PM Eastern time.

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Eastern Asia, China, Oceania		
Australia (Scoresby, Victoria)	**61 3 9730 8600**	**61 3 9730 8799**
China (Beijing)	**86 10 64106608** or **86 800 8100497**	**86 10 64106617**
Hong Kong	**852 2756 6928**	**852 2756 6968**
India (New Delhi)	**91 11 653 3743/3744**	**91 11 653 3138**
Korea (Seoul)	**82 2 593 6470/6471**	**82 2 593 6472**
Malaysia (Petaling Jaya)	**60 3 79588268**	**60 3 79549043**
Singapore	**65 896 2168**	**65 896 2147**
Taiwan (Taipei Hsien)	**886 2 2358 2838**	**886 2 2358 2839**
Thailand (Bangkok)	**66 2 719 6405**	**66 2 319 9788**
Europe		
Austria (Wien)	**43 (0)1 867 35 75 0**	**43 (0)1 867 35 75 11**
Belgium	**32 (0)2 532 4484**	**32 (0)2 582 1886**
Denmark (Naerum)	**45 45 58 60 00**	**45 45 58 60 01**
Finland (Espoo)	**358 (0)9 251 24 250**	**358 (0)9 251 24 243**
France (Paris)	**33 (0)1 69 59 85 85**	**33 (0)1 69 59 85 00**
Germany (Weiterstadt)	**49 (0)6150 101 0**	**49 (0)6150 101 101**
Italy (Milano)	**39 (0)39 83891**	**39 (0)39 838 9492**
Norway (Oslo)	**47 23 12 06 05**	**47 23 12 05 75**
Portugal (Lisboa)	**351.(0)22.605.33.14**	**351.(0)22.605.33.15**
Spain (Tres Cantos)	**34.(0)91.806.1210**	**34.(0)91.806.12.06**
Sweden (Stockholm)	**46 (0)8 619 4400**	**46 (0)8 619 4401**
Switzerland (Rotkreuz)	**41 (0)41 799 7777**	**41 (0)41 790 0676**
The Netherlands (Nieuwerkerk a/d IJssel)	**31 (0)180 392400**	**31 (0)180 392409** or **31 (0)180 392499**
United Kingdom (Warrington, Cheshire)	**44 (0)1925 825650**	**44 (0)1925 282502**

Region	Telephone	Fax
European Managed Territories (EMT)		
Africa, English speaking (Johannesburg, South Africa)	**27 11 478 0411**	**27 11 478 0349**
Africa, French speaking (Paris, France)	**33 1 69 59 85 11**	**33 1 69 59 85 00**
India (New Delhi)	**91 11 653 3743** **91 11 653 3744**	**91 11 653 3138**
Poland, Lithuania, Latvia, and Estonia (Warszawa)	**48 22 866 40 10**	**48 22 866 40 20**
For all other EMT countries not listed (Central and southeast Europe, CIS, Middle East, and West Asia)	**44 1925 282481**	**44 1925 282509**
Japan		
Japan (Hacchobori, Chuo-Ku, Tokyo)	**81 3 5566 6230**	**81 3 5566 6507**
Latin America		
Caribbean countries, Mexico, and Central America	**52 55 35 3610**	**52 55 66 2308**
Brazil	**0 800 704 9004** or **55 11 5070 9654**	**55 11 5070 9694/95**
Argentina	**800 666 0096**	**55 11 5070 9694/95**
Chile	**1230 020 9102**	**55 11 5070 9694/95**
Uruguay	**0004 055 654**	**55 11 5070 9694/95**

To Reach Technical Support Through the Applied Biosystems Web Site

At the Applied Biosystems web site, you can search through frequently asked questions (FAQs) or a solution database, or you can submit a question directly to Technical Support.

Search FAQs

To search for FAQs:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Frequently Asked Questions**.
3	Click you geographic region for the product area of interest.
4	Follow the instructions under the **Frequently Asked Questions** section (1) to display a list of FAQs for your area of interest.

Search the Solution Database

To search for solutions to problems using the Solution Database:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Frequently Asked Questions**.
3	Follow the instructions under the **Search the Solution Database** section (2) to find a solution to your problem.

Submit a Question

To submit a question directly to Technical Support:

1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Frequently Asked Questions**.
3	In the **Personal Assistance – E-Mail Support** section (3), click **Ask Us RIGHT NOW**.
4	In the displayed form, enter the requested information and your question, then click **Ask Us RIGHT NOW**. Within 24 to 48 hours, you will receive an e-mail reply to your question from an Applied Biosystems technical expert.

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- Through the Applied Biosystems web site

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2	Follow the voice instructions to order documents (for delivery by fax). **Note** There is a limit of five documents per fax request.

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To view, download, or order documents through the Applied Biosystems web site:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, then click **Documents on Demand**.
3	In the search form, enter and select search criteria, then click **Search** at the bottom of the page.
4	In the results screen, do any of the following: ♦ Click the pdf icon to view a PDF version of the document. ♦ Right-click the pdf icon, then select **Save Target As** to download a copy of the PDF file. ♦ Select the **Fax** check box, then click **Deliver Selected Documents Now** to have the document faxed to you. ♦ Select the **Email** check box, then click **Deliver Selected Documents Now** to have the document (PDF format) e-mailed to you. **Note** There is a limit of five documents per fax request, but no limit on the number of documents per e-mail request.

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Step	Action
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Toll Free: +1 800.345.5224
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