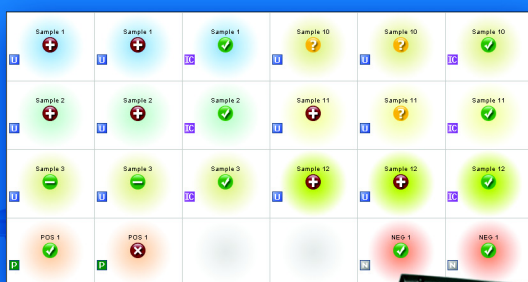


AccuSEQ™ Real-Time PCR Detection Software Mycoplasma SEQ Experiments



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Safety information

Note: For general safety information, see this Preface and [Appendix B, “Safety” on page 89](#). When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the “Safety” Appendix for the complete alert on the chemical or instrument.

Safety alert words Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT**, **CAUTION**, **WARNING**, **DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.



CAUTION! – Indicates a potentially hazardous situation that, 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 that, if not avoided, could result in death or serious injury.



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

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document appears with an open triangle figure that contains a hazard symbol. *These hazard symbols are identical to the hazard symbols that are affixed to Applied Biosystems instruments* (see [“Safety symbols” on page 90](#)).

MSDSs The MSDSs for any chemicals supplied by Applied Biosystems or Ambion are available to you free 24 hours a day. For instructions on obtaining MSDSs, see [“MSDSs” on page 97](#).

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion contact the chemical manufacturer.

Safety labels on instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Applied Biosystems instruments in combination with the safety symbols described in the preceding section.

Hazard symbol	English	Français
	CAUTION! Hazardous chemicals. Read the Material Safety Data Sheets (MSDSs) before handling.	ATTENTION! Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	CAUTION! Hazardous waste. Refer to MSDS(s) and local regulations for handling and disposal.	ATTENTION! Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la réglementation locale associées à la manipulation et l'élimination des déchets.
	WARNING! This instrument is designed for 12 V, 75 W Halogen lamps only.	AVERTISSEMENT! Cet instrument est conçu pour des lampes d'halogène de 12 V et 75 W seulement.
	WARNING! Hot lamp.	AVERTISSEMENT! Lampe brûlante.
	WARNING! Hot. Do not remove lamp until 15 min after disconnecting supply.	AVERTISSEMENT! Lampe brûlante, après avoir déconnecté le câble d'alimentation de l'appareil, attendre environ 15 minutes avant d'effectuer un remplacement de la lampe.
	WARNING! Hot. Replace lamp with an Applied Biosystems lamp.	AVERTISSEMENT! Composants brûlants. Remplacer la lampe par une lampe Applied Biosystems.
	CAUTION! Hot surface.	ATTENTION! Surface brûlante.
	DANGER! High voltage.	DANGER! Haute tension.
	WARNING! To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Applied Biosystems qualified service personnel.	AVERTISSEMENT! Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié venant de chez Applied Biosystems.
	CAUTION! Moving parts. Crush/pinch hazard.	ATTENTION! Pièces en mouvement, risque de pincement et/ou d'écrasement.

About this guide

Assumptions

This guide assumes that you:

- Are familiar with the Microsoft Windows® operating system.
- Are familiar with the Internet and Internet browsers.
- Know how to handle DNA samples and prepare them for PCR.
- Understand data storage, file transfer, and copying and pasting.
- Have networking experience, if you plan to integrate the 7500 Fast system into your existing laboratory data flow.

How to use this guide

- Text conventions** This guide uses the following conventions:
- **Bold** text indicates user action. For example:
Type **0**, then press **Enter** for each of the remaining fields.
 - *Italic* text indicates new or important words and is also used for emphasis.
For example:
Before analyzing, *always* prepare fresh matrix.
 - A right arrow symbol (▶) separates successive commands you select from a drop-down or shortcut menu. For example:
Select **File ▶ Open ▶ Spot Set**.
Right-click the sample row, then select **View Filter ▶ View All Runs**.

User attention words Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: – Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

How to obtain support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

1

Get Started

Chapter 1 Get Started

Chapter 2 Set Up the Experiment

Chapter 3 Prepare the Reactions

Chapter 4 Run the Experiment

Chapter 5 Review the Results Summary

Chapter 6 (Optional) Review the Results

Chapter 7 (Optional) Investigate the Results

This chapter covers:

- About the MicroSEQ Mycoplasma detection system 2
- About the AccuSEQ Software 2
- About Mycoplasma SEQ experiments 2
- How to use this guide 4
- About the example Mycoplasma SEQ experiment 5
- Example experiment workflow 8

Note: For more information about any of the topics discussed in this guide, access the Help system by pressing **F1**, by clicking  in the toolbar of the AccuSEQ Software window, or by selecting **Help ▶ Contents and Index**.

About the MicroSEQ *Mycoplasma* detection system

Components of the MicroSEQ® *Mycoplasma* Detection System include:

- **Software** – AccuSEQ™ Real-Time PCR Detection Software
- **Instrument** – Applied Biosystems 7500 Fast Real-Time PCR System
- **Reagents** –
 - PrepSEQ™ *Mycoplasma* Nucleic Acid Extraction Kit
 - MicroSEQ® *Mycoplasma* Real-Time PCR Detection Kit

About the AccuSEQ Software

Applied Biosystems AccuSEQ Software is detection and analysis software used with Real-Time PCR (polymerase chain reaction) assays for impurity and contaminant analysis in pharmaceutical quality control and process development environments.

The Applied Biosystems AccuSEQ Software v1.0 features include:

- **Support for Applied Biosystems 7500 Fast Real-Time PCR Systems** – The AccuSEQ software allows you to set up, run, and analyze experiments using the 7500 Fast instrument with two workflows:
 - **SEQ Experiment Workflow** – To set up, run, and analyze *Mycoplasma* experiment samples prepared with the MicroSEQ® *Mycoplasma* Real-Time PCR Detection Kit.
 - **Custom Experiment Workflows** – To set up, run, and analyze your own standard curve, melt curve, or presence/absence experiments.
- **Quality and results summaries** – Provide results in graphical and tabular format.
- **Security, Audit, and Electronic Signature (SAE) Module** – Allows the AccuSEQ Software Administrator to control access to the software and determine auditing and electronic signature requirements.
- **Instrument Maintenance Manager** – Provides step-by-step instructions for performing instrument calibration and verification runs.

About *Mycoplasma* SEQ experiments

Mycoplasma SEQ experiments are presence/absence experiments that include a melt curve. Perform a *Mycoplasma* SEQ experiment to detect the presence of *Mycoplasma* species DNA in a sample.

Note: In this guide, the term *experiment* refers to the entire process of performing a run using the 7500 Fast system, including setup, run, and analysis.

About the *Mycoplasma* SEQ experiment workflow

The *Mycoplasma* SEQ experiment workflow is designed to set up, run, and analyze samples prepared with the MicroSEQ *Mycoplasma* Real-Time PCR Detection Kit.

The *Mycoplasma* SEQ experiment workflow:

- Creates an experiment to detect the presence of *Mycoplasma* species DNA in a sample.
- Allows you to:
 - Specify the number of samples and controls
 - Enter sample names
 - Specify sample volume per reaction
- Provides a suggested plate layout, but allows you to modify the plate layout.
- Generates a reaction set-up worksheet and plate layout diagram that includes reagent volumes needed and optional instructions for preparing samples.
- Includes optimized thermal-cycling settings.
- Provides a summary of sample and control results.
- Provides detailed call assessments and troubleshooting suggestions for inconclusive or unexpected results.
- Provides results reports that you can print, or save as pdf (Adobe® Acrobat®), html, ppt (Microsoft PowerPoint®), or xls (Microsoft Excel®).

About the Mycoplasma Real - Time PCR Detection Kit

The *Mycoplasma* Real-Time PCR Detection Kit is designed for routine screening and detection of *Mycoplasma* species (mycoplasmas) and other closely related species such as *Acholeplasma laidlawii* and *Spiroplasma citri*. Based on SYBR® Green dye technology, this assay is optimized for broad detection of mycoplasmas. When used with the recommended sample preparation, this assay provides the specificity and sensitivity needed for routine use in the monitoring of cell lines, raw materials, and biopharmaceutical product-manufacturing.

About the PCR reactions

For a *Mycoplasma* SEQ experiment, you prepare PCR reactions that contain multiple primers designed to amplify the mycoplasma targets and a reagent to detect amplification of the targets in unknown samples.

The reactions required in a *Mycoplasma* SEQ experiment are:

- **Unknown** – A DNA sample from media, cell culture, or other source that you are testing for the presence of mycoplasmas.
- **Negative Control** – A reaction solution that contains water or buffer instead of sample template.
- **Positive Control (*Mycoplasma* Real-Time PCR DNA Control)** – A specially designed plasmid DNA used as the positive control whose amplification mimics the expected amplification of a target.
- **Inhibition Control** – A reaction solution that includes the *Power* SYBR® Green PCR master mix, the unknown sample, and the positive control (*Mycoplasma* Real-Time PCR DNA control).

About the run method

In *Mycoplasma* SEQ experiments, the run method is pre-determined by the AccuSEQ software and is not editable. The run method includes the following stages:

- **Holding** – In the thermal profile for a *Mycoplasma* SEQ experiment, a stage that includes a single step. The holding stage is performed on the 7500 Fast instrument before PCR amplification to activate the AmpliTaq Gold® enzyme.

- **Cycling (Amplification)** – Part of the run method in which PCR produces amplification of the target. The amplification stage is performed on the 7500 Fast instrument.
- **Melt (Dissociation) curve** – In the thermal profile for a Mycoplasma SEQ experiment, a stage with a temperature increment to generate a melt curve that is required to determine the results for the experiment. The melt curve stage is performed on the 7500 Fast instrument after PCR amplification to collect endpoint fluorescence data.

Fluorescence data collected during the run method are stored in an experiment data file (*.eds).

About the analysis

Data collected from the run method are used to automatically determine Mycoplasma SEQ experiment presence/absence calls. The results are reported in the following screens:

- **Results Summary** – Provides a summary of expected, unexpected, present, absent, and inconclusive results, and displays results in plate view or table view.
- **Quality Summary** – Provides a description and troubleshooting information for inconclusive results in the Results Summary.
- **Plot Analysis** – Amplification Plot, Melt Curve, Multicomponent Plot, Raw Data Plot, and Multiple Plots View can be used to further investigate results, modify C_T settings, and re-analyze the data.

For more information

For information on:

- Mycoplasmas, and the *Mycoplasma* assay and detection kit – Refer to the *MicroSEQ[®] Mycoplasma Real-Time PCR Detection Kit Protocol*.
- The 7500 Fast system and supported consumables – Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Maintenance Guide*.
- SYBR[®] Green dye technology – Refer to the *Applied Biosystems Real-Time PCR Systems Reagent Guide*.
- Custom experiments (standard curve, presence/absence, and melt curve) – Refer to the *AccuSEQ Real-Time PCR Detection Software Custom Experiments Quick Reference Card*.

How to use this guide

Using this guide as a tutorial

Using example experiment data provided with the AccuSEQ software, you can use this guide as a tutorial for performing a Mycoplasma SEQ experiment on a 7500 Fast system. For the example experiment, follow the procedures in chapters 2 through 7:

Chapter	Procedure
2	Set up the experiment using the SEQ Experiment workflow in the AccuSEQ software.

Chapter	Procedure
3	Prepare the experiment, using the reagents and volumes calculated by the SEQ Experiment workflow in Chapter 2 .
4	Run the experiment on a 7500 Fast instrument.
5	Review the Results Summary.
6	(Optional) Review the analysis results.
7	(Optional) Investigate the analysis results.

For more information, see “[About the example Mycoplasma SEQ experiment](#)” on [page 5](#).

Using this guide with your own experiments

After completing the tutorial exercises in chapters [2](#) through [7](#), use this guide to lead you through your own Mycoplasma SEQ experiments. Each procedure in Chapters [2](#) through [7](#) includes a set of guidelines, marked with the heading *Guidelines for your experiment*, that you can use to perform your own experiments.

Additionally, you can use one of the other workflows provided in the AccuSEQ software to perform your experiments. The table below provides a summary of all the workflows available in the AccuSEQ software.

Workflow	Description	See...
SEQ Experiment	Set up a new experiment for specific Applied Biosystems SEQ assay(s) with guidance from the software, recommended for new users. The SEQ Experiment workflow calculates reagent volumes, determines optimum plate setup, guides you through experiment set up, uses optimized thermal cycling conditions, and performs data analysis.	Chapter 2
SEQ Template	Set up a new SEQ experiment using setup information from a template.	Appendix A
Custom Experiment	Set up a new experiment using advanced options, recommended for experienced users. The Custom Experiment workflow allows design flexibility as you create your own experiment.	Documentation

Note: An experiment you create in the SEQ experiment workflow cannot be accessed from the custom experiment workflow.

About the example Mycoplasma SEQ experiment

To illustrate how to perform Mycoplasma SEQ experiments, this guide leads you through the process of designing, preparing, running and analyzing an example experiment. The example experiment represents a typical setup that you can use to quickly familiarize yourself with a 7500 Fast system running AccuSEQ software.

Description The objective of the example Mycoplasma SEQ experiment (*example experiment*) is to detect the presence of *Mycoplasma* species DNA in each test sample.

In the example experiment:

- DNA is extracted from samples using the PrepSEQ™ *Mycoplasma* Nucleic Acid Extraction Kit.
- The target is the bacteria being tested (mycoplasmas).
- Each reaction in the experiment contains multiple primer sets that are designed to amplify the same target sequence from different species and SYBR® Green I dye to detect the dsDNA products generated during PCR.

Reaction plate layout

The AccuSEQ software displays the 96-well reaction plate layout as shown below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1 U Mycoplasma ...	Sample 1 U Mycoplasma ...	Sample 1 IC Mycoplasma ...	Sample 4 U Mycoplasma ...	Sample 4 U Mycoplasma ...	Sample 4 IC Mycoplasma ...	Sample 7 U Mycoplasma ...	Sample 7 U Mycoplasma ...	Sample 7 IC Mycoplasma ...	Sample 10 U Mycoplasma ...	Sample 10 U Mycoplasma ...	Sample 10 IC Mycoplasma ...
B	Sample 2 U Mycoplasma ...	Sample 2 U Mycoplasma ...	Sample 2 IC Mycoplasma ...	Sample 5 U Mycoplasma ...	Sample 5 U Mycoplasma ...	Sample 5 IC Mycoplasma ...	Sample 8 U Mycoplasma ...	Sample 8 U Mycoplasma ...	Sample 8 IC Mycoplasma ...	Sample 11 U Mycoplasma ...	Sample 11 U Mycoplasma ...	Sample 11 IC Mycoplasma ...
C	Sample 3 U Mycoplasma ...	Sample 3 U Mycoplasma ...	Sample 3 IC Mycoplasma ...	Sample 6 U Mycoplasma ...	Sample 6 U Mycoplasma ...	Sample 6 IC Mycoplasma ...	Sample 9 U Mycoplasma ...	Sample 9 U Mycoplasma ...	Sample 9 IC Mycoplasma ...	Sample 12 U Mycoplasma ...	Sample 12 U Mycoplasma ...	Sample 12 IC Mycoplasma ...
D												
E												
F												
G												
H	POS 1 P Mycoplasma ...	POS 1 P Mycoplasma ...									NEG 1 N Mycoplasma ...	NEG 1 N Mycoplasma ...

Wells: U Unknown 24 Negative Control 2 Positive Control 2 Inhibition Control 12 56 Empty

About the example experiment data files

In this getting started guide, you will use two files:

- In [Chapter 2](#), you will create an example *Mycoplasma* SEQ experiment that contains setup data, then you will save the file to your computer.
- In [Chapter 5](#), you will view results in an example *Mycoplasma* SEQ experiment file that contains run data. The data file for the example experiment installs with the AccuSEQ software.

You can find the data file for the example experiment on your computer at `<drive>:\Applied Biosystems\AccuSEQ\experiments\Mycoplasma SEQ Example.eds`, where `<drive>` is the computer hard drive on which the AccuSEQ software is installed.

The experiments folder of the AccuSEQ software contains several example files that you can reference when analyzing your own data. The following example files install with the AccuSEQ software:

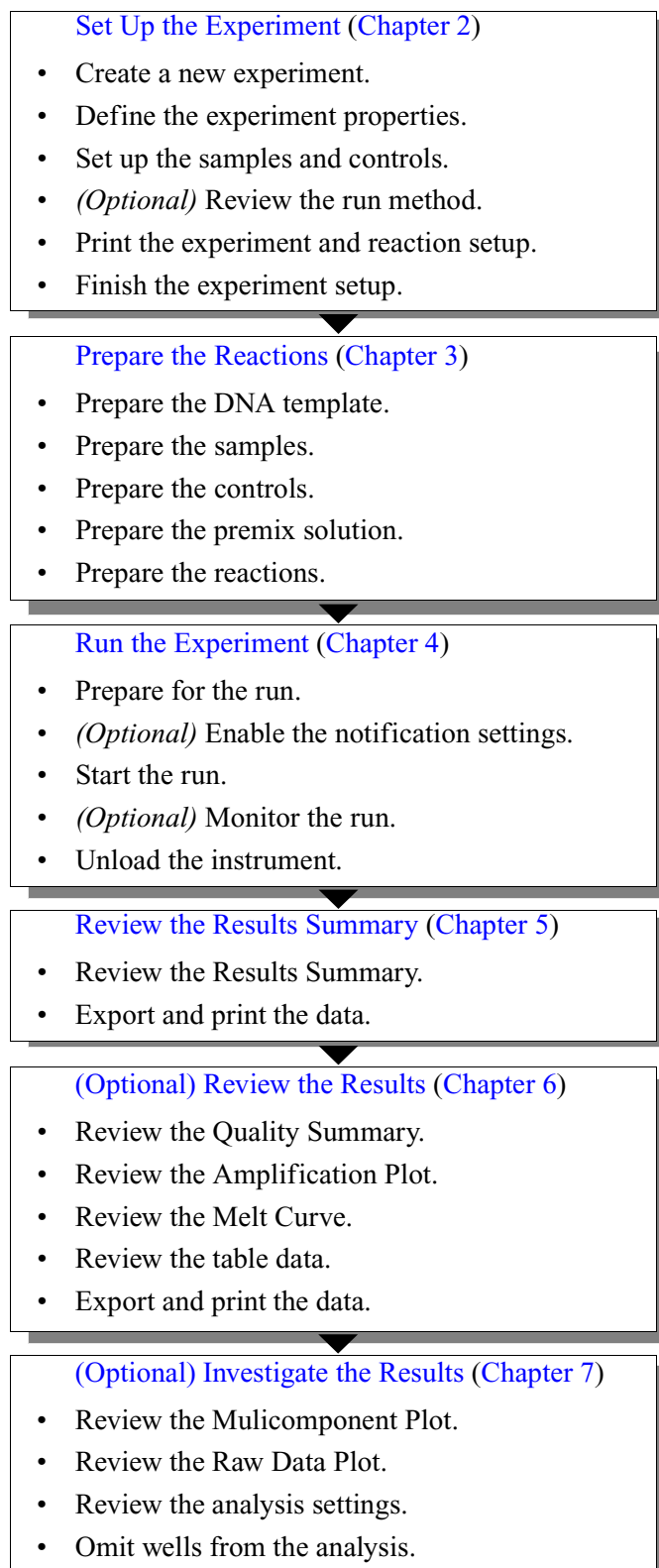
- *Mycoplasma* SEQ Example.eds

- Presence Absence Example.eds
- Standard Curve Example.eds

Note: Be sure to use the *Mycoplasma SEQ Example.eds* file when you perform the procedures in this guide.

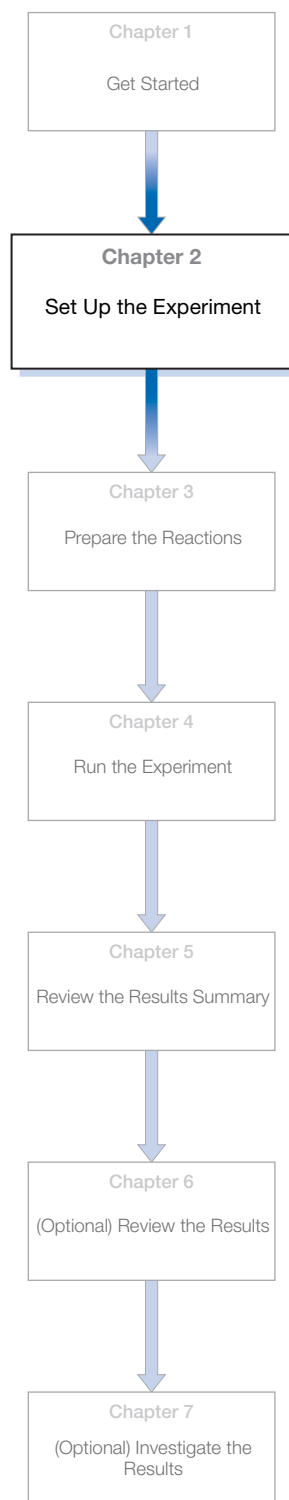
Example experiment workflow

The figure below shows the workflow for the example Mycoplasma SEQ experiment.



2

Set Up the Experiment



This chapter covers:

■ Chapter overview	10
■ Create a new experiment.	12
■ Define the experiment properties	13
■ Set up the samples and controls	15
■ (Optional) Review the run method	20
■ Print the experiment and reaction setup	21
■ Finish the experiment setup	22

Note: For more information about any of the topics discussed in this guide, access the Help system by pressing **F1**, by clicking  in the toolbar of the AccuSEQ Software window, or by selecting **Help ▶ Contents and Index**.

Chapter overview

This chapter explains how to use the SEQ Experiment workflow in the AccuSEQ software to set up the example Mycoplasma SEQ experiment. The SEQ Experiment workflow provides a step-by-step workflow for a specific Applied Biosystems SEQ assay. The SEQ experiment workflow calculates reagent volumes, determines optimum plate setup, guides you through experiment set up, uses optimized thermal cycling conditions, and performs data analysis.

Note: An experiment you create in the SEQ experiment workflow cannot be accessed from the custom experiment workflow. When you design your own experiments, you can select alternate workflows (see [“Using this guide with your own experiments” on page 5](#)).

About predefined settings

For the example experiment, the SEQ workflow guides you through Applied Biosystems recommended best practices as you enter design parameters to set up, run, and analyze Mycoplasma experiment samples prepared with the MicroSEQ[®] *Mycoplasma* Real-Time PCR Detection Kit. Based on the Mycoplasma SEQ assay you select, the following settings are pre-defined by the software and are not editable:

- The experiment type is presence/absence.
- The run method includes three stages: holding, cycling, and melt curve.
- The reaction volume per well is 30 µL.
- SYBR[®] Green dye is the reporter dye.
- A minimum of one of each of the following sample types are included in the plate layout: unknown sample, inhibition control (one per sample), positive control, and negative control.

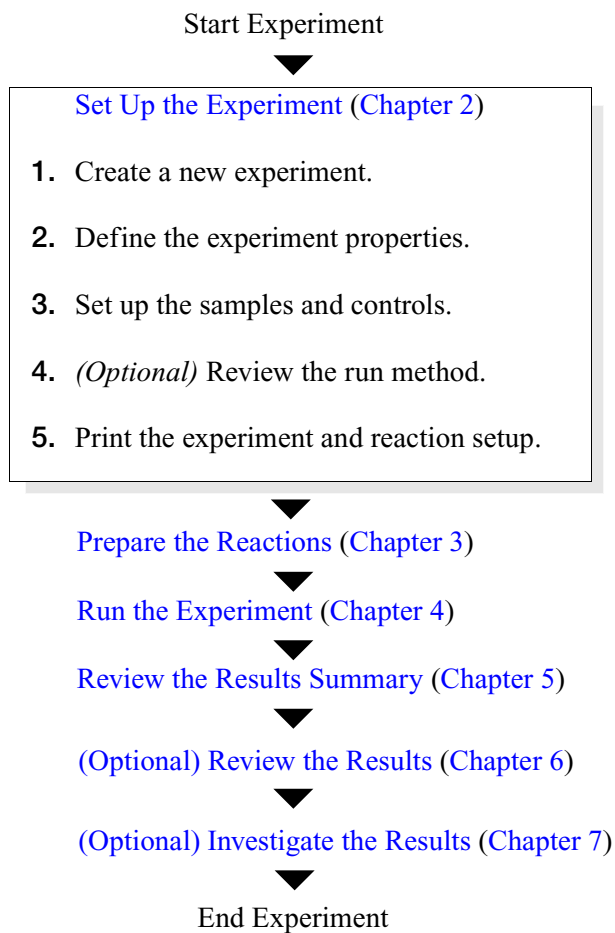
For more information

For more information on:

- Consumables – Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Maintenance Guide*.
- SYBR Green reagents and PCR – Refer to the *Applied Biosystems Real-Time PCR Systems Reagent Guide*.
- Using the Custom Experiment workflow – Refer to the *AccuSEQ Real-Time PCR Detection Software Custom Experiments Quick Reference Card*.

**Example
experiment
workflow**

The workflow for setting up an experiment similar to the example experiment provided with this getting started guide is shown below.



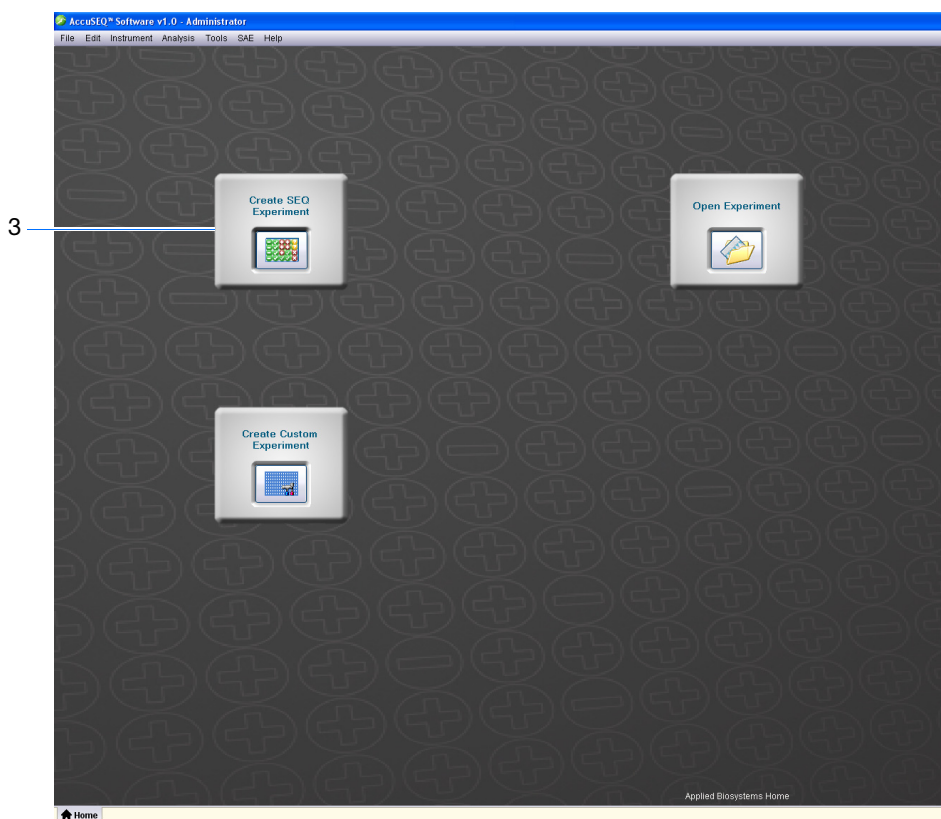
Create a new experiment

Create a new experiment using the SEQ Experiment workflow in the AccuSEQ software.

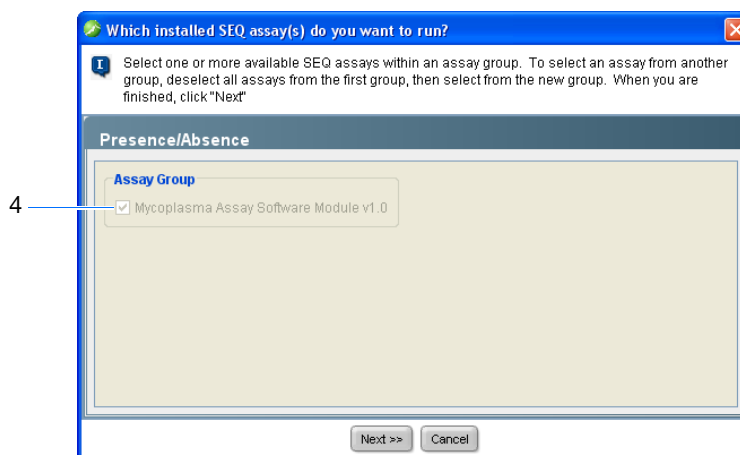
1. Double-click  (**AccuSEQ Software**).
2. Enter your user name and password, then click **OK**.

Note: See your system administrator for user name and password.

3. In the Home screen, click **Create SEQ Experiment** to open the SEQ Experiment workflow.



4. In the SEQ assay selection dialog, verify that **Mycoplasma Assay Software Module v1.0** is selected from the presence/absence assay group, then click **Next**.



Note: The SEQ assays shown in this dialog may vary, depending on the valid SEQ assays installed on your system.

Define the experiment properties

In the Experiment Properties screen, enter identifying information for the experiment, and verify the assay information to use.

About the example experiment

In the example Mycoplasma SEQ experiment:

- The experiment is identified as an example.
- The SEQ experiment type is presence/absence.
- The SEQ assay used is Mycoplasma Assay Software Module v1.0.

Note: The SEQ experiment workflow automatically specifies the setup information (experiment type, reagents, targets) and an optimized run method for the selected assay.

Complete the Experiment Properties screen

1. Click the **Experiment Name** field, then enter **Mycoplasma SEQ Example Setup**.

Note: The experiment header updates with the experiment name you entered.

2. Leave the Barcode field empty.
3. Click the **Comment** field, then enter **Mycoplasma Getting Started Guide Example**.
4. Verify the SEQ experiment type and assay to use in the example experiment:

- **SEQ Experiment Type – Presence/Absence.** The SEQ experiment type indicates the category of experiment automatically specified when you select the SEQ assay to use. A SEQ presence/absence experiment detects the amplification of targets.

IMPORTANT! The SEQ presence/absence experiment is customized for detection of specific organisms and species and may yield different results than a custom presence/absence experiment.

- **SEQ Assay(s) Used – Mycoplasma Assay Software Module v1.0.** The SEQ assay used indicates the organism detected by the assay.

5. Click **Next**.

Experiment: Mycoplasma SEQ Examp...

Experiment Properties

Enter an experiment name, enter a comment (optional), then click "Next" to continue.

How do you want to identify this experiment?

1 Experiment Name: Mycoplasma SEQ Example Setup

2 Barcode (Optional):

3 Comment (Optional): Mycoplasma Getting Started Guide Example

4 SEQ Experiment Type: Presence/Absence
SEQ Assay(s) Used: Mycoplasma Assay Software Module v1.0

Guidelines for your experiment

When you set up your own Mycoplasma SEQ experiment:

- Enter an experiment name that is descriptive and easy to remember.

Note: The experiment name is used as the default file name.

- (Optional) Enter a barcode to identify the barcode on the reaction plate.
- (Optional) Enter comments to describe the experiment.

For more information

For more information on:

- Completing the Experiment Properties screen – Access the *AccuSEQ Software Help* by clicking or pressing **F1**.
- Mycoplasma experiments – Refer to the *MicroSEQ® Mycoplasma Real-Time PCR Detection Kit Protocol*.

Set up the samples and controls

In the Sample Setup screen, enter the number of samples, replicates, and controls to include in the reaction plate, enter the sample and control names, then select which plot color to assign to each sample and control.

The AccuSEQ software uses the sample information that you enter in the Sample Setup screen to fill the wells in the plate layout (see [page 16](#)) and to calculate the required reaction component volumes for each sample type, based on the *MicroSEQ Mycoplasma Real-Time PCR Detection Kit Protocol* guidelines.

About the example experiment

The example Mycoplasma SEQ experiment uses:

- Twelve samples, where the presence/absence of the target (a mycoplasma contaminant) is unknown.
- Two Unknown sample replicates. Replicates are identical to the sample reactions, containing identical components and volumes.
- One Inhibition Control replicate for each sample. Each Inhibition Control replicate contains positive control template (*Mycoplasma* Real-Time PCR DNA Control) and sample template.
- Two Positive Control replicates. Replicates contain positive control template (*Mycoplasma* Real-Time PCR DNA Control) instead of sample template.
- Two Negative Control replicates. Replicates contain water instead of sample template.

Complete the Sample Setup screen

1. In the *Mycoplasma Module v1.0* column of the *How many samples and controls do you want to run?* table, specify the number of samples and sample volume.

- a. Specify the number of samples and replicates:

For Number of... [‡]	Enter... [§]
Samples	12
Sample replicates	2
Inhibition control replicates for each sample	1
Positive control replicates	2
Negative control replicates	2

[‡] At least one positive control, one negative control, and one inhibition control are required.

[§] The number that you specify is listed below in the sample type list (see [step 2](#)).

- b. Set the Sample volume per reaction (2-10µL) to **10**.

2. Leave the defaults in the *Define Samples for Assay: Mycoplasma Module v1.0* table:

- a. Sample Names for samples with Sample Type = UNKNOWN:

- **Sample 1**
- **Sample 2**

- ...
- **Sample 12**

Sample Type	Sample Name	Name Fill	Plot Color	Number of Wells - Sample	Number of Wells - Inhibition Controls
U UNKNOWN	Sample 1		 ▼	2	1
U UNKNOWN	Sample 2		 ▼	2	1
U UNKNOWN	Sample 3		 ▼	2	1
U UNKNOWN	Sample 4		 ▼	2	1
U UNKNOWN	Sample 5		 ▼	2	1
U UNKNOWN	Sample 6		 ▼	2	1
U UNKNOWN	Sample 7		 ▼	2	1

b. Sample Names for samples with Sample Type = POS and NTC:

- **POS 1**
- **NEG 1**

c. Colors in the Plot Color drop-down lists.

Note: The plot color represents the amplification plot data in the software and does not correspond to the dye color in the chemistry.

3. Click **Next**.

View the plate layout

The AccuSEQ software automatically selects locations for the wells in the reaction plate based on the sample setup information you entered in the Sample Setup screen (see [page 15](#)). You can view and edit the plate layout before starting an instrument run. For the example experiment, use the following procedure to view the plate layout and move pairs of samples to new well positions by swapping their well locations.

1. Click  (**Setup ► Plate Layout**) in the navigation pane, if not already selected.
2. Review the initial well selections in the Plate Layout screen. In the Mycoplasma SEQ experiment, the wells are arranged as shown:

2

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1 U Mycoplasma ...	Sample 2 U Mycoplasma ...	Sample 3 U Mycoplasma ...	Sample 4 U Mycoplasma ...	Sample 5 U Mycoplasma ...	Sample 6 U Mycoplasma ...	Sample 7 U Mycoplasma ...	Sample 8 U Mycoplasma ...	Sample 9 U Mycoplasma ...	Sample 10 U Mycoplasma ...	Sample 11 U Mycoplasma ...	Sample 12 U Mycoplasma ...
B	Sample 1 U Mycoplasma ...	Sample 2 U Mycoplasma ...	Sample 3 U Mycoplasma ...	Sample 4 U Mycoplasma ...	Sample 5 U Mycoplasma ...	Sample 6 U Mycoplasma ...	Sample 7 U Mycoplasma ...	Sample 8 U Mycoplasma ...	Sample 9 U Mycoplasma ...	Sample 10 U Mycoplasma ...	Sample 11 U Mycoplasma ...	Sample 12 U Mycoplasma ...
C	Sample 1 IC Mycoplasma ...	Sample 2 IC Mycoplasma ...	Sample 3 IC Mycoplasma ...	Sample 4 IC Mycoplasma ...	Sample 5 IC Mycoplasma ...	Sample 6 IC Mycoplasma ...	Sample 7 IC Mycoplasma ...	Sample 8 IC Mycoplasma ...	Sample 9 IC Mycoplasma ...	Sample 10 IC Mycoplasma ...	Sample 11 IC Mycoplasma ...	Sample 12 IC Mycoplasma ...
D												
E												
F												
G												
H	POS 1 P Mycoplasma ...	POS 1 P Mycoplasma ...									NEG 1 N Mycoplasma ...	NEG 1 N Mycoplasma ...

3 Wells: U Unknown 24 N Negative Control 2 P Positive Control 2 IC Inhibition Control 12 56 Empty

By default, the software:

- Groups Unknown sample and Inhibition Control replicates in columns, starting with well A1.
- Assigns Positive Control and Negative Control replicates to the last well row (wells H1-H12).

3. In the Wells pane, make sure there are:

- 24 Unknowns **U**
- 12 Inhibition Controls **IC**
- 2 Positive Controls **P**
- 2 Negative Controls **N**

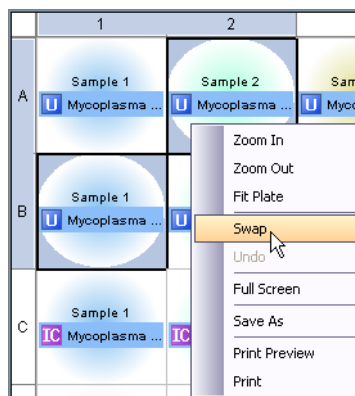
Note: If the plate layout is incorrect, click **Previous** and check your entered values.

4. In the Plate Layout pane, swap well contents:

- In the Group Wells By drop-down list, verify that **Columns** is selected.
- Swap the well contents for the following pairs of wells:

For Samples...	Swap well...	With well...
1 to 3	A2	B1
	A3	C1
	B3	C2
4 to 6	A5	B4
	A6	C4
	B6	C5
7 to 9	A8	B7
	A9	C7
	B9	C8
10 to 12	A11	B10
	A12	C10
	B12	C11

To swap the well contents for a pair of discontinuous wells in the plate layout, press the **Ctrl** key while you click the desired wells, right-click the plate layout, then select **Swap**.



c. View the final plate layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1 U Mycoplasma ...	Sample 1 U Mycoplasma ...	Sample 1 IC Mycoplasma ...	Sample 4 U Mycoplasma ...	Sample 4 U Mycoplasma ...	Sample 4 IC Mycoplasma ...	Sample 7 U Mycoplasma ...	Sample 7 U Mycoplasma ...	Sample 7 IC Mycoplasma ...	Sample 10 U Mycoplasma ...	Sample 10 U Mycoplasma ...	Sample 10 IC Mycoplasma ...
B	Sample 2 U Mycoplasma ...	Sample 2 U Mycoplasma ...	Sample 2 IC Mycoplasma ...	Sample 5 U Mycoplasma ...	Sample 5 U Mycoplasma ...	Sample 5 IC Mycoplasma ...	Sample 8 U Mycoplasma ...	Sample 8 U Mycoplasma ...	Sample 8 IC Mycoplasma ...	Sample 11 U Mycoplasma ...	Sample 11 U Mycoplasma ...	Sample 11 IC Mycoplasma ...
C	Sample 3 U Mycoplasma ...	Sample 3 U Mycoplasma ...	Sample 3 IC Mycoplasma ...	Sample 6 U Mycoplasma ...	Sample 6 U Mycoplasma ...	Sample 6 IC Mycoplasma ...	Sample 9 U Mycoplasma ...	Sample 9 U Mycoplasma ...	Sample 9 IC Mycoplasma ...	Sample 12 U Mycoplasma ...	Sample 12 U Mycoplasma ...	Sample 12 IC Mycoplasma ...
D												
E												
F												
G												
H	POS 1 P Mycoplasma ...	POS 1 P Mycoplasma ...									NEG 1 N Mycoplasma ...	NEG 1 N Mycoplasma ...
Wells: U Unknown 24 N Negative Control 2 P Positive Control 2 IC Inhibition Control 12 56 Empty												

5. (Optional) Review the run method (see [page 20](#)).

Note: The SEQ experiment workflow automatically specifies an optimized run method for the selected assay.

6. Click **Next**.

Guidelines for your experiment

When you set up your own Mycoplasma SEQ experiment:

- Identify each sample with a unique name and color.
- Enter the number of samples and controls to set up in the reaction plate.

IMPORTANT! Use at least one negative and one positive control per run, and at least one inhibition control per sample.

- If the number of reactions required exceeds the number of wells in the reaction plate, reduce the number of samples, Unknown replicates, Negative Control replicates, Positive Control replicates, and/or Inhibition Controls.

IMPORTANT! A SEQ experiment provides a default plate layout. To modify the default plate layout, refer to the *Mycoplasma Real-Time PCR Detection Kit Protocol* for plate layout suggestions.

- If you use Fast reaction tube strips (PN 4358293) for your reactions, a maximum of 6 tube strips can be used in the 7500 Fast instrument (see [page 40](#) for reaction loading guidelines).
- Enter a sample volume per reaction. Applied Biosystems recommends a sample volume per reaction of 10 µL for Mycoplasma SEQ experiments. The 7500 Fast system supports total reaction volumes from 10 to 30 µL.

For more information

For more information on completing the **Sample Setup** screen, access the *AccuSEQ Software Help* by clicking  or pressing **F1**.

(Optional) Review the run method

In the SEQ experiment workflow, the AccuSEQ software selects a thermal profile for the instrument run that is optimized for the SEQ assay you select in [step 4 on page 12](#). You can review, but not edit, the pre-defined thermal profile in the Run Method screen.

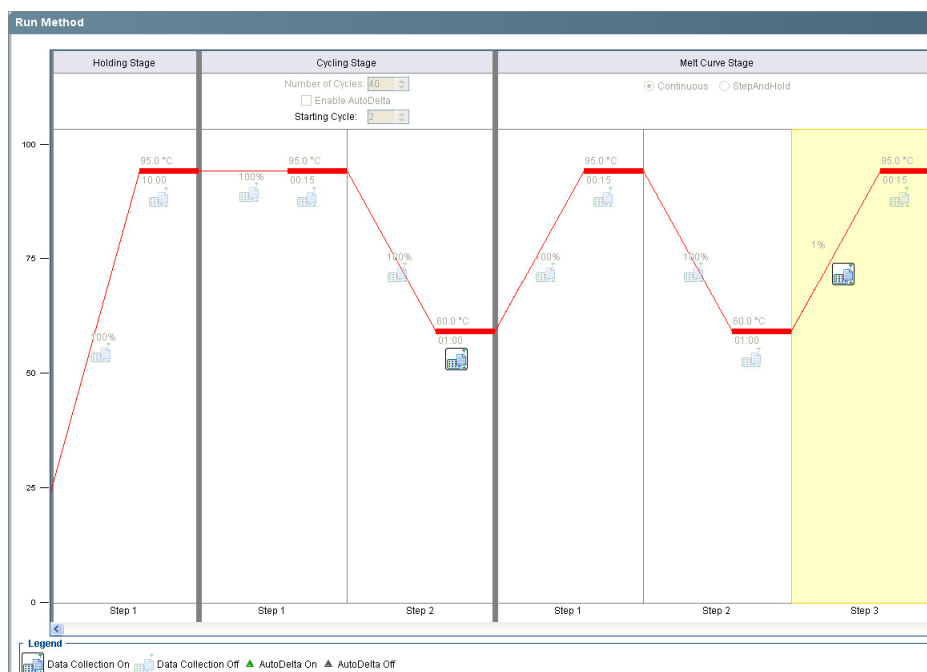
Note: The SEQ experiment workflow automatically specifies the setup information (experiment type, reagents, targets) and an optimized run method for the selected assay.

About the example experiment

For the example Mycoplasma SEQ experiment, the pre-defined run method is optimized for use with the Mycoplasma v1.0 SEQ assay.

Review the Run Method screen

Click  **Run** in the navigation pane, then click  **Run Method**. The thermal profile displays the holding, cycling, and melt curve stages shown below.



Note: For the example experiment, do not perform the run at this time.

For more information

For more information on the Run Method screen, access the *AccuSEQ Software Help* by clicking [?](#) or pressing **F1**.

Print the experiment and reaction setup

In the Print Experiment Setup screen, save and print the plate layout and the detailed reaction setup instructions (the calculated volumes for preparing the samples, controls, and PCR reactions) for the example experiment.

About the example experiment

Based on the setup information you entered in the Sample Setup screen (see [page 15](#)), the reaction setup instructions for the example Mycoplasma SEQ experiment includes the following information:

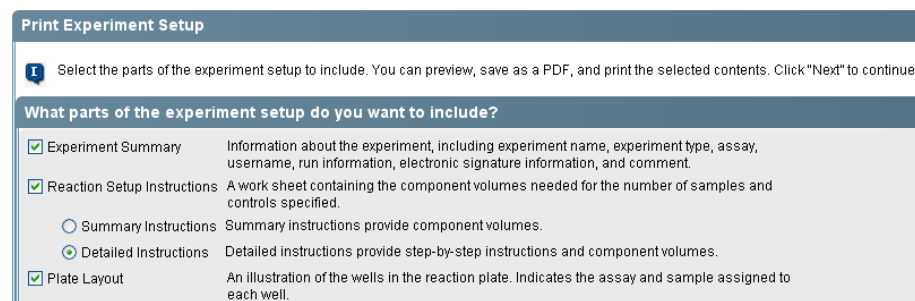
- The SEQ assay type is Mycoplasma Assay Software Module v1.0.
- The reaction volume per well is 30 μ L with a 10% excess.
- The reaction components are:
 - Power SYBR[®] Green PCR Master Mix (2X)
 - Mycoplasma Real-Time PCR Primer Mix (10X)
 - Mycoplasma Real-Time PCR DNA Control (positive control)
 - Negative Control (water)
 - Unknown sample DNA
- Volume for all samples:

- The premix solution volume per well is 18 µL.
- The final sample and control volume per well is 12 µL.

Print the experiment setup

Print the experiment setup (plate layout and detailed reaction setup instructions) for the example experiment, then save the instructions for [Chapter 3](#).

1. Click **Setup** in the navigation pane, then click  **Print Experiment Setup**.
2. In the Print Experiment Setup screen, select the elements to include in the report:
 - Experiment Summary
 - Detailed Instructions
 - Plate Layout



Print Experiment Setup

Select the parts of the experiment setup to include. You can preview, save as a PDF, and print the selected contents. Click "Next" to continue.

What parts of the experiment setup do you want to include?

☒ Experiment Summary	Information about the experiment, including experiment name, experiment type, assay, username, run information, electronic signature information, and comment.
☒ Reaction Setup Instructions	A work sheet containing the component volumes needed for the number of samples and controls specified.
☐ Summary Instructions	Summary instructions provide component volumes.
☐ Detailed Instructions	Detailed instructions provide step-by-step instructions and component volumes.
☒ Plate Layout	An illustration of the wells in the reaction plate. Indicates the assay and sample assigned to each well.

3. Click **Print** to print the report, or click **Preview** to display the report on-screen.
4. Specify a reason for change, if prompted. For more information, see [“Security, Audit and E-Signature \(SAE\) for users”](#) on page 84.
5. Set the printed reaction setup instructions aside until you prepare the reactions (see [page 25](#)).

For more information

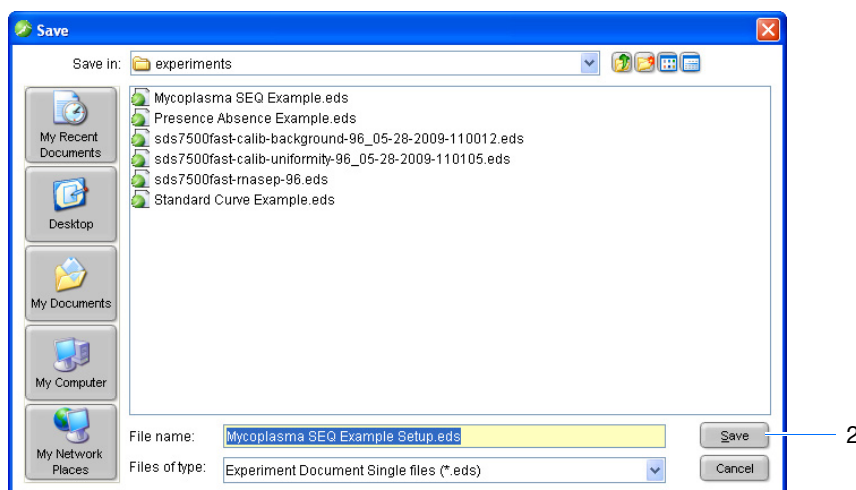
For more information on printing the plate layout and reaction setup instructions, access the *AccuSEQ Software Help* by clicking  or pressing **F1**.

Finish the experiment setup

To finish the experiment setup, select a save option.

Save the experiment

1. At the bottom of the AccuSEQ software screen, click **Save & Finish**.
2. In the Save Experiment dialog box, verify the *Mycoplasma SEQ Example Setup.edx* file name, then click **Save**.



3. Specify a reason for change and sign the experiment, if prompted. For more information, see [“Security, Audit and E-Signature \(SAE\) for users”](#) on page 84.
4. By default, the example experiment is saved to the <drive>:\Applied Biosystems\AccuSEQ\experiments folder, and you are returned to the Run Method screen. For the example experiment, do not perform the run at this time.

To continue with:

- Examining the results of the example experiment, go to [Chapter 5](#).
- Instructions for running your own Mycoplasma SEQ experiment using the example experiment as a guide, go to [Chapter 3](#).

Guidelines for your experiment

When you finish your own Mycoplasma SEQ experiment:

- In the SEQ Experiment screen, select the appropriate save option:

Select/Click	To...
File ► Save (or  in the toolbar)	Save the experiment using the current file name and save location.
File ► Save As...	Save the experiment using a new file name and save location.
File ► Save as Template...	Save the experiment as an SEQ template (*.edt file type).
Save & Finish	Save the experiment without making any further changes and prepare to start the run.

- By default, experiments are saved to:
<drive>:\Applied Biosystems\AccuSEQ\experiments

To change the:

- Save location for a specific experiment – Navigate to the desired location using the Save Experiment dialog box.

- Default save location – Select **Tools ► Preferences**, then select the **Defaults** tab. In the Data Folder field, browse to then select the desired location.

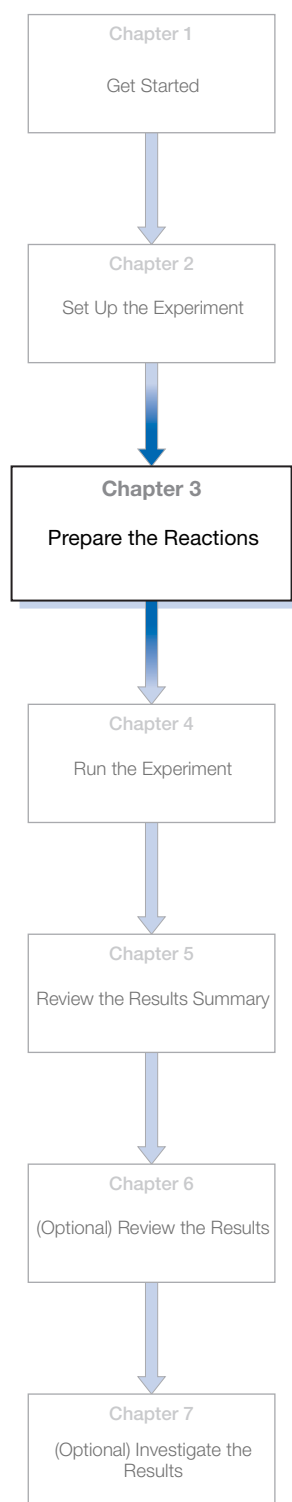
IMPORTANT! Do not manipulate the experiment files stored on the hard drive of the computer. If you do so, you will corrupt the experiment file and will not be able to open it in the AccuSEQ software.

**For more
information**

For more information on using SEQ templates, see [“SEQ template workflow” on page 87](#).

3

Prepare the Reactions



This chapter covers:

■ Chapter overview	26
■ Prepare the DNA template	27
■ Prepare the samples	27
■ Prepare the controls	28
■ Prepare the premix solution	30
■ Prepare the reactions	32
■ Guidelines for your experiment	34

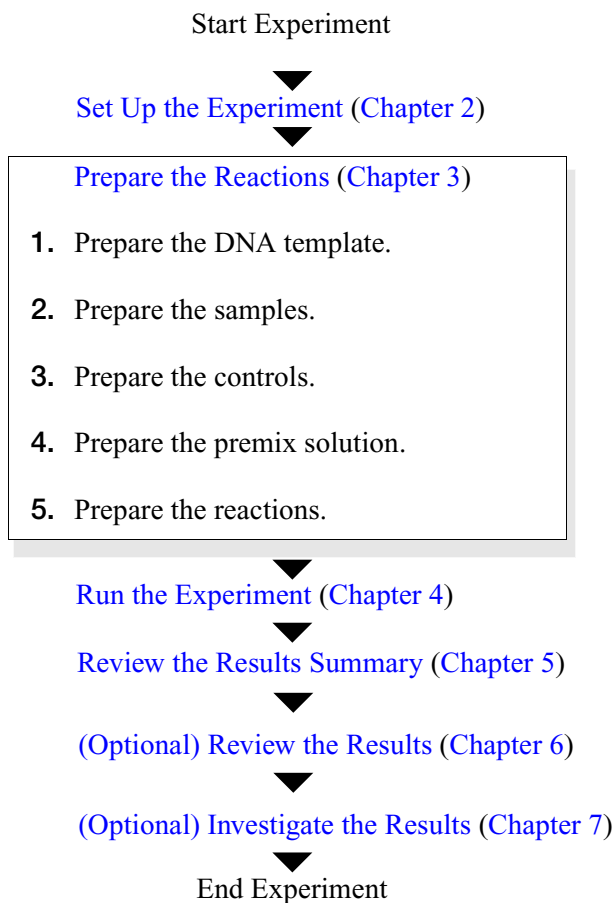
Note: For more information about any of the topics discussed in this guide, access the Help system by pressing **F1**, by clicking  in the toolbar of the AccuSEQ Software window, or by selecting **Help ▶ Contents and Index**.

Chapter overview

This chapter explains how to prepare the PCR reactions for the example Mycoplasma SEQ experiment and provides guidelines for preparing the PCR reactions for your own Mycoplasma SEQ experiment.

Example experiment workflow

The workflow for preparing the PCR reactions for the example experiment provided with this getting started guide is shown below.



Prepare the DNA template

In this section, prepare the DNA template for the PCR reactions using the PrepSEQ™ *Mycoplasma* Nucleic Acid Extraction Kit.

About the example experiment

For the example *Mycoplasma* SEQ experiment, the template for the PCR reactions is DNA extracted from bacteria found in test samples. There are 12 test samples to analyze for the presence or absence of mycoplasma bacterial contaminants.

Required materials

- The PrepSEQ™ *Mycoplasma* Nucleic Acid Extraction Kit for DNA isolation.
- Media, cell culture, or other source that you are testing for the presence of mycoplasmas.

Prepare the template

Refer to the *PrepSEQ™ Mycoplasma Nucleic Acid Extraction Kit Protocol* for details on DNA isolation.

Prepare the samples

In this section, prepare the DNA samples (Unknowns) using the volumes that were calculated by the AccuSEQ software (see [page 21](#)).

About the example experiment

For the example *Mycoplasma* SEQ experiment:

- The sample volume per reaction is 10 µL.
- The excess reaction volume is 10%.

Required materials

- Unknown sample DNA
- Negative Control (water)
- Microcentrifuge tubes
- Pipettors
- Pipette tips
- Vortexer
- Centrifuge

Prepare the sample dilutions

1. Label a separate microcentrifuge tube for each unknown sample:
 - **Sample 1**
 - **Sample 2**
 - ...
 - **Sample 12**
2. Add the required volumes of each component to the appropriate sample tube:

Component	Volume (μL) for 2 Reactions [‡]
Unknown sample DNA	22.0
Negative Control (water)	4.4
Total Sample Volume	26.4

[‡] Includes 10% excess to compensate for pipetting errors.

- Mix each sample by gently pipetting up and down, then cap each tube.
- Vortex each sample for 3 to 5 seconds, then centrifuge the tubes briefly.
- Place the samples on ice until you prepare the reactions.

Prepare the controls

In addition to unknown samples, the control reactions required in a Mycoplasma SEQ experiment are:

- Inhibition control** – A minimum of one for each unknown sample
- Positive control** – A minimum of one per plate
- Negative control** – A minimum of one per plate

In this section, prepare the control reactions using the volumes that were calculated by the AccuSEQ software (see [page 21](#)).

About the example experiment

For the example Mycoplasma SEQ experiment:

- 12 μL of each control (inhibition, positive, negative) is added to 18 μL of Premix Solution.
- The final reaction volume per well is 30 μL.
- The excess reaction volume is 10%.
- The number of control wells in the reaction plate are:

Control Type	Number of Wells
Inhibition	12 [‡]
Positive	2
Negative	2

[‡] One inhibition control well for each unknown sample (see [page 3](#))

Required materials

- Unknown sample DNA
- Mycoplasma* Real-Time PCR DNA Control (positive control)
- Negative Control (water)
- Microcentrifuge tubes
- Pipettors

- Pipette tips
- Vortexer
- Centrifuge

Prepare the inhibition control

1. Thaw the *Mycoplasma* Real-Time PCR DNA Control (positive control) completely. Applied Biosystems recommends thawing the positive control at 37 °C for 5 minutes to ensure consistent results.
2. Vortex, then spin down the positive control.
3. Label a separate microcentrifuge tube for each inhibition control (one tube for each unknown sample):

Sample Name	Control Name
Sample 1	INHC 1
Sample 2	INHC 2
...	...
Sample 12	INHC 12

4. Add the required volumes of each component to the appropriate inhibition control tube:

Component	Volume (µL) for 1 Reaction [‡]
Unknown sample DNA	11.0
<i>Mycoplasma</i> Real-Time PCR DNA Control	2.2
Total Inhibition Control Volume	13.2

[‡] Includes 10% excess to compensate for pipetting errors.

5. Mix each inhibition control by gently pipetting up and down, then cap each tube.
6. Vortex each inhibition control for 3 to 5 seconds, then centrifuge the tubes briefly.
7. Place the inhibition controls on ice until you prepare the reactions.

Prepare the positive control

1. Make sure the positive control is thawed completely, then vortex and spin down the tube (see [step 1](#) above).
2. Label an appropriately sized tube for the positive control: **POS 1**.
3. Add the required volumes of each component to the tube:

Component	Volume (µL) for 2 Reactions [‡]
<i>Mycoplasma</i> Real-Time PCR DNA Control	4.4
Negative Control (water)	22.0

Component	Volume (μL) for 2 Reactions [‡]
Total Positive Control Volume	26.4

[‡] Includes 10% excess to compensate for pipetting errors.

- Mix the positive control by gently pipetting up and down, then cap the tube.
- Vortex the positive control for 3 to 5 seconds, then centrifuge the tube briefly.
- Place the diluted positive control on ice until you prepare the reactions.

Prepare the negative control

- Label an appropriately sized tube for the negative control: **NEG 1**.
- Prepare the negative control according to the following table:

Component	Volume (μL) for 2 Reactions [‡]
Negative Control (water)	26.4
Total Negative Control Volume	26.4

[‡] Includes 10% excess to compensate for pipetting errors.

- Centrifuge the tube briefly to spin down the contents and eliminate air bubbles.
- Set the negative control aside until you prepare the reactions.

Prepare the premix solution

Prepare the premix solution using the components and volumes that were calculated by the AccuSEQ software (see [page 21](#)). For a Mycoplasma SEQ experiment, you prepare a premix solution that contains multiple primers designed to amplify the mycoplasma targets and a reagent to detect amplification of the targets.

Note: The premix solution includes all components *except* unknown sample, positive control DNA and negative control (water).

About the example experiment

All samples contain the same primers for the bacterial target (mycoplasmas); therefore, only one premix solution is required.

The premix solution components and volumes calculated in the software are:

Component	Volume (µL) for 1 Reaction
<i>Power</i> SYBR® Green PCR Master Mix (2X)	15.0
<i>Mycoplasma</i> Real-Time PCR Primer Mix (10X)	3.0
Total Premix Solution Volume	18.0

Note: The sample is not added at this time.

Required materials

- Premix solution components (listed above)
- Microcentrifuge tubes
- Pipettors
- Pipette tips
- Vortexer
- Centrifuge

Prepare the premix solution

For the following hazard, see the complete safety alert descriptions in [Appendix B on page 89](#).



WARNING! CHEMICAL HAZARD. *Power* SYBR® Green PCR Master Mix.

1. Thaw all kit reagents completely.
2. Vortex, then spin down the reagents.
3. Label an appropriately sized tube for the premix solution: **Premix Solution**.
4. Prepare the premix solution according to the following table.

IMPORTANT! Use a separate pipette tip for the *Power* SYBR® Green PCR Master Mix and the *Mycoplasma* Real-Time PCR Primer Mix.

Component	Volume (µL) for 40 Reactions [‡]
<i>Power</i> SYBR® Green PCR Master Mix (2X)	660.0
<i>Mycoplasma</i> Real-Time PCR Primer Mix (10X)	132.0
Total Premix Solution Volume	792.0

[‡] Includes 10% excess to compensate for pipetting errors.

5. Mix the premix solution by gently pipetting up and down, then cap the tube.
6. Centrifuge the tube briefly.

- Place the premix solution on ice until you prepare the reactions.

Prepare the reactions

Prepare the reactions using the volumes that were calculated by the AccuSEQ software (see [page 21](#)).

About the example experiment

For the example Mycoplasma SEQ experiment:

- A MicroAmp® Fast Optical 96-Well Reaction Plate is used.
- The reaction volume is 30 µL/well.
- The reaction plate contains:
 - 24 Unknowns **U**
 - 12 Inhibition Controls **IC**
 - 2 Positive Controls **P**
 - 2 Negative Controls **N**
- The following plate layout is used:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1 U Mycoplasma ...	Sample 1 U Mycoplasma ...	Sample 1 IC Mycoplasma ...	Sample 4 U Mycoplasma ...	Sample 4 U Mycoplasma ...	Sample 4 IC Mycoplasma ...	Sample 7 U Mycoplasma ...	Sample 7 U Mycoplasma ...	Sample 7 IC Mycoplasma ...	Sample 10 U Mycoplasma ...	Sample 10 U Mycoplasma ...	Sample 10 IC Mycoplasma ...
B	Sample 2 U Mycoplasma ...	Sample 2 U Mycoplasma ...	Sample 2 IC Mycoplasma ...	Sample 5 U Mycoplasma ...	Sample 5 U Mycoplasma ...	Sample 5 IC Mycoplasma ...	Sample 8 U Mycoplasma ...	Sample 8 U Mycoplasma ...	Sample 8 IC Mycoplasma ...	Sample 11 U Mycoplasma ...	Sample 11 U Mycoplasma ...	Sample 11 IC Mycoplasma ...
C	Sample 3 U Mycoplasma ...	Sample 3 U Mycoplasma ...	Sample 3 IC Mycoplasma ...	Sample 6 U Mycoplasma ...	Sample 6 U Mycoplasma ...	Sample 6 IC Mycoplasma ...	Sample 9 U Mycoplasma ...	Sample 9 U Mycoplasma ...	Sample 9 IC Mycoplasma ...	Sample 12 U Mycoplasma ...	Sample 12 U Mycoplasma ...	Sample 12 IC Mycoplasma ...
D												
E												
F												
G												
H	POS 1 P Mycoplasma ...	POS 1 P Mycoplasma ...									NEG 1 N Mycoplasma ...	NEG 1 N Mycoplasma ...

Wells: **U** Unknown 24 **N** Negative Control 2 **P** Positive Control 2 **IC** Inhibition Control 12 56 Empty

Required materials

- Samples and controls (from [page 27](#))
- Premix solution (from [page 30](#))
- Negative Control (water)
- MicroAmp® Fast Optical 96-Well Reaction Plate (PN 4346906, also called a *Fast reaction plate*)

- MicroAmp® Optical Adhesive Film (PN 4311971)
- Pipettors
- Pipette tips
- Centrifuge

Prepare the reactions

Use the plate layout and instructions to prepare the PCR reactions. Make sure the arrangement of PCR reactions matches the plate layout in the software:

1. Add 18 μ L of premix solution to the following wells: 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, H1, H2, H11, H12.
2. Prepare Sample reactions (Sample Type = UNKNOWN): Add 12 μ L of unknown sample DNA mixture (see [“Prepare the samples” on page 27](#)) to the appropriate wells.

Sample Name	Add 12 μ L Sample to Wells
Sample 1	A1, A2
Sample 2	B1, B2
Sample 3	C1, C2
Sample 4	A4, A5
Sample 5	B4, B5
Sample 6	C4, C5
Sample 7	A7, A8
Sample 8	B7, B8
Sample 9	C7, C8
Sample 10	A10, A11
Sample 11	B10, B11
Sample 12	C10, C11

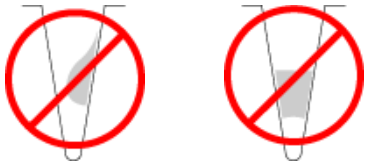
3. Prepare Inhibition Control reactions (Sample Type = INHC): Add 12 μ L of each unknown sample and positive control DNA mixture (see [“Prepare the inhibition control” on page 29](#)) to the appropriate wells.

Inhibition Control Name	Add 12 μ L Inhibition Control to Well
INHC 1	A3
INHC 2	B3
INHC 3	C3
INHC 4	A6
INHC 5	B6
INHC 6	C6
INHC 7	A9

Inhibition Control Name	Add 12 μ L Inhibition Control to Well
INHC 8	B9
INHC 9	C9
INHC 10	A12
INHC 11	B12
INHC 12	C12

4. Prepare Positive Control reactions (Sample Type = POS): Add 12 μ L of positive control DNA dilution (see “[Prepare the positive control](#)” on page 29) to wells H1 and H2.
5. Prepare Negative Control reactions (Sample Type = NTC): Add 12 μ L of Negative Control (water) to wells H11 and H12.
6. Seal the reaction plate with optical adhesive film (see [page 35](#)).
7. Centrifuge the reaction plate briefly to remove air bubbles.
8. Confirm that the liquid is at the bottom of each well of the reaction plate. If not, centrifuge the reaction plate again at a higher speed and for a longer period of time.

IMPORTANT! Do not allow the bottom of the reaction plate to become dirty. Fluids and other contaminants that adhere to the bottom of the reaction plate can contaminate the sample block and cause an abnormally high background signal.

Correct	Incorrect
	
Liquid is at the bottom of the well.	Not centrifuged with enough force, or Not centrifuged for enough time

9. Until you are ready to perform the run, place the reaction plate on ice in the dark.

Guidelines for your experiment

Reaction preparation guidelines

When you prepare your own Mycoplasma presence/absence reactions:

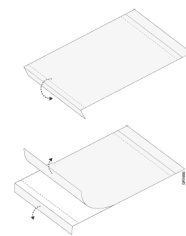
- Include excess volume in your calculations to compensate for the loss that occurs during reagent transfers.
- Include all required components.

- Keep the *Power SYBR*[®] Green PCR Master Mix (2X) protected from light, in the freezer, until you are ready to use it. Excessive exposure to light may affect the fluorescent SYBR[®] Green I dye.
- Prior to use:
 - Mix the master mix thoroughly by swirling the bottle.
 - Resuspend the primer mix by vortexing, then centrifuge the tube briefly.
 - Thaw the positive control DNA at 37 °C for 5 minutes to ensure consistent results.
 - Thaw frozen samples by placing them on ice. When thawed, resuspend the samples by vortexing, then centrifuge the tubes briefly.

Experiment preparation guidelines

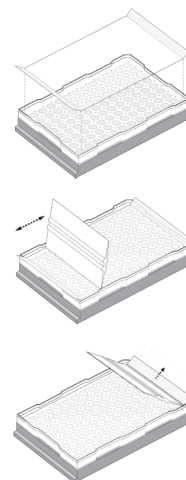
When you prepare your own Mycoplasma SEQ experiment:

- Wear appropriate protective eyewear, clothing, and gloves.
- Make sure you use the appropriate consumables. If you use:
 - **Fast reaction plates** (PN 4346906) – Seal the reaction plate with optical adhesive film (PN 4311971)
 - **Fast reaction tube strips** (PN 4358293) – Cover the tubes with optical caps (PN 4323032)
- Make sure the arrangement of the PCR reactions matches the plate layout in the AccuSEQ software. You can either:
 - Accept the plate layout automatically generated by the AccuSEQ software.
 - or*
 - Use the Swap and Click-Drag controls available in the Plate Layout screen to move samples to new well positions in the plate layout.
- If you use optical adhesive film, seal each reaction plate as follows:
 - a. Place the reaction plate onto the center of the 96-well base (PN N8010531).
 - b. Load the reaction plate as desired.
 - c. Remove a single optical adhesive film (film) from the box. Fold back one of the end-tabs. Hold the film with its backing side up.
 - d. In one continuous movement, peel back the white protective backing from the center sealing surface. Do not touch the center sealing surface.



IMPORTANT! Improper peeling of the optical adhesive film may result in haziness, but it does not affect results. Haziness disappears when the film comes into contact with the heated cover in the instrument.

- e. While holding the film by the end-tabs, lower the film onto the reaction plate (adhesive side facing the reaction plate). Be sure the film completely covers all wells of the reaction plate.
- f. While applying firm pressure, move the applicator (PN 4333183) slowly across the film, horizontally and vertically, to ensure good contact between the film and the entire surface of the reaction plate.
- g. While using the applicator to hold the edge of the film in place, grasp one end of the end-tab and pull up and away sharply. Repeat for the other end-tab.
- h. Repeat [step f](#) to ensure a tight, evaporation-free seal. While applying firm pressure, run the edge of the applicator along all four sides of the outside border of the film.



Note: Optical adhesive films do not adhere on contact. The films require the application of pressure to ensure a tight seal.

- i. Inspect the reaction plate to be sure all wells are sealed. You should see an imprint of all wells on the surface of the film.

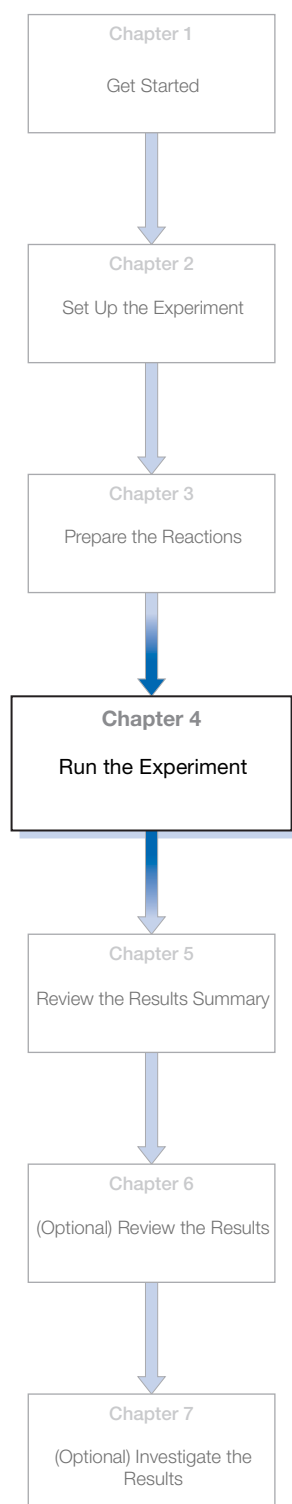
For more information

For more information on:

- Consumables – Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Maintenance Guide*.
- Using Swap and Click-Drag controls to change the plate layout – Access the *AccuSEQ Software Help* by clicking  or pressing **F1**.

4

Run the Experiment



This chapter covers:

■ Chapter overview	38
■ Prepare for the run	39
■ (Optional) Enable the notification settings	40
■ Start the run.	42
■ (Optional) Monitor the run	42
■ Unload the instrument.	47

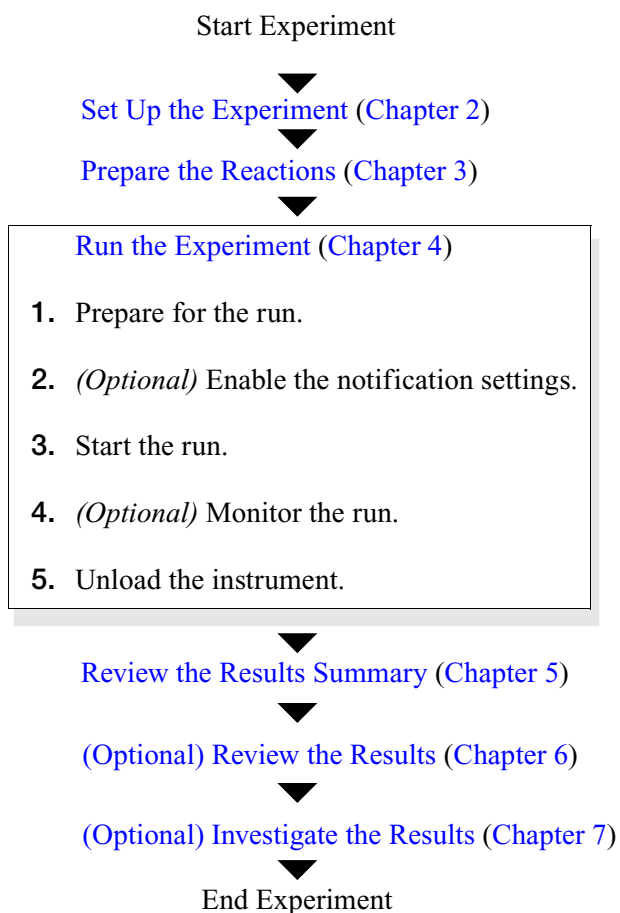
Note: For more information about any of the topics discussed in this guide, access the Help system by pressing **F1**, by clicking  in the toolbar of the AccuSEQ Software window, or by selecting **Help ▶ Contents and Index**.

Chapter overview

This chapter explains how to perform a run on the Applied Biosystems 7500 Fast Real-Time PCR System.

Example experiment workflow

The workflow for running the example experiment provided with this getting started guide is shown below.



Prepare for the run

Prepare for the run by opening the example experiment file you created in [Chapter 2](#), then loading the sealed reaction plate into the 7500 Fast instrument.

Open the example experiment

1. Double-click  (AccuSEQ Software).
2. Enter your user name and password, then click **OK**.

Note: See your system administrator for user name and password.

3. In the Home screen, click **Open Experiment**.
4. In the Open dialog box, navigate to the **experiments** folder (default):
<drive>:\Applied Biosystems\AccuSEQ\experiments
5. Double-click **Mycoplasma SEQ Example Setup.eds** to open the example experiment file you created in [Chapter 2](#).

Load the reaction plate into the instrument

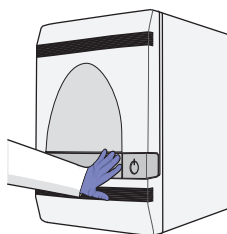


CAUTION! PHYSICAL INJURY HAZARD. During operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

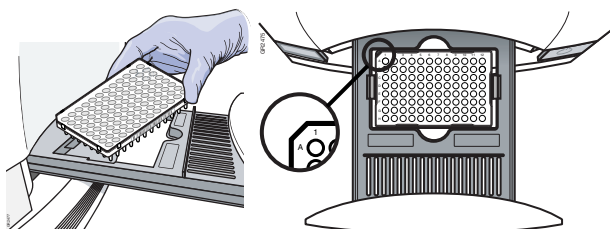


IMPORTANT! Wear powder-free gloves when you handle the reaction plate.

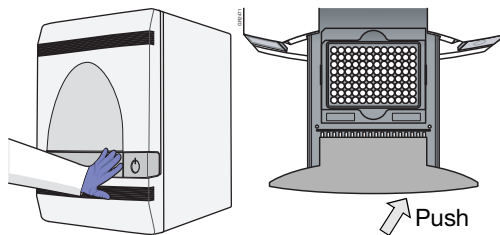
1. Push the tray door to open it.



2. Load the plate into the plate holder in the instrument with the notched A1 position at the top-left of the tray. Ensure that the plate is properly aligned in the holder.



3. Push the tray door to close it. Apply pressure to the right side of the tray door at an angle.



Guidelines for your experiment

IMPORTANT! Standard reaction plates and tube strips will not properly function and might be crushed when using the Fast sample block.

When you load the reactions into the instrument, if you use:

- **Fast reaction plates** – Place the reaction plate in the plate holder with well A1 at the back-left corner.
- **Fast reaction tube strips** – Place the tube strips in the plate holder for tube strips.

Note: For optimal performance with partial loads on a 7500 Fast instrument:

- Place empty tube strips in columns 1 and 12 to prevent crushing of tubes containing samples.
 - Place tube strips with samples in the plate holder vertically, starting in columns 6 and 7 and moving outward.
 - A maximum of 6 tube strips can be used in the 7500 Fast instrument. Leave columns 2, 3, 10, and 11 empty.
- **Reaction tubes** – Place the tubes in the plate holder.

IMPORTANT! Do not use MicroAmp® Fast Reaction Tubes (PN 4358297) in the 7500 Fast system.

(Optional) Enable the notification settings

Enable the notification settings so that the AccuSEQ software sends you e-mail when the 7500 Fast instrument begins and completes the run, or if an error occurs during the run. Enabling the notifications settings feature is optional and does not affect the performance of the 7500 Fast system or the duration of the run.

IMPORTANT! The notification settings feature is available only if the computer that you are using is running the 7500 Fast instrument *and* is connected to an Ethernet network.

Note: Run notifications are saved per user.

About the example experiment

In the example experiment:

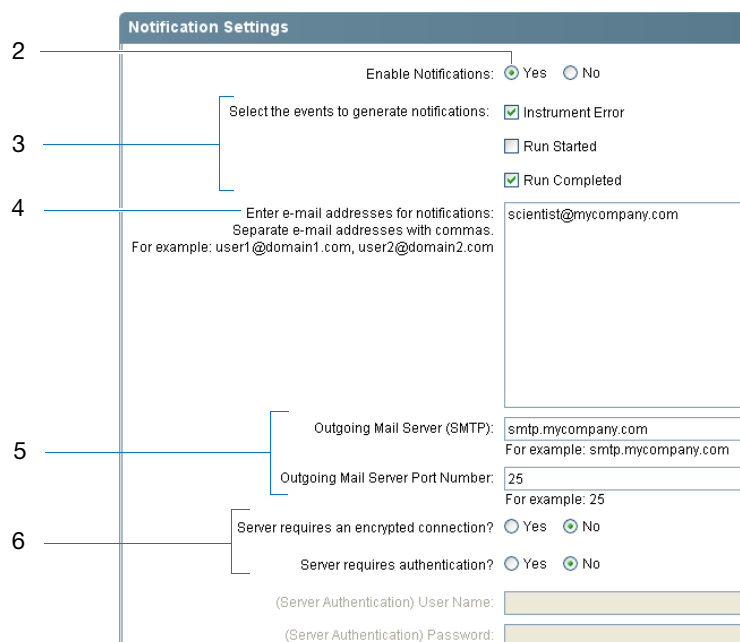
- The AccuSEQ software is set up to send notifications when the 7500 Fast system ends the run and if it encounters any errors during operation.
- The example outgoing mail server (SMTP) is not set up for secure sockets layer (SSL) encryption and does not require authentication for use.

Set up notifications

1. Click  **Run** in the navigation pane, then click  **Notification Settings**.
2. Select **Yes** to Enable Notifications.
3. Select the events that will trigger notifications:
 - **Instrument Error**
 - **Run Completed**
4. Enter e-mail addresses for notifications.
For example: **scientist@mycompany.com**.

Note: Separate addresses with a comma (,).

5. Set the outgoing mail server settings:
 - a. Enter the name of the Outgoing Mail Server (SMTP).
For example: **smtp.mycompany.com**.
 - b. Leave the default Outgoing Mail Server Port Number (25).
6. Set the server authentication settings:
 - a. Select **No** for Server requires an encrypted connection.
 - b. Select **No** for Server requires authentication.



The screenshot shows the 'Notification Settings' dialog box. On the left, a vertical line has numbers 2 through 6 with brackets pointing to specific settings. The settings are as follows:

- 2:** 'Enable Notifications:' with radio buttons for 'Yes' (selected) and 'No'.
- 3:** 'Select the events to generate notifications:' with checkboxes for 'Instrument Error' (checked), 'Run Started' (unchecked), and 'Run Completed' (checked).
- 4:** 'Enter e-mail addresses for notifications:' with a text field containing 'scientist@mycompany.com'. Below the field is a note: 'Separate e-mail addresses with commas. For example: user1@domain1.com, user2@domain2.com'.
- 5:** 'Outgoing Mail Server (SMTP):' with a text field containing 'smtp.mycompany.com'. Below it is a note: 'For example: smtp.mycompany.com'.
- 5:** 'Outgoing Mail Server Port Number:' with a text field containing '25'. Below it is a note: 'For example: 25'.
- 6:** 'Server requires an encrypted connection?' with radio buttons for 'Yes' (unchecked) and 'No' (selected).
- 6:** 'Server requires authentication?' with radio buttons for 'Yes' (unchecked) and 'No' (selected).
- Below the authentication settings are two empty text fields labeled '(Server Authentication) User Name:' and '(Server Authentication) Password:'.

Guidelines for your experiment

When you set up the 7500 Fast system for automatic notification:

- Your system must be set up for network use. Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Maintenance Guide*.
- Contact your systems administrator or information technology department if you need:
 - E-mail addresses for users who will receive notifications
 - A network address for a simple mail transfer protocol (SMTP) server on the LAN
 - A port number for the server, if different from the default (25)
 - A user name and password for the server, if required for access
 - The Secure Sockets Layer (SSL) setting of the server (on or off)

Start the run

IMPORTANT! While the 7500 Fast instrument is performing a run, do not create experiments, perform maintenance, print experiment data, or allow the computer to run antivirus software or to enter hibernation mode. Performing such activities while the instrument is running an experiment causes gaps in data collection.

To start your 7500 Fast instrument:

1. Click  **Run** in the navigation pane.
2. Click **START RUN**  at the top of any run screen.
3. Specify a reason for change, if prompted. For more information, see [“Security, Audit and E-Signature \(SAE\) for users” on page 84](#).

(Optional) Monitor the run

You can view the progress of the run in real time as described below. During the run, periodically view all three available plots (Amplification, Melt Curve, Temperature) from the AccuSEQ software for potential problems.

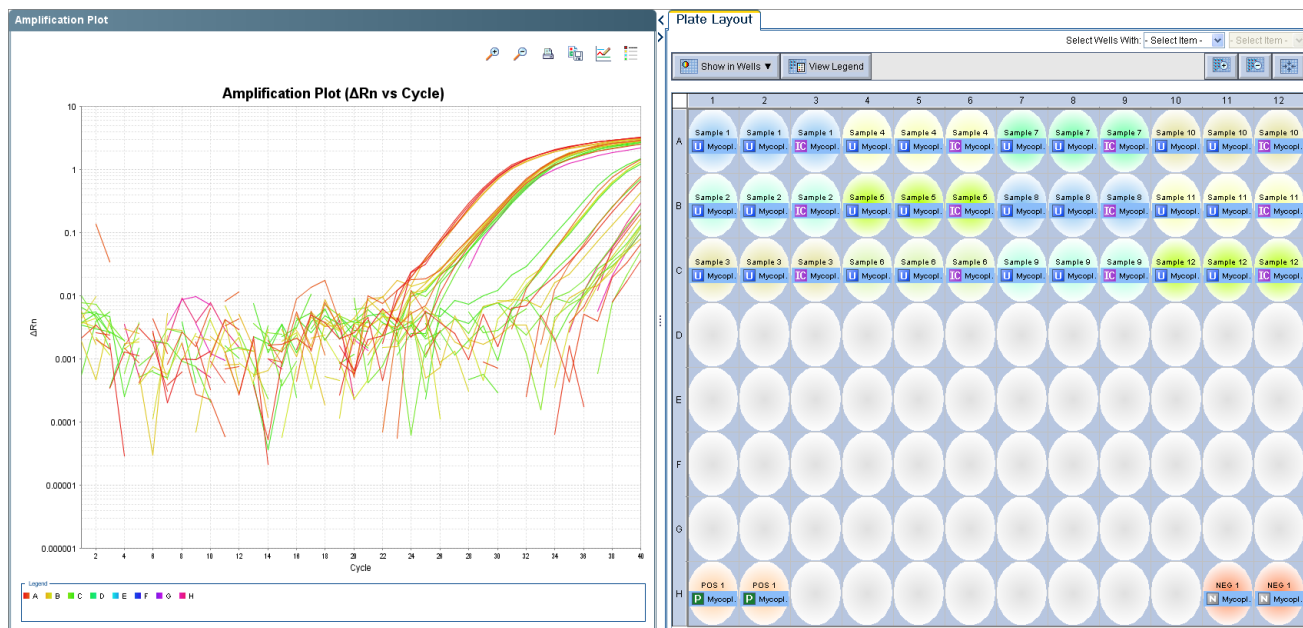
To...	Action
Stop the run	1. In the AccuSEQ software, click STOP RUN. 2. In the Stop Run dialog box, click one of the following: • Stop Immediately to stop the run immediately.. • Stop after Current Cycle/Hold to stop the run after the current cycle or hold. • Cancel to continue the run. IMPORTANT! You cannot resume a run after you stop it.
View amplification data in real time	Select  Amplification Plot . See “About the Amplification Plot screen” on page 43.
View the melt curve in real time	Select  Melt Curve . See “About the Melt Curve screen” on page 44.
View the temperature plot during the run	Select  Temperature Plot .
View progress of the run in the Run Method screen	Select  Run Method . See “About the Run Method screen” on page 45.
Enable/disable notifications in the Notification Settings screen	Select or deselect Enable Notifications . See “(Optional) Enable the notification settings” on page 40.

Note: You cannot change the sample setup or plate layout during a run.

About the Amplification Plot screen

The screen displays sample amplification (reporter signal) as your instrument collects fluorescence data during a run. If a method is set up to collect real-time data, the Amplification Plot screen displays the data for the wells selected in the Plate Layout tab. The plot contrasts the change in normalized reporter signal (ΔR_n) and cycle number. The figure below shows the Amplification Plot screen as it appears during the example experiment.

To view data in the Amplification Plot screen, select the wells to view in the Plate Layout tab.



The Amplification Plot screen is useful for identifying and examining abnormal amplification. Abnormal amplification can include the following:

- Increased fluorescence in negative control wells.
- Absence of detectable fluorescence at an expected cycle (determined from previous similar experiments run using the same reagents under the same conditions).

If you notice abnormal amplification or no signal in a well, troubleshoot the error as explained in the *AccuSEQ Software Help* (click  or press **F1**).

About the Melt Curve screen

The screen displays sample amplification products (reporter signal) as your instrument collects fluorescence data during a melt curve run, at the end of the cycling stage. If a method is set up to collect melt curve data, the Melt Curve screen displays the data for the wells selected in the Plate Layout tab. The plot contrasts normalized reporter signal (Rn) and temperature (°C). The figure below shows the Melt Curve screen as it appears during the example experiment.

To view data in the Melt Curve screen, select the wells to view in the Plate Layout tab.



The Melt Curve provides the melting temperature (T_m) of each target (Target T_m). The T_m information is used by the AccuSEQ software, along with the cycle threshold (C_T) and the derivative signal, to define the presence or absence of mycoplasma in the Unknown sample.

The Melt Curve screen is also useful for identifying and examining non-specific amplification. Non-specific amplification can include the following:

- Increased fluorescence in negative control wells.
- A melting temperature in the no-target range (No Target T_m).

If you notice non-specific amplification, troubleshoot the error as explained in the *AccuSEQ Software Help* (click  or press **F1**).

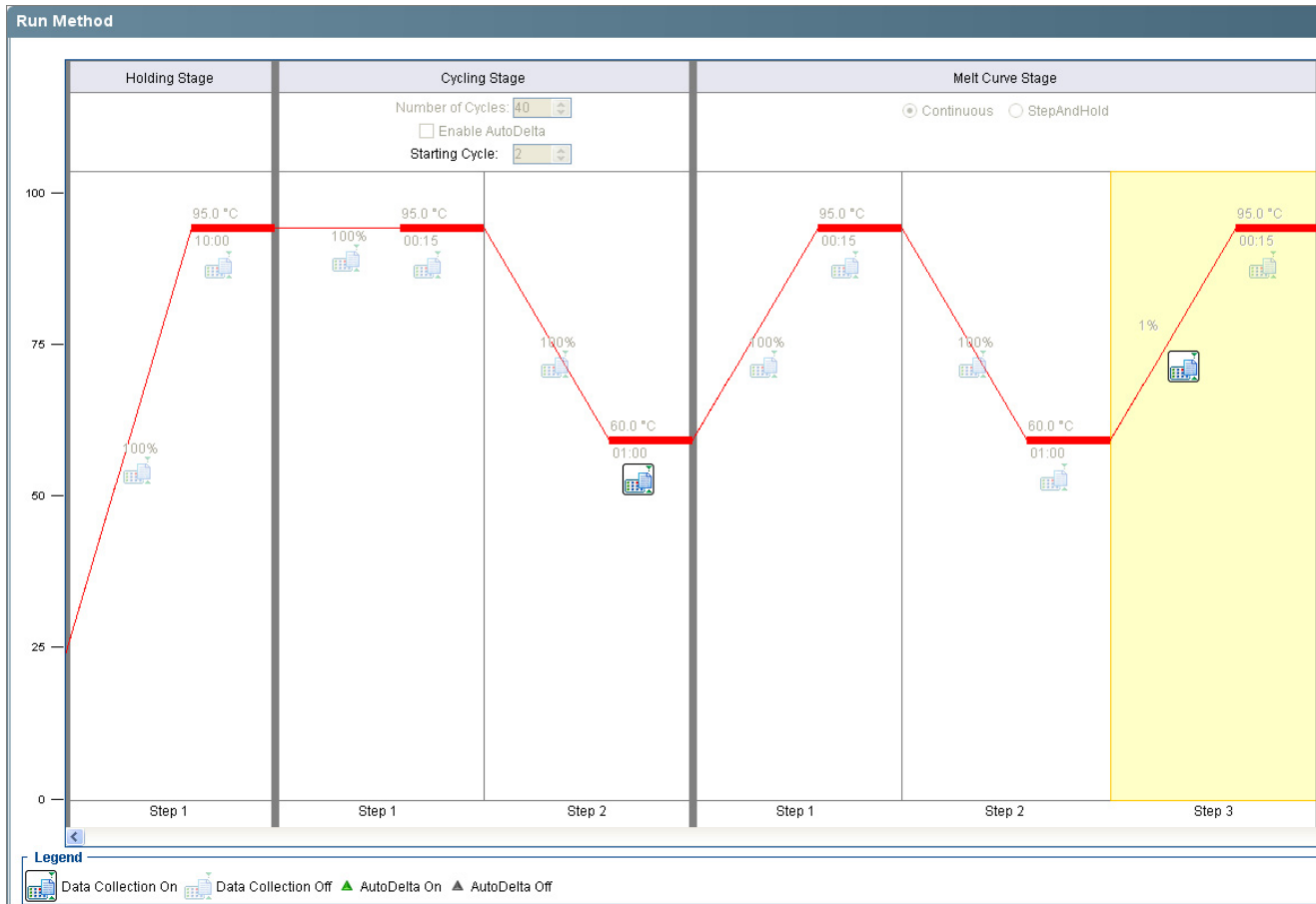
About the Run Method screen

The screen displays the run method selected for the run in progress. The software updates the Run Status field and displays progress bars throughout the run.

For Mycoplasma SEQ experiments:

- A pre-defined run method is used and cannot be edited.
- During the third stage, there is a delay between melting and completion of the third stage while the system analyzes the data.
- The Results Summary displays after the run is complete (see [Chapter 5](#)).

The figure below shows the Run Method screen as it appears in the example experiment.



If an alert appears, click the error for more information and troubleshoot the problem as explained in the *AccuSEQ Software Help* (click  or press **F1**).

Unload the instrument

When your 7500 Fast system displays the Run Complete message, unload the reaction plate from the instrument.

Note: When the instrument completes a run, the system saves the details of the run to the instrument log.

Unload the reaction plate

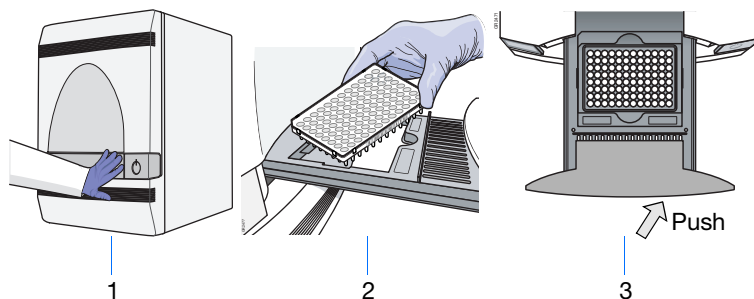


CAUTION! PHYSICAL INJURY HAZARD. During operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.



IMPORTANT! Wear powder-free gloves when you handle the reaction plate.

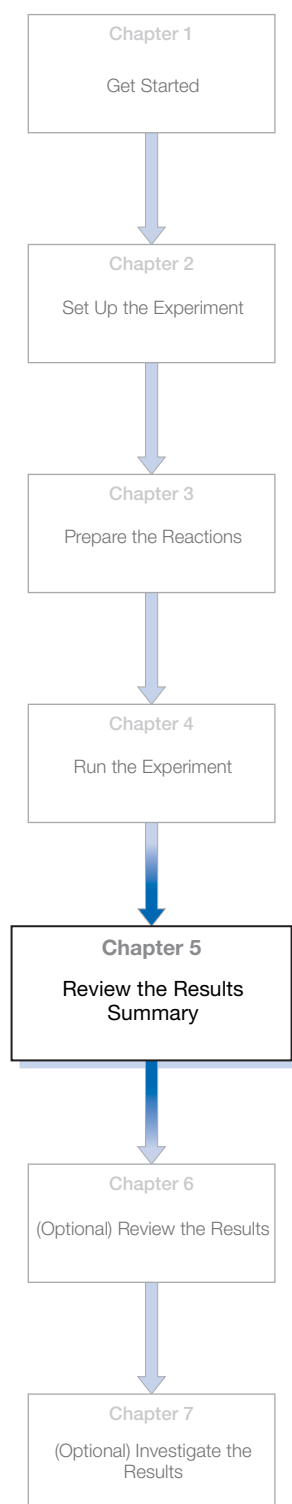
1. Push the tray door to open it.
2. Remove the reaction plate.
3. Push the tray door to close it.



4. Discard the reactions as appropriate.

5

Review the Results Summary



This chapter covers:

- Chapter overview 50
- About the analysis results 51
- Review the Results Summary 52
- Export and print the data. 55

Note: For more information about any of the topics discussed in this guide, access the Help system by pressing **F1**, by clicking  in the toolbar of the AccuSEQ Software window, or by selecting **Help ▶ Contents and Index**.

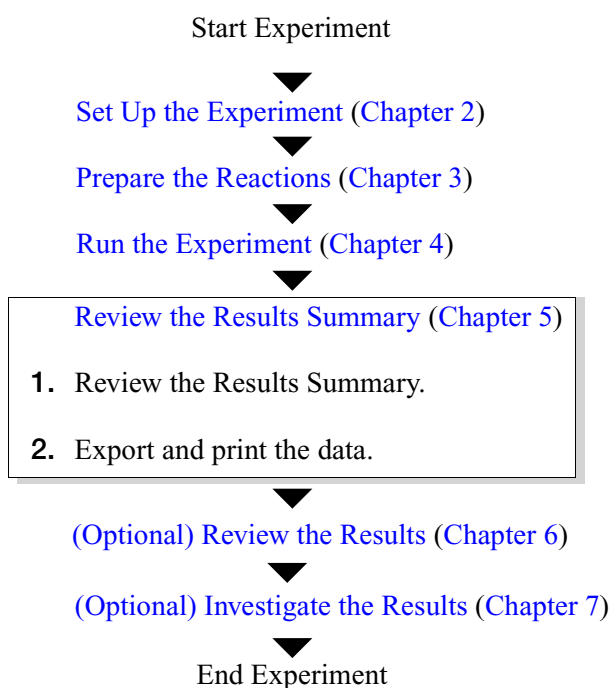
Chapter overview

The AccuSEQ software automatically analyzes your data using information from the instrument run. This chapter explains how to review the analysis results for the example experiment using the Results Summary screen and how to export and print data. If you receive questionable results, [Chapter 6](#) explains how to further review the results.

See “[Navigation tips](#)” on [page 86](#) for information on navigating within the analysis results screens.

Example experiment workflow

The workflow for reviewing the example experiment data provided with this getting started guide is shown below.



About the analysis results

About the analysis Immediately after the instrument run is complete, the AccuSEQ software automatically analyzes the SEQ experiment data using pre-defined analysis settings optimized for the selected SEQ assay, then displays presence/absence calls in the Results Summary on your computer.

About the example experiment The AccuSEQ software automatically analyzes the example experiment data using the default analysis settings for the mycoplasma target. To view the example Mycoplasma SEQ experiment results, open the data file that installs with the AccuSEQ software. The data file was created with the same design parameters that are provided in [Chapter 2](#), then run and analyzed on a 7500 Fast system.

You can find the data file for the example experiment on your computer:

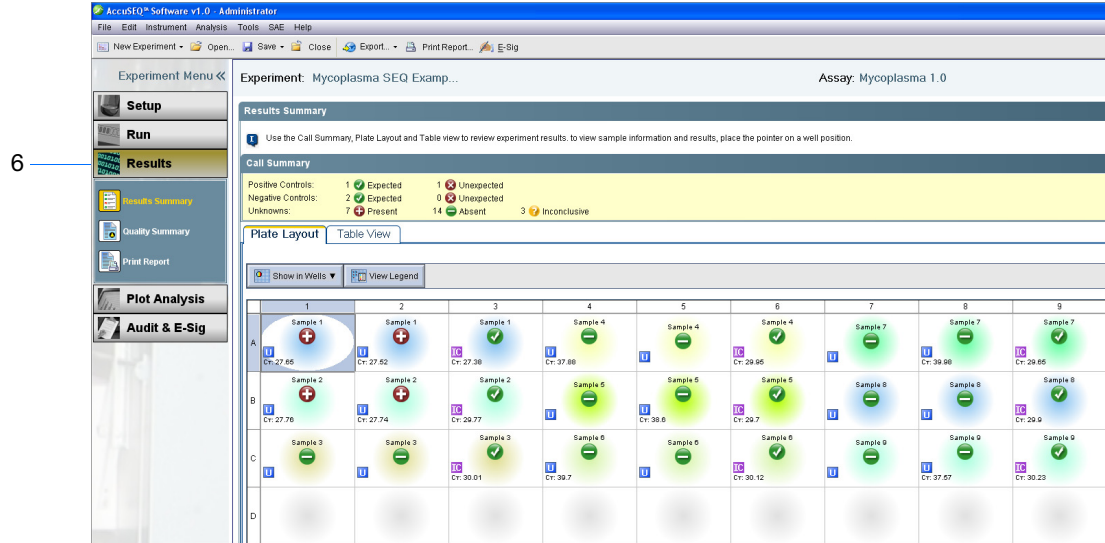
1. Double-click  (**AccuSEQ Software**).
2. Enter your user name and password, then click **OK**.

Note: See your system administrator for user name and password.

3. In the Home screen, click **Open Experiment**.
4. In the Open dialog box, navigate to the **experiments** folder (default):
<drive>:\Applied Biosystems\AccuSEQ\experiments
5. Double-click **Mycoplasma SEQ Example.eds** to open the example experiment data file.

Note: The experiments folder contains several data files; be sure to select *Mycoplasma SEQ Example.eds*.

6. Select  **Results** in the navigation pane to view the run results.



Guidelines for your experiment

When you review your own Mycoplasma SEQ experiment:

- Open a Mycoplasma SEQ experiment that contains run data.
- Review the run results in the Results Summary (see [page 53](#)).

Note: If a well is incorrectly identified when you review results (for example, you pipetted Sample 1 into the well labeled as Sample 2), you can move samples to the correct well positions, then re-analyze. See the *AccuSEQ Software Help* for more information.

- (Optional) To further review results, view the Quality Summary (see [page 59](#)).

Review the Results Summary

Use the Call Summary, Plate Layout and Table views in the Results Summary screen to review the experiment results.

About the example experiment

The presence/absence calls for the samples in the example Mycoplasma SEQ experiment are:

- 24 Unknowns **U** – 7 Present, 14 Absent, 3 Inconclusive
- 12 Inhibition Controls **IC** – 12 Expected
- 2 Positive Controls **P** – 1 Expected, 1 Unexpected
- 2 Negative Controls **N** – 2 Expected

For presence/absence call definitions, see [page 53](#).

IMPORTANT! The Mycoplasma SEQ experiment performs a presence/absence assay. The SEQ presence/absence assay is customized for detection of specific organisms and species and may yield different results than a custom presence/absence assay.

Review the Call Summary

The Call Summary at the top of the Results Summary screen lists a summary of results for the wells in the reaction plate.

- From the navigation pane, select  **Results** ►  **Results Summary**.
- Review the Call Summary for results:

Sample Type	Call [‡]	Description
Positive Control, Inhibition Control	 Expected	Target detected
	 Unexpected	No target detected (target should be detected)
Negative Control	 Expected	No target detected
	 Unexpected	Target detected (no target should be detected)
Unknown	 Present	Target signal is present and is within the temperature range, derivative range, and C _T range for the assay.
	 Absent	Target signal is not present within the temperature range, derivative range, and C _T range for the assay.
	 Inconclusive	The AccuSEQ software is unable to determine a result.

[‡] Causes of an unexpected or inconclusive result are displayed in the Results Summary (see [step 3 on page 54](#)) and the Quality Summary (see [page 59](#)).

Review the results in the plate layout

Use the Plate Layout to display Mycoplasma SEQ assay presence/absence calls, and to select the data displayed in the Table View.

- Select the **Plate Layout** tab in the Results Summary screen. The plate layout displays call information about each well in the reaction plate in an illustration (, , , , or ).

1 — **Plate Layout** | Table View

Select Wells With:

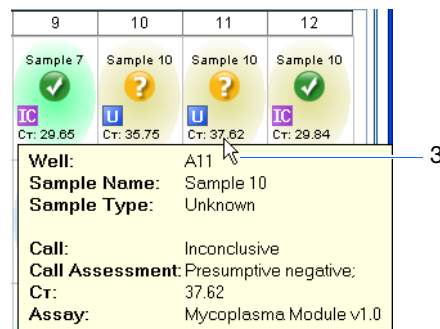
	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1  U Ct: 27.65	Sample 1  U Ct: 27.62	Sample 1  IC Ct: 27.38	Sample 4  U Ct: 37.88	Sample 4  U Ct: 29.95	Sample 4  IC Ct: 29.95	Sample 7  U Ct: 39.98	Sample 7  U Ct: 39.98	Sample 7  IC Ct: 29.65	Sample 10  U Ct: 35.75	Sample 10  U Ct: 37.62	Sample 10  IC Ct: 29.84
B	Sample 2  U Ct: 27.76	Sample 2  U Ct: 27.74	Sample 2  IC Ct: 29.77	Sample 5  U Ct: 38.6	Sample 5  U Ct: 29.7	Sample 5  IC Ct: 29.7	Sample 8  U Ct: 35.97	Sample 8  U Ct: 35.97	Sample 8  IC Ct: 29.9	Sample 11  U Ct: 35.97	Sample 11  U Ct: 36.03	Sample 11  IC Ct: 29.8
C	Sample 3  U Ct: 30.01	Sample 3  U Ct: 30.01	Sample 3  IC Ct: 30.01	Sample 6  U Ct: 39.7	Sample 6  U Ct: 30.12	Sample 6  IC Ct: 30.12	Sample 9  U Ct: 37.57	Sample 9  U Ct: 37.57	Sample 9  IC Ct: 30.23	Sample 12  U Ct: 35.21	Sample 12  U Ct: 36.11	Sample 12  IC Ct: 30.34
D												

2. In the Select Wells With drop-down list, select **Sample Type**, then select **Inhibition Control**. The AccuSEQ software selects the associated wells in the Plate Layout.



While not present in the example experiment, an inhibition control (IC) well can display an Unexpected result (?), indicating that inhibitors are present in the unknown sample.

3. To display the call assessment for an unexpected or inconclusive result, place the mouse pointer on a well. For the example experiment, display wells: **A10**, **A11**, **B11** and **H2**. The call assessment for well A11 is shown below.



4. (Optional) To further review unexpected or inconclusive results, view the Quality Summary (see [page 59](#)).

Review the results in the table view

Use the Table View to select, group or sort the results data for each well in the reaction plate (see [page 70](#)).

Export and print the data

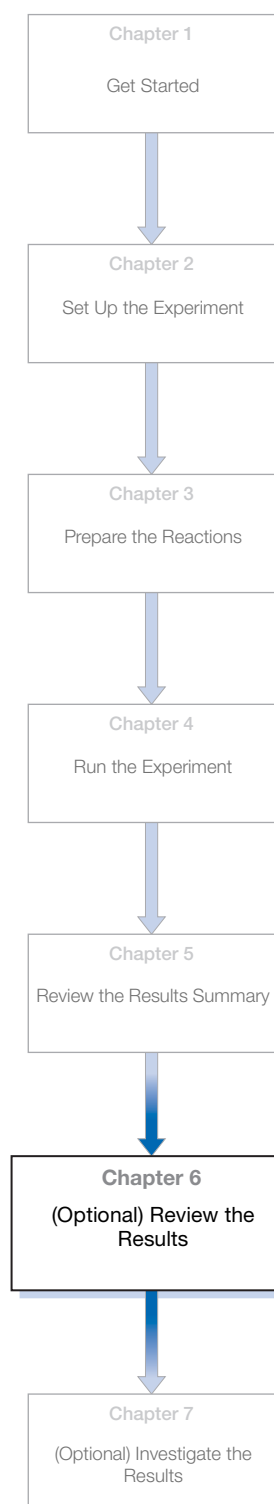
You can publish the experiment data in several ways:

- Export data
- Print the plate layout
- Create a slide of the plate layout
- Print a report

For more information

For information on performing these procedures, access the *AccuSEQ Software Help* by clicking  or pressing **F1**.

6

(Optional) Review the Results

This chapter covers:

■ Chapter overview	58
■ About the analysis results	59
■ Review the Quality Summary	59
■ Review the Amplification Plot	62
■ Review the Melt Curve	67
■ Review the table data	70
■ Export and print the data.	72

Note: For more information about any of the topics discussed in this guide, access the Help system by pressing **F1**, by clicking  in the toolbar of the AccuSEQ Software window, or by selecting **Help ▶ Contents and Index**.

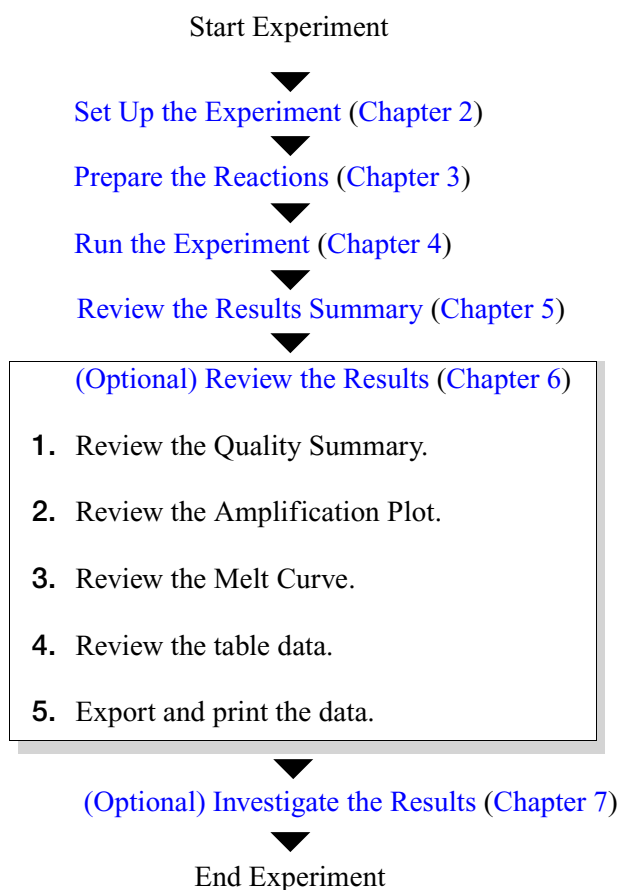
Chapter overview

In addition to reporting the presence/absence calls in the Results Summary immediately after an instrument run completes, the AccuSEQ software provides a call assessment and troubleshooting information for any questionable results in the Quality Summary. This chapter explains how to further review the analysis results of the example experiment using the Quality Summary and several of the plot analysis screens, and how to export and print data. If further investigation is required, [Chapter 7](#) explains how to review the remaining plots and the analysis settings.

See “[Navigation tips](#)” on [page 86](#) for information on navigating within the analysis results screens.

Example experiment workflow

The workflow for reviewing the analysis results for the example experiment data provided with this getting started guide is shown below.



About the analysis results

About the results	The Quality Summary provides a call assessment description and troubleshooting information for any inconclusive or unexpected results shown in the Results Summary. Use the Quality Summary table, the Amplification Plot and Melt Curve screens, and the Table View tab to review the call assessments for the plate.
About the example experiment	To review the example Mycoplasma SEQ experiment results, open the data file that installs with the AccuSEQ software (see page 6). For the example experiment, investigate the results for wells: A10 , A11 , B11 and H2 .
Guidelines for your experiment	When you review the results for your own Mycoplasma SEQ experiment: • Open a Mycoplasma SEQ experiment that contains run data. • Review the run results in the Results Summary (see Chapter 5). • Review questionable results using the Quality Summary (see page 60), then review the appropriate plots (see page 62). • <i>(Optional)</i> To further investigate questionable results, see Chapter 7.

Review the Quality Summary

The Quality Summary displays a list of the AccuSEQ software call assessments. This summary includes the call assessment frequency and location for the open experiment.

Possible call assessments	For Mycoplasma SEQ experiments, the call assessments listed below may be triggered by the experiment data.
----------------------------------	--

Call Assessment	Description
Unknown DNA in negative control	Unknown DNA signal is detected in negative-control wells.
No expected DNA in positive and/or inhibition control	No expected DNA signal is detected in positive-control wells and/or inhibition-control wells.
Presumptive positive [‡]	Sample is determined to be presumptive positive.
Presumptive negative [‡]	Sample is determined to be presumptive negative.
Unexpected result in inhibition control	The corresponding inhibition control(s) yield Unexpected result.
Sample inhibits amplification	The unknown sample is negative and the inhibition control shows a $\Delta C_T > 3$ when compared to the positive control.
High background signal	The background fluorescence signal is higher than 500,000 fluorescent standard units (FSU).
Baseline algorithm failed	The software cannot calculate the best fit baseline for the data.
Thresholding algorithm failed	The software cannot calculate a threshold.
Bad passive reference signal	Passive reference signal is abnormal.
Fluorescence is offscale	Fluorescence exceeds the instrument's maximum detectable range for one or more cycles.
All positive controls failed	All positive controls yield Unexpected result.
All negative controls failed	All negative controls yield Unexpected result.
No inhibition control found	No inhibition control(s) found for the unknown sample.
Unable to determine the SYBR call	The SYBR signal cannot be interpreted.
No signal in well	The well produced very low or no fluorescence.
C_T algorithm failed	The software cannot calculate C_T .
Exponential algorithm failed	The software cannot identify the exponential region of the amplification plot.

[‡] A sample that is determined to be presumptive must be repurified, and the assay must be repeated. See the *MicroSEQ Mycoplasma Real-Time PCR Detection Kit Protocol* for more information.

IMPORTANT! The Mycoplasma SEQ experiment performs a presence/absence assay. The SEQ presence/absence assay is customized for detection of specific organisms and species and may yield different results than a custom presence/absence assay.

About the example experiment

In the example Mycoplasma SEQ experiment, you review the Quality Summary for any call assessments generated by the experiment data. In the example experiment, call assessments are generated for wells: **A10**, **A11**, **B11** and **H2**.

View the Quality Summary

1. Click  **Results** in the navigation pane, then click  **Quality Summary**.

- In the Quality Summary table, look in the Frequency and Wells columns to determine which call assessments appear in the experiment.
- Click a call assessment in the table to display more information about the assessment. The call assessment for well A11 in the example experiment is shown below.

Quality Summary

Call Assessment	Frequency	Wells
Unknown DNA in negative control	0	
No expected DNA in positive and/or inhibition control	1	H2
Presumptive positive	2	A10 , B11
Presumptive negative	1	A11
Unable to determine SYBR call	0	
Unexpected result in inhibition control	0	
Sample inhibits amplification	0	
High background signal	0	
Baseline algorithm failed	0	
Thresholding algorithm failed	0	
Bad passive reference signal	0	
Exponential algorithm failed	0	
All positive controls failed	0	
All negative control failed	0	

Call Assessment Presumptive negative

Description Sample is determined to be presumptive negative.

Wells A11

[View Troubleshooting Information](#)

- Click a troubleshooting link below the table to view information on correcting the call assessment. The troubleshooting information for well A11 in the example experiment is shown below.

Applied Biosystems AccuSEQ Software v1.0 Help

Presumptive positive	Sample is determined to be presumptive positive.	Low concentrations of <i>Mycoplasma</i> in the samples.	Regrow the culture for an additional 24 hours. Repurify the sample and repeat the experiment using properly stored assay components.
Presumptive negative	Sample is determined to be presumptive negative.		
Unexpected result in inhibition control	The corresponding inhibition control(s) yield Unexpected result.	The unknown sample is negative and the inhibition control shows a $\Delta CT > 3$ when compared to the positive control. Inhibitors were carried over from the original sample.	Repurify the sample and repeat the experiment.
		No positive control was added to the inhibition control well	Repeat the experiment with positive control correctly added to inhibition control well.
		Incorrect volume of . . .	

- To investigate the data for results, review plots (see [page 62](#)).

Guidelines for your experiment

When you review your own Mycoplasma SEQ experiment:

- Click each call assessment in the Flag Details table with a frequency > 0 to display detailed information about the call assessment.
- If needed, click the troubleshooting link to view information on correcting the call assessment.

For more information

For more information on the Quality Summary screen or on call assessments, access the *AccuSEQ Software Help* by clicking  or pressing **F1**.

Review the Amplification Plot

The amplification plot displays amplification of all samples in the selected wells. There are three amplification plot views available:

- **ΔR_n vs Cycle** – ΔR_n is the magnitude of the normalized fluorescence signal generated by the reporter at each cycle during the PCR amplification. This plot displays ΔR_n as a function of cycle number. You can use this plot to identify and examine irregular amplification and to view threshold and baseline values for the run.
- **R_n vs Cycle** – R_n is the fluorescence signal from the reporter dye normalized to the fluorescence signal from the passive reference. This plot displays R_n as a function of cycle number. You can use this plot to identify and examine irregular amplification.
- **C_T vs Well** – C_T is the PCR cycle number at which the fluorescence signal equals the threshold in the amplification plot. This plot displays C_T as a function of well position. You can use this plot to locate outlying amplification (outliers).

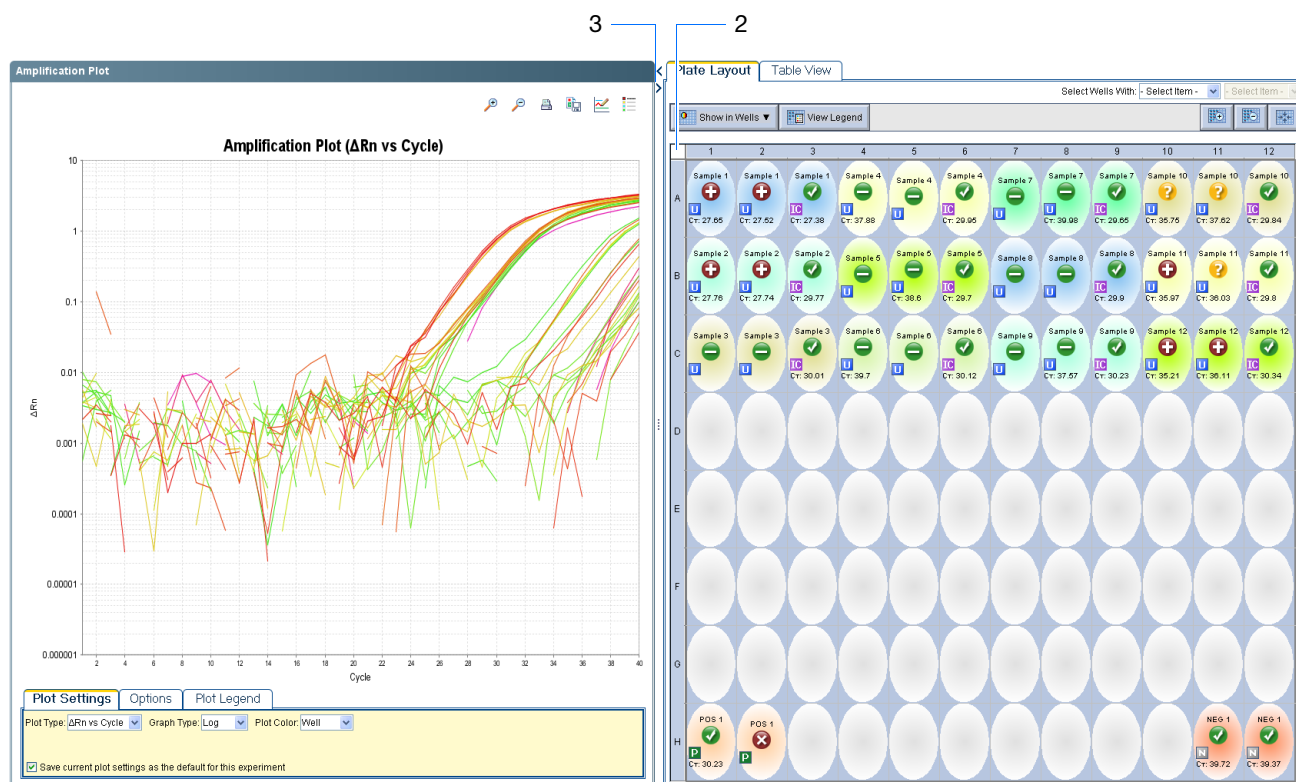
About the example experiment

You can examine the amplification plot to help with troubleshooting and quality control. In the example Mycoplasma SEQ experiment, you review the amplification plot for:

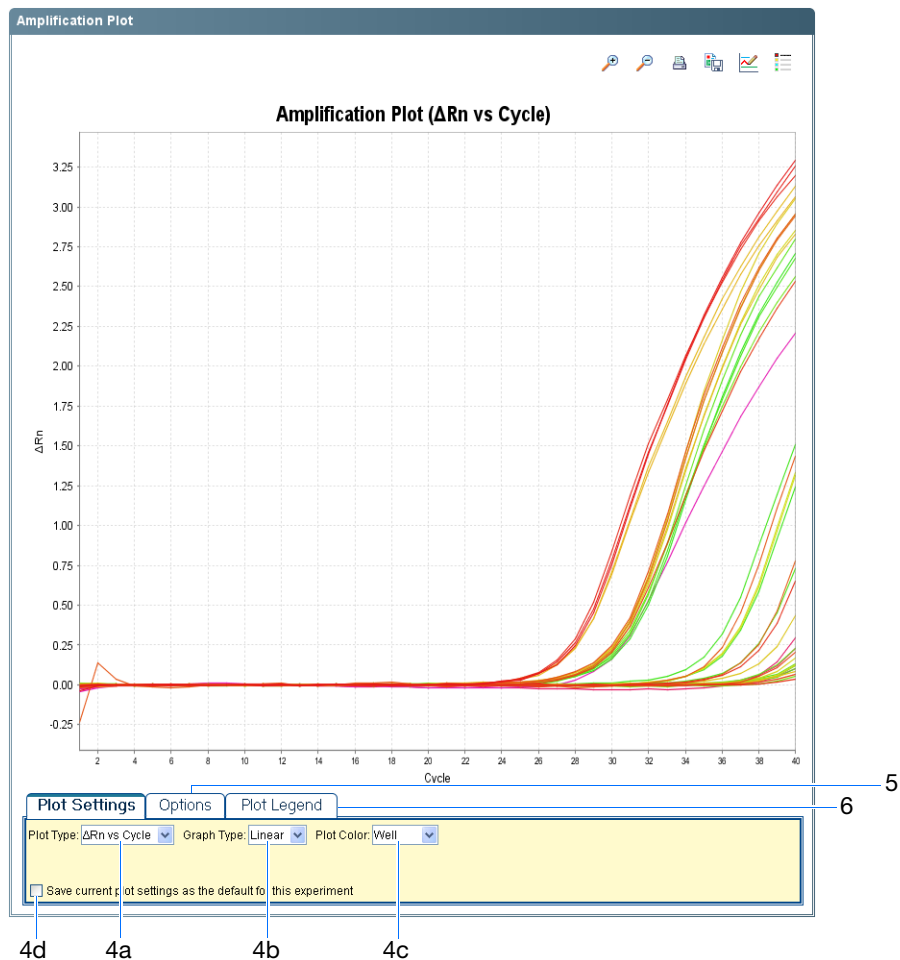
- C_T values
- Irregular amplification
- Outliers

View the Amplification Plots

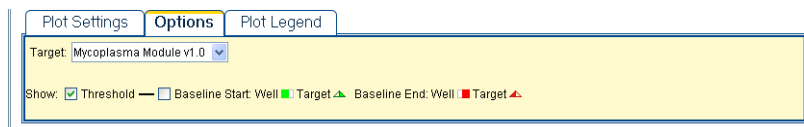
1. From the navigation pane, select **Plot Analysis**  **Amplification Plot**.
2. Display all 96 wells in the amplification plot by clicking the upper left corner of the plate layout in the Plate Layout tab.
3. (Optional) For an expanded display of the Amplification Plot, click  in the upper left corner of the plate layout in the Plate Layout tab.



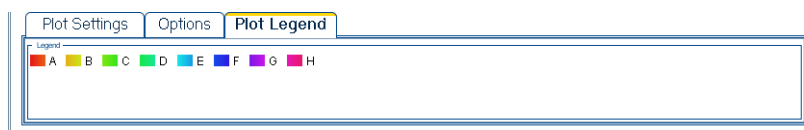
4. Select the **Plot Settings** tab, and adjust the plot display:
 - a. From the Plot Type drop-down list, select **ΔRn vs Cycle**.
 - b. From the Graph Type drop-down list, select **Linear**.
 - c. From the Plot Color drop-down list, select **Well**.
 - d. (Optional) Deselect the **Save current plot settings as the default for this experiment** checkbox.



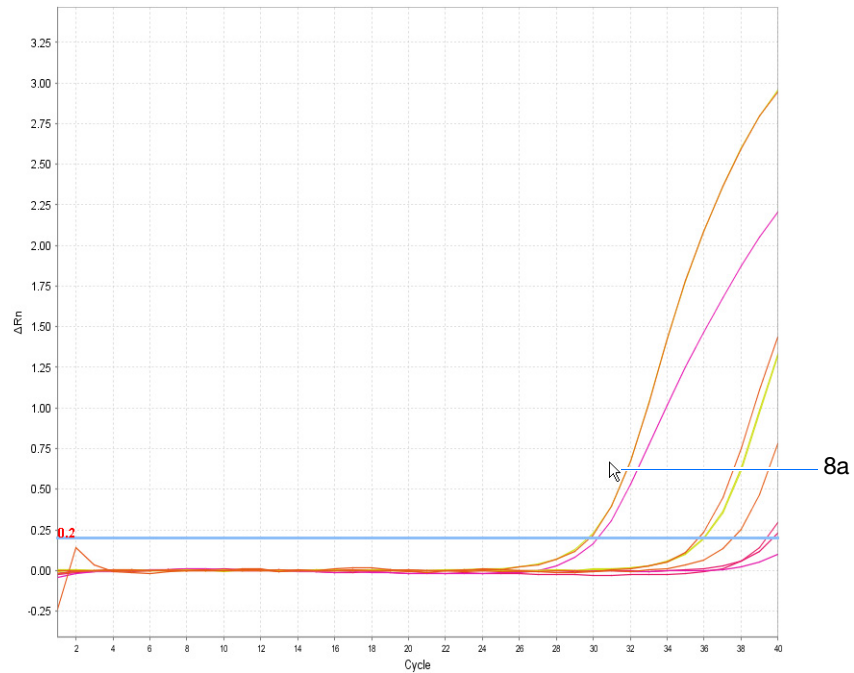
5. Select the **Options** tab, then select to show the **Threshold (0.2)**.



6. (Optional) Select the **Plot Legend** tab to view the well colors assigned to each row in the plate layout.

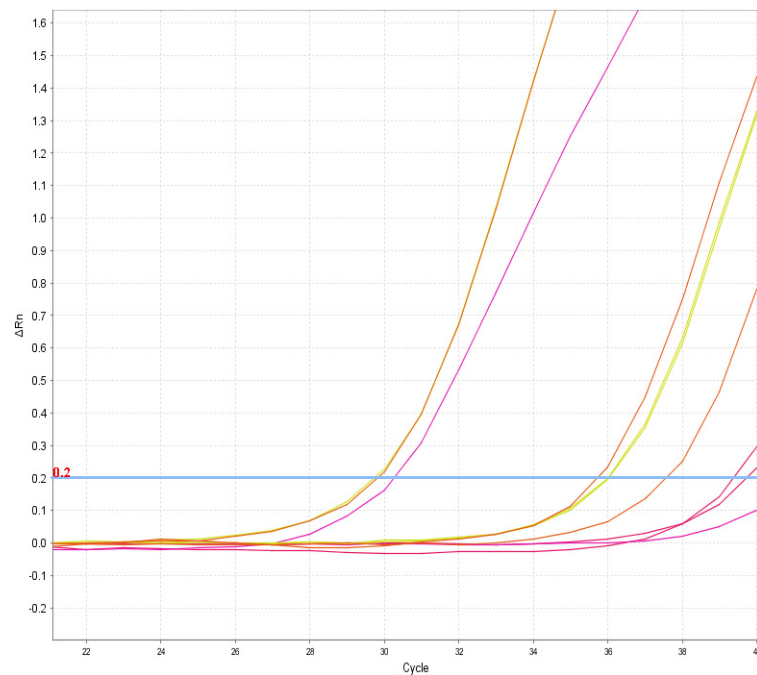


7. Select wells in the plate layout to view in the amplification plot (see [page 86](#) for tips). For the example experiment, display the following replicate wells: **A10-A12** (Sample 10), **B10-B12** (Sample 11), **H1-H2** (POS 1), and **H11-H12** (NEG 1). The amplification plots for wells A10-A12, H1-H2, and H11-H12 are shown below.



While not present in the example experiment, an inhibition control (IC) well can display a delay in amplification (C_T value) as compared to the positive control (POS) well(s), indicating that inhibitors are present in the unknown sample.

8. (Optional) To view an area of the amplification plot in detail, click:
- A quadrant in the plot to the left of the area you want to review.
 -  (Zoom in).



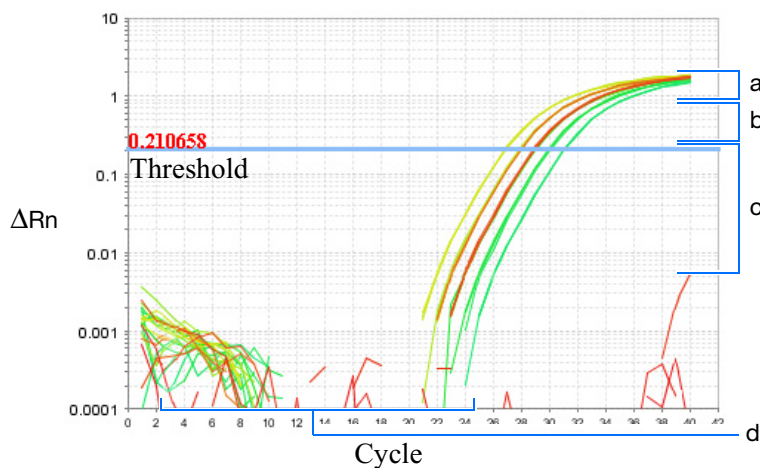
9. (Optional) Click  (Hide the plot legend).

Note: This is a toggle button. When the legend is hidden, the button changes to Show a legend for the plot.

Guidelines for your experiment

When you review the amplification plot for your own Mycoplasma SEQ experiment, look for:

- Outliers
- A typical amplification plot with four distinct sections:
 - a. Plateau phase
 - b. Linear phase
 - c. Exponential (geometric phase)
 - d. Baseline



- Correct baseline and threshold values

IMPORTANT! Experimental error (such as contamination or pipetting errors) or other reaction conditions can produce atypical amplification curves that can result in incorrect baseline and threshold value calculations. Therefore, Applied Biosystems recommends that you examine the Amplification Plot and review the assigned baseline and threshold values for each unexpected and inconclusive well after analysis completes.

If your experiment does not meet the guidelines above, you can:

- Adjust the baseline and/or threshold (see [page 80](#)).
- or
- Omit wells (see [page 81](#)).

For more information

For more information on the Amplification Plot screen, access the *AccuSEQ Software Help* by clicking  or pressing **F1**.

Review the Melt Curve

About the Melt Curve

The Melt Curve plot displays the fluorescence peak data collected at each well position during the melt curve stage of an instrument run. For Mycoplasma SEQ experiments, the AccuSEQ software determines the melting temperature (T_m) and reporter signal (T_m height) for each peak.

Peaks in the melt curve can indicate the T_m of the target (Target T_m) or can identify non-specific PCR amplification (No Target T_m), where:

- The Target T_m is the temperature at which 50% of the DNA is double-stranded and 50% of the DNA is dissociated into single-stranded DNA.
- The No Target T_m is a T_m in the no-target range.

You can view the melt curve as normalized reporter (Rn) vs. temperature, or as derivative reporter (-Rn') vs. temperature.

About the example experiment

In the example Mycoplasma SEQ experiment:

- For the 24 UNKNOWN wells:
 - The mycoplasma target is present in Samples 1, 2, 11 (one replicate), and 12.
 - The mycoplasma target is absent in Samples 3, 4, 5, 6, 7, 8, and 9.
 - The AccuSEQ software is unable to determine a result for Samples 10 and 11 (one replicate).
- There are no inhibited INHC wells.
- One POS well produced an unexpected result.
- All NTC wells produced expected results.

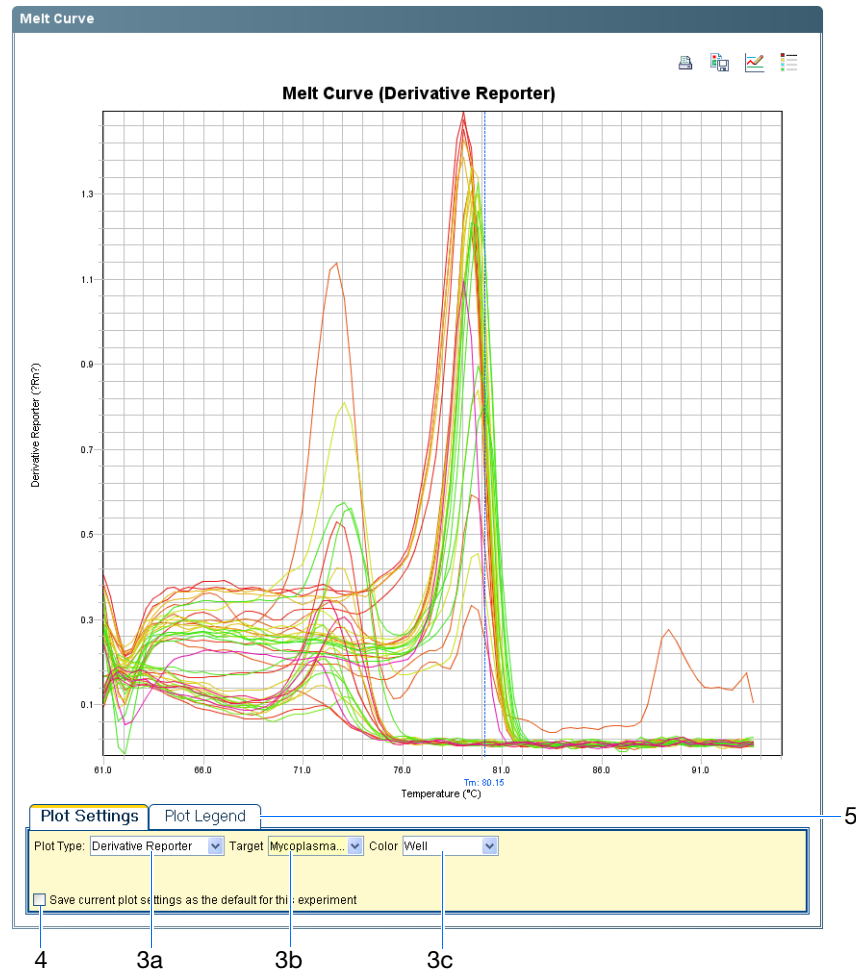
View the Melt Curve

Click a sample in the plate layout or the well table to display the corresponding plots.

1. From the navigation pane, select **Plot Analysis**  **Melt Curve**.
2. (Optional) For an expanded display of the 96 wells of the Melt Curve, click  in the upper left corner of the plate layout in the Plate Layout tab.



3. In the Plot Settings tab, select:
 - a. **Plot Type** – Derivative Reporter
 - b. **Target** – Mycoplasma Module v1.0
 - c. **Color** – Well



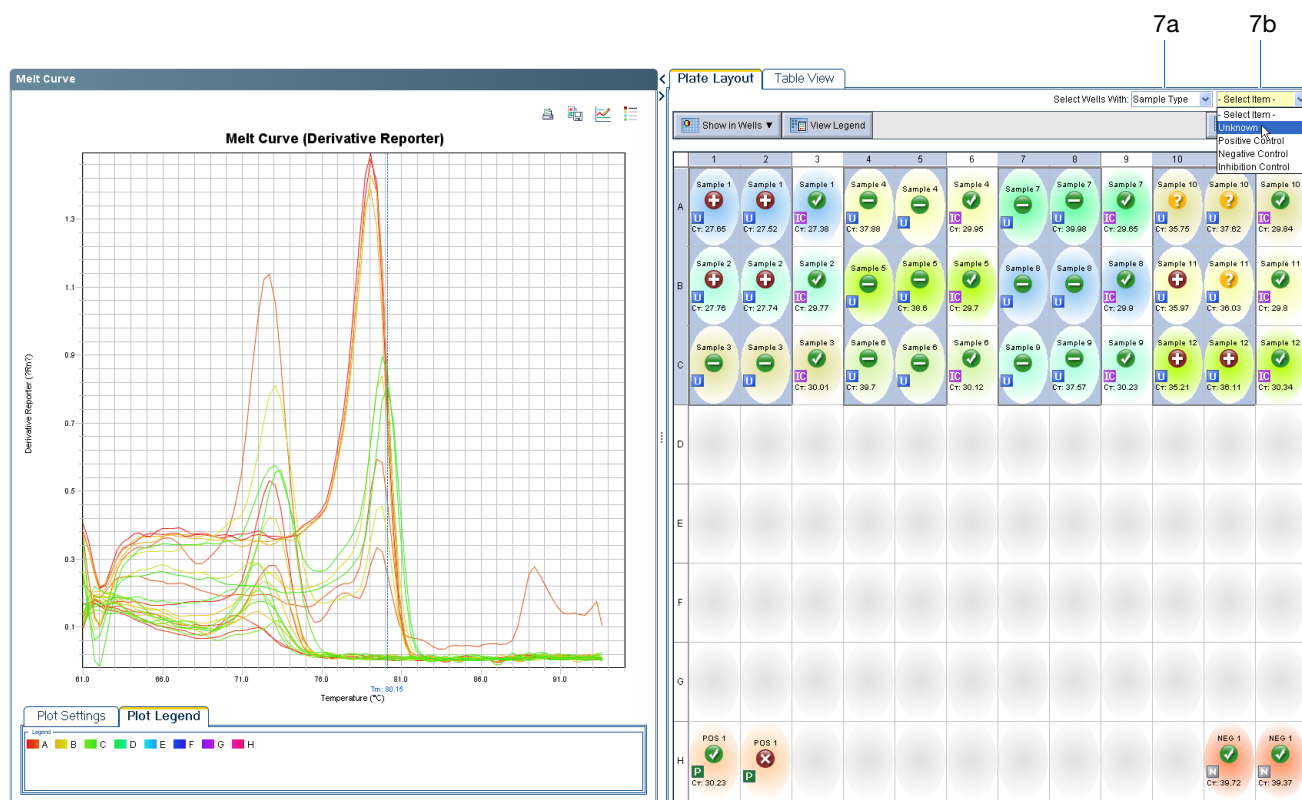
4. (Optional) Deselect the **Save current plot settings as the default for this experiment** checkbox.
5. (Optional) Select the **Plot Legend** tab to view the well colors assigned to each row in the plate layout.
6. (Optional) Click  (Hide the plot legend).

Note: This is a toggle button. When the legend is hidden, the button changes to Show a legend for the plot.

7. Select wells in the plate layout to view in the melt curve plot:
 - a. In the first Select Wells With drop-down list, select **Sample Type**.
 - b. In the second Select Wells With drop-down list, select:
 - **Negative Control** to view only NTC wells. The example experiment contains expected results for both NTC wells.
 - **Positive Control** to view only POS wells. The example experiment contains an unexpected result for one of the POS replicate wells.

- **Inhibition Control** to view only INHC wells. The example experiment contains expected results for all of the INHC wells.
- **Unknown** to view only UNKNOWN wells. The example experiment contains inconclusive results for three UNKNOWN wells.

The melt curve plot for the UNKNOWN wells in the example experiment is shown below.



The T_m value displayed below the melt curve plot is the T_m associated with the largest peak in the data, which may be the Target T_m or the No Target T_m . You can determine the Target T_m by viewing the table data (see [page 70](#)).

Note: The T_m value displayed below the plot may not exactly match the values displayed in the well table because of rounding.

Guidelines for your experiment

When you review your own Mycoplasma SEQ experiment, review the Melt Curve plot for:

- **The Target T_m** – The T_m is in the expected range.
- **The No Target T_m** – The T_m is in the no-target range, which may indicate non-specific amplification.
- **Unknown Calls:**
 - Present
 - Absent
 - Inconclusive

- **Control Well Calls:**
 -  Expected
 -  Unexpected

For more information

For more information on the Melt Curve screen, access the *AccuSEQ Software Help* by clicking  or pressing **F1**.

Review the table data

The Table View displays results data for each well in the reaction plate, including:

- The well number, sample name, sample type, assay, call, call assessment, and cycle 1 fluorescence (FSU)
- The calculated values: C_T , target T_m , target T_m height, no-target T_m , no-target T_m height, and ΔR_n

Note: The cycle 1 fluorescence and ΔR_n values are only available for display in the Table View when it is accessed from the Plot Analysis screens.

About the example experiment

For the example Mycoplasma SEQ experiment, group the wells in the Table View by:

- Call
- Call assessment

View the Table View

1. From the navigation pane, select **Plot Analysis**, then select the **Table View** tab.
2. (Optional) For an expanded display of the Table View, click  in the upper left corner of the table in the Table View tab.
3. Use the Group By drop-down list to group wells by a specific category.

Note: You can select only one category at a time.

For the example experiment:

- a. From the Group By drop-down list, select **Call**. Wells are listed in the order:
 - Absent
 - Expected
 - Inconclusive
 - Present
 - Unexpected
 - Empty Well (no call)

3a

Plate Layout Table View

Select Wells With: - Select Item - - Select Item -

Show in Table Group By

Well Position (Row)
Well Position (Column)
Call

#	Well Position	Sample Name	Sample Type	Ct	Call	Call Assessment	Target Tm	Target Tm Height	No-Target Tm	No-Target Tm Height
1	Absence									
2	A4	Assay		37.884	Absence				72.879	0.53
3	A5				Absence				61.709	0.171
4	A7				Absence				61.709	0.18
5	A8			39.982	Absence				72.879	0.284
6	B4				Absence				61.886	0.193
7	B5	Sample 5	Unknown	38.605	Absence				72.879	0.425
8	B7	Sample 8	Unknown		Absence				72.347	0.234
9	B8	Sample 8	Unknown		Absence				72.879	0.262
10	C1	Sample 3	Unknown		Absence				71.992	0.208
11	C2	Sample 3	Unknown		Absence				61.709	0.178
12	C4	Sample 6	Unknown	39.7	Absence				71.992	0.288
13	C5	Sample 6	Unknown		Absence				71.815	0.224
14	C7	Sample 9	Unknown		Absence				61.709	0.217
15	C8	Sample 9	Unknown	37.573	Absence				73.234	0.565
16	Expected									
17	A3	Sample 1	Inhibition Control	27.378	Expected		79.084	1.452	72.347	0.327
18	A6	Sample 4	Inhibition Control	29.947	Expected		79.439	1.213	64.546	0.256
19	A9	Sample 7	Inhibition Control	29.65	Expected		79.439	1.347	64.014	0.293
20	A12	Sample 10	Inhibition Control	29.842	Expected		79.439	1.345	72.524	0.344
21	B3	Sample 2	Inhibition Control	28.773	Expected		79.439	1.307	64.369	0.293
22	B6	Sample 5	Inhibition Control	29.703	Expected		79.616	1.389	63.482	0.302
23	B9	Sample 8	Inhibition Control	29.904	Expected		79.616	1.321	64.901	0.287
24	B12	Sample 11	Inhibition Control	29.796	Expected		79.439	1.354	71.815	0.323
25	C3	Sample 3	Inhibition Control	30.012	Expected		79.439	1.22	65.964	0.27
26	C6	Sample 6	Inhibition Control	30.119	Expected		79.794	1.327	65.255	0.3
27	C9	Sample 9	Inhibition Control	30.226	Expected		79.794	1.259	67.56	0.274
28	C12	Sample 12	Inhibition Control	30.343	Expected		79.616	1.259	65.964	0.273
29	H1	POS 1	Positive Control	30.226	Expected		79.084	1.096	66.319	0.23
30	H11	NEG 1	Negative Control	39.723	Expected				73.056	0.307
31	H12	NEG 1	Negative Control	39.37	Expected				72.17	0.349
32	Inconclusive									
33	A10	Sample 10	Unknown	35.746	Inconclusive	Presumptive positive;	79.616	0.336	72.524	1.142
34	A11	Sample 10	Unknown	37.616	Inconclusive	Presumptive negative;	79.616	0.603	63.659	0.249
35	B11	Sample 11	Unknown	36.035	Inconclusive	Presumptive positive;	79.616	0.465	73.056	0.811
36	Presence									
37	A1	Sample 1	Unknown	27.65	Presence		79.084	1.493	67.028	0.392
38	A2	Sample 1	Unknown	27.521	Presence		79.084	1.475	71.992	0.382
39	B1	Sample 2	Unknown	27.763	Presence		79.084	1.387	66.319	0.371
40	B2	Sample 2	Unknown	27.743	Presence		79.084	1.428	67.028	0.372
41	B10	Sample 11	Unknown	35.975	Presence		79.616	0.841	71.638	0.291
42	C10	Sample 12	Unknown	35.208	Presence		79.794	0.896	73.056	0.574
43	C11	Sample 12	Unknown	36.112	Presence		80.148	0.805	65.964	0.264
44	Unexpected									
45	H2	POS 1	Positive Control		Unexpected	No expected DNA in ...			71.638	0.206

In the example experiment:

- **Inconclusive** – Unknown sample wells A10, A11, and B11
- **Unexpected** – Positive Control well H2

b. From the Group By drop-down list, select **None**. In the table, click the column heading **Call Assessment**. Wells are sorted by the assigned call assessment. Click the column heading again to reverse the sort order and bring the wells with call assessments to the top of the table.

#	Well Position	Sample Name	Sample Type	Ct	Call	Call Assessment	Target Tm	Target Tm Height	No-Target Tm	No-Target Tm Height
1	A10	Sample 10	Unknown	35.746	Inconclusive	Presumptive positive;	79.616	0.336	72.524	1.142
2	B11	Sample 11	Unknown	36.035	Inconclusive	Presumptive positive;	79.616	0.465	73.056	0.811
3	A11	Sample 10	Unknown	37.616	Inconclusive	Presumptive negative;	79.616	0.603	63.659	0.249
4	H2	POS 1	Positive Control		Unexpected	No expected DNA in positive and/or inhibition control;			71.638	0.206
5	A1	Sample 1	Unknown	27.65	Presence		79.084	1.493	67.028	0.392

In the example experiment:

- **Presumptive positive** – Unknown sample wells A10 and B11
- **Presumptive negative** – Unknown sample well A11

- **No expected DNA in positive and/or inhibition control** – Positive Control well H2

Note: A sample that is determined to be presumptive must be repurified, and the assay must be repeated. See the *MicroSEQ Mycoplasma Real-Time PCR Detection Kit Protocol* for more information.

Guidelines for your experiment

When you review your own Mycoplasma SEQ experiment using the Table View:

- Group the wells by:
 - **Call** – The software groups the called and uncalled wells. For a description of the Mycoplasma presence/absence calls, see [page 53](#).
 - **Call Assessment** – The software groups the wells by call assessment. For a description of the possible Mycoplasma call assessments, see [page 59](#).
- Review the following values for wells with inconclusive or unexpected results:
 - Target T_m and Target T_m height
 - No-target T_m and No-target T_m height
 - C_T
- (Optional) To further investigate questionable results, see [Chapter 7](#).

For more information

For more information on the Table View, access the *AccuSEQ Software Help* by clicking  or pressing **F1**.

Export and print the data

You can publish the experiment data in several ways:

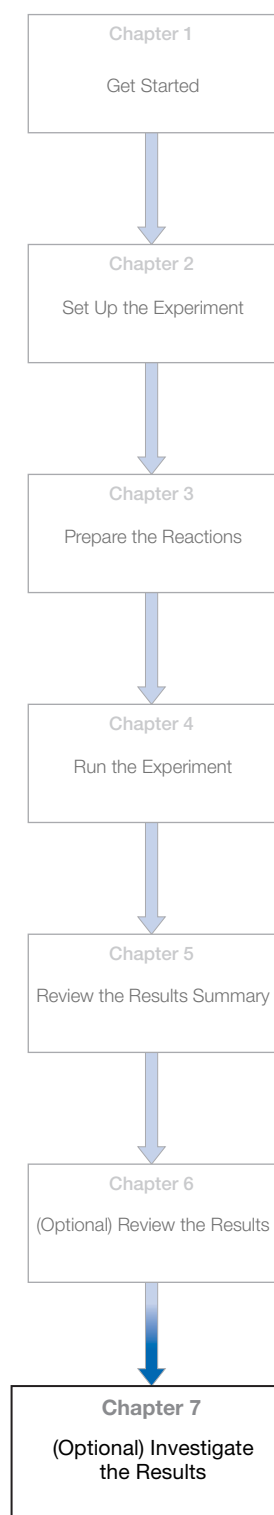
- Export data
- Save a plot as an image file
- Print a plot
- Print the plate layout
- Create slides
- Print a report

For more information

For information on performing these procedures, access the *AccuSEQ Software Help* by clicking  or pressing **F1**.

7

(Optional) Investigate the Results



This chapter covers:

■ Chapter overview	74
■ About the analysis results	75
■ Review the Multicomponent Plot	75
■ Review the Raw Data Plot	77
■ Review the analysis settings	80
■ Omit wells from the analysis	81

Note: For more information about any of the topics discussed in this guide, access the Help system by pressing **F1**, by clicking  in the toolbar of the AccuSEQ Software window, or by selecting **Help ▶ Contents and Index**.

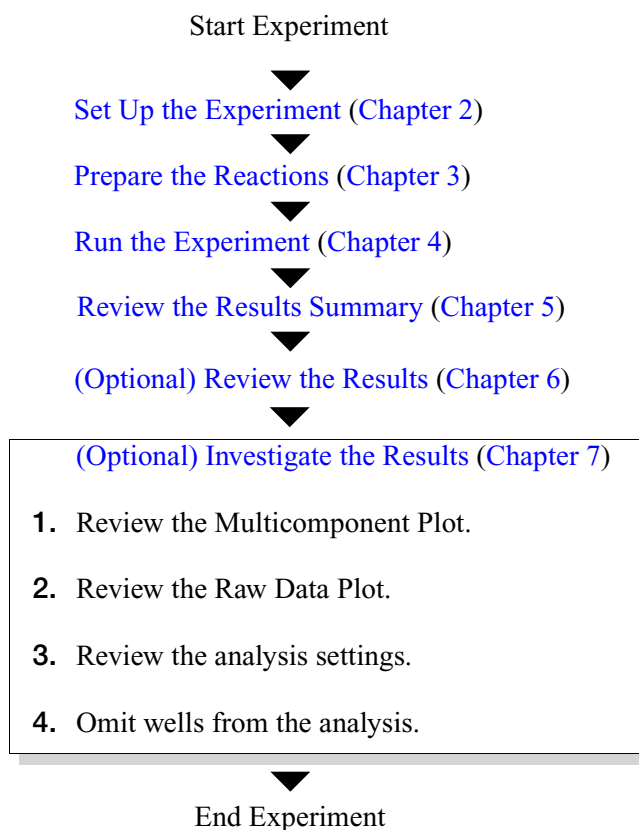
Chapter overview

After you review any questionable results in the Quality Summary, Amplification plot, and Melt Curve plot as explained in [Chapter 6](#), you can investigate any questionable results that require further examination using the remaining plot analysis screens. This chapter explains how to further investigate the analysis results of the example experiment using the Multicomponent and Raw Data plots, and how to review the analysis settings and omit wells from the analysis.

See “[Navigation tips](#)” on [page 86](#) for information on navigating within the analysis results screens.

Example experiment workflow

The workflow for investigating the analysis results for the example experiment data provided with this getting started guide is shown below.



About the analysis results

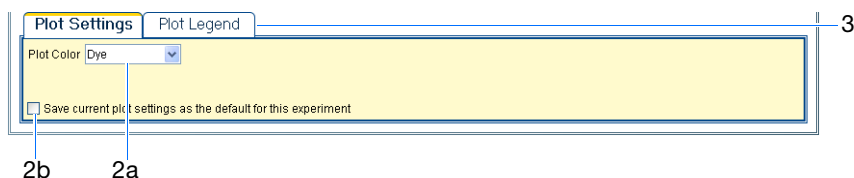
About the results	If you cannot determine the source of results identified as questionable in the Quality Summary, use the Multicomponent and Raw Data plots to review the amplification data for the plate, then edit the analysis (C_T) settings and omit wells from the analysis as needed.
About the example experiment	To investigate the example Mycoplasma SEQ experiment results, open the data file that installs with the AccuSEQ software (see page 6). For the example experiment, investigate wells: A10 , A11 , B11 and H2 .
Guidelines for your experiment	To investigate the example experiment and your own Mycoplasma SEQ experiment: • Open a Mycoplasma SEQ experiment that contains run data. • Review the run results in the Results Summary (see Chapter 5). • If needed, review any questionable results in the Quality Summary (see page 59), then review plots as needed (see page 62). • As needed, investigate questionable results: – Review the Multicomponent and Raw Data plots (see page 75). – Review the analysis (C_T) settings (see page 80). – Omit wells from the analysis (see page 81).

Review the Multicomponent Plot

The Multicomponent Plot screen displays the complete spectral contribution of each dye in a selected well over the duration of a PCR run.

About the example experiment	In the example Mycoplasma SEQ experiment, you review the Multicomponent Plot for: • SYBR[®] Green dye • ROX[™] dye • Spikes, dips, and/or sudden changes. • Amplification in the negative control wells.
View the Multicomponent Plot	1. From the navigation pane, select Plot Analysis ►  Multicomponent Plot. 2. Select the Plot Settings tab, and adjust the plot display: a. From the Plot Color drop-down list, select Dye. b. (Optional) Deselect the Save current plot settings as the default for this experiment checkbox.

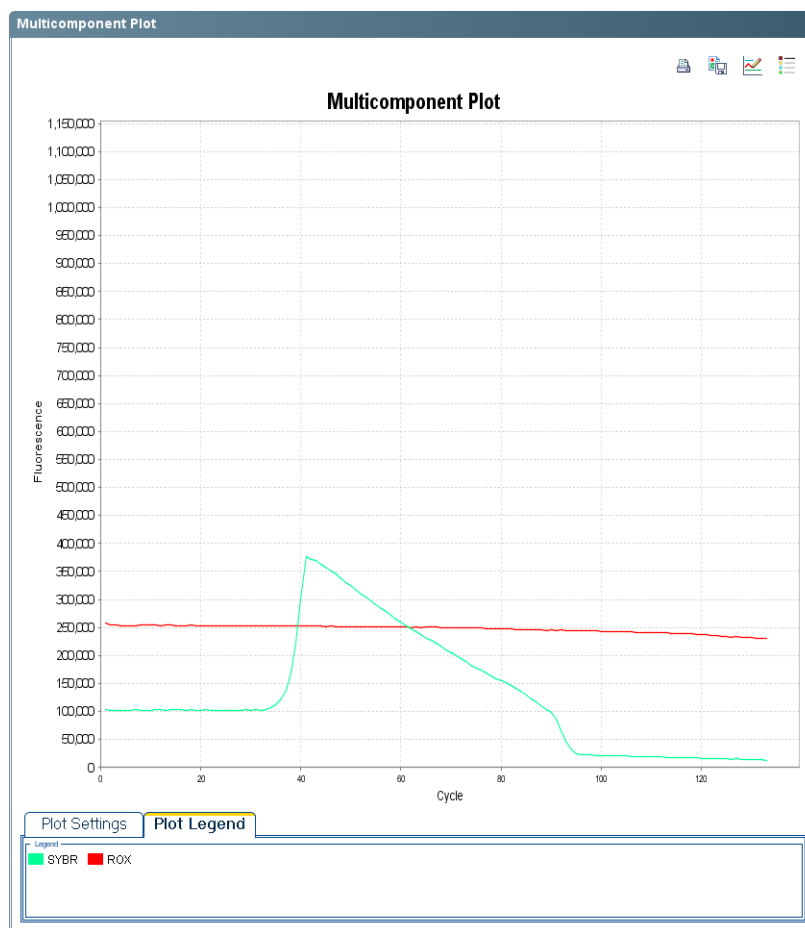
3. (Optional) Select the **Plot Legend** tab to view the color assigned to each dye in the experiment.



4. (Optional) Click  (Hide the plot legend).

Note: This is a toggle button. When the legend is hidden, the button changes to Show a legend for the plot.

5. Display the wells one at a time in the Multicomponent Plot screen:
- Click the **Plate Layout** tab.
 - Select one well in the plate layout; the well is shown in the Multicomponent Plot screen. The plot for well A11 (= Inconclusive UNKNOWN) in the example experiment is shown below.



6. (Optional) For an expanded display of the Amplification Plot, click ➤ in the upper left corner of the plate layout in the Plate Layout tab.
7. Check the ROX dye signal. In the example experiment, the ROX dye signal remains constant throughout the run.
8. Check the SYBR dye signal. In the example experiment, the SYBR dye signal should not increase for NTC wells, if the call for an UNKNOWN well is Absent, or if the call for a POS well is Unexpected. The SYBR dye signal should increase for POS and INHC wells, or if the call for an UNKNOWN well is Present.

Guidelines for your experiment

When you review your own Mycoplasma SEQ experiment, look for:

- **Passive reference (ROX dye)**– The passive reference dye fluorescence level should remain relatively constant throughout the PCR process.
- **Reporter (SYBR dye)** – The reporter dye fluorescence level should display a flat region corresponding to the baseline. If target is present in the sample (a Present call is made), the baseline will be followed by a rapid rise in fluorescence as the amplification proceeds.
- **Irregularities in the signal** – There should not be any spikes, dips, and/or sudden changes in the fluorescence.
- **Negative control wells** – There should not be any amplification in the negative control wells.

For more information

For more information on the Multicomponent Plot screen, access the *AccuSEQ Software Help* by clicking  or pressing **F1**.

Review the Raw Data Plot

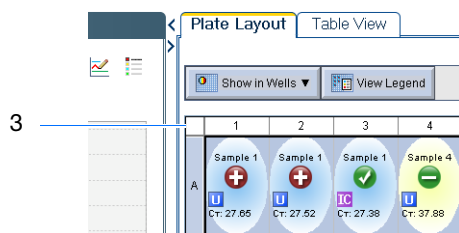
The Raw Data Plot screen displays the raw fluorescence (not normalized) for each optical filter for the selected wells during each cycle of the real-time PCR.

About the example experiment

In the example Mycoplasma SEQ experiment, you review the Raw Data Plot screen for a stable increase in signal (no abrupt changes or dips) from the appropriate filter.

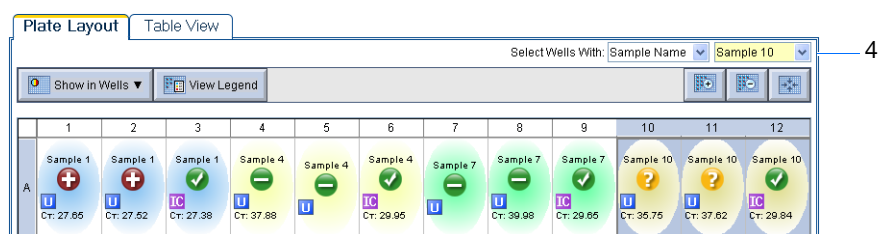
View the Raw Data Plot

1. From the navigation pane, select **Analysis** ▶  **Raw Data Plot**.
2. (Optional) Select the **Plot Legend** tab to view the well colors assigned to each row in the plate layout.
3. Display all 96 wells in the Raw Data Plot screen by clicking the upper left corner of the plate layout in the Plate Layout tab.



4. In the Plate Layout tab, select wells corresponding to sample replicates. For the example experiment:

- Unknown sample wells (including Inhibition Control wells) – From the Select Wells with drop-down lists, select **Sample Name** and then select a sample from the list (**Sample 1 – Sample 12**). For example, select Sample 10 from the list:

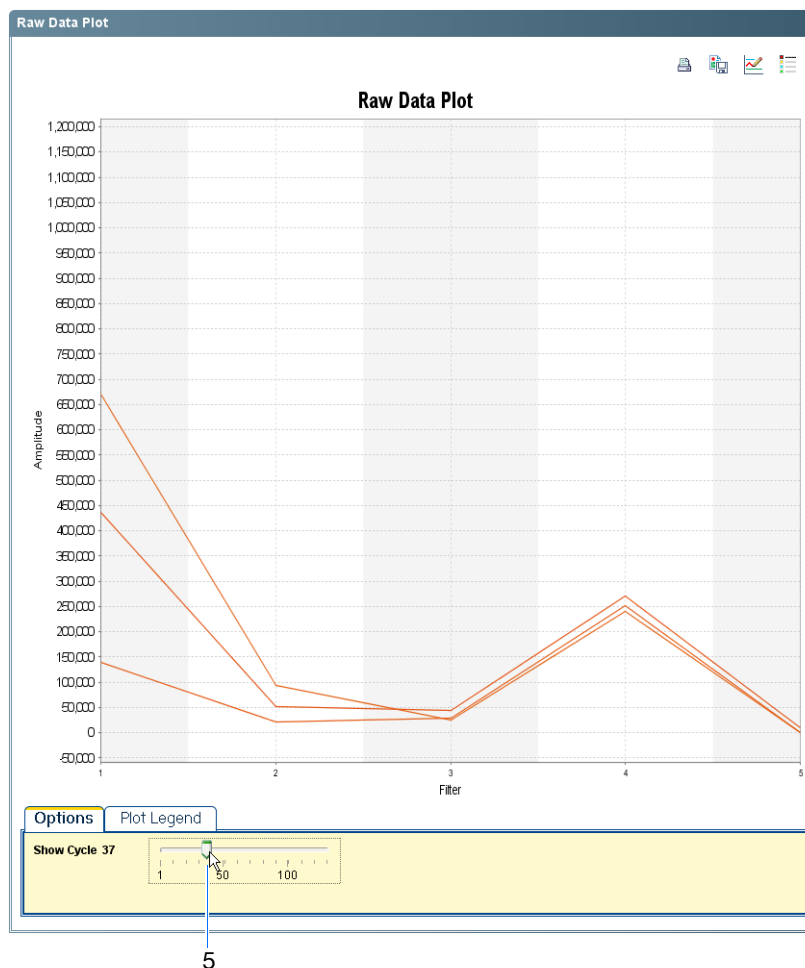


- Negative Control wells – From the Select Wells with drop-down lists, select **Sample Name** and **NEG 1**.
 - Positive Control wells – From the Select Wells with drop-down lists, select **Sample Name** and **POS 1**.
5. Click and drag the Show Cycle pointer from cycle 1 to cycle 133, and review the data from each filter:
 - The data from filter 1 correspond to the signal from SYBR[®] Green dye, the reporter for the Mycoplasma target. Increase indicates amplification of the target.
 - The data from filter 4 correspond to the signal from ROX[™] dye, which is used as the passive reference.

The filters are:

Filter	1	2	3	4	5
Dye(s)	FAM [™] dye SYBR [®] Green dye	JOE [™] dye VIC [®] dye	TAMRA [™] dye NED [™] dye Cy [™] 3 dye	ROX [™] dye Texas Red [®] dye	Cy [™] 5 dye

The filter data from cycle 37 for Sample 10 (wells A11-A12) in the example experiment are shown below.



6. Repeat [step 4](#) and [step 5](#) for each sample replicate.

7. (Optional) Click  (Hide the plot legend).

Note: This is a toggle button. When the legend is hidden, the button changes to Show a legend for the plot.

Guidelines for your experiment

When you review the Mycoplasma SEQ experiment, look for the following in each filter:

- Characteristic signal growth
- No abrupt changes or dips

For more information

For more information on the Raw Data Plot screen, access the *AccuSEQ Software Help* by clicking  or pressing **F1**.

Review the analysis settings

The Analysis Settings dialog box displays the pre-defined threshold cycle (C_T) analysis settings for Mycoplasma SEQ experiments, optimized in the AccuSEQ software for use with the MicroSEQ *Mycoplasma* Real-Time PCR Detection Kit.

Note: For details on the Mycoplasma assay and detection kit, refer to the *MicroSEQ Mycoplasma Real-Time PCR Detection Kit Protocol*.

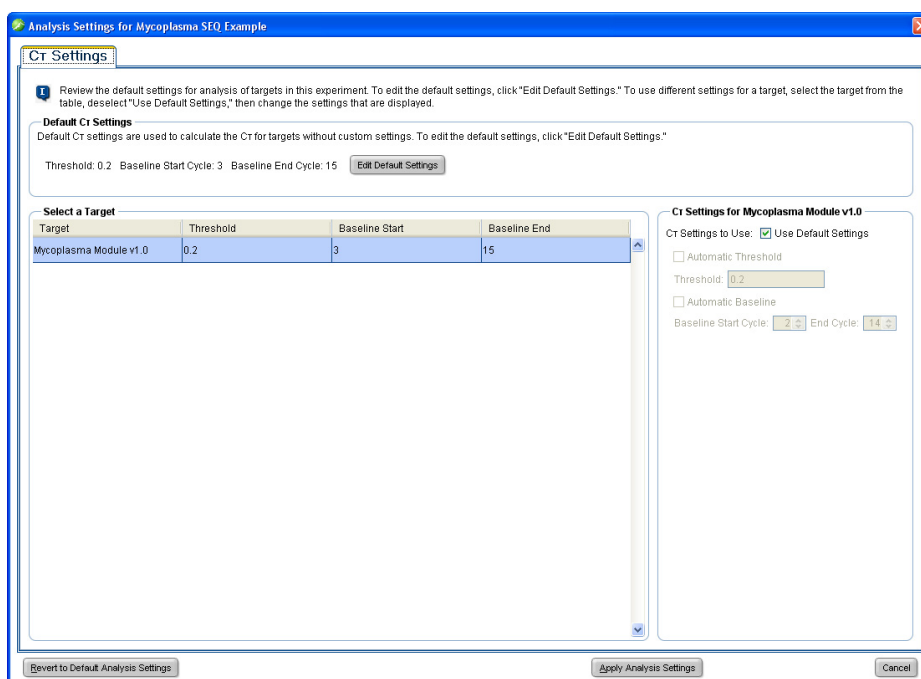
About the example experiment

In the example Mycoplasma SEQ experiment, the pre-defined analysis settings are used without changes.

View the analysis settings

Select **Analysis ► Analysis Settings** to open the Analysis Settings dialog box.

For the example experiment, the pre-defined analysis settings are displayed in the C_T Settings tab:



Guidelines for your experiment

If the pre-defined analysis settings are not suitable for your experiment, you can change the C_T settings in the Analysis Settings dialog box, then re-analyze your experiment. Use the C_T Settings tab to set the threshold and baseline either automatically or manually.

IMPORTANT! C_T settings are optimized for each assay. If you change settings, particularly autobaseline, assay results may be affected.

Note: You must have administrator or scientist privileges to access these functions.

When you reset the threshold and baseline, keep the following Applied Biosystems recommendations in mind:

Setting	Recommendation
Threshold	Enter a value for the threshold so that the threshold is: • Above the background. • Below the plateau and linear regions of the amplification curve. • Within the exponential phase of the amplification curve.
Baseline	Select Start Cycle and End Cycle values so that the baseline ends before significant fluorescence is detected.

For more information

For more information on the analysis settings, access the *AccuSEQ Software Help* by clicking  or pressing **F1** when the Analysis Settings dialog box is open.

Omit wells from the analysis

You may omit wells from analysis if you do not want to consider data generated by the well.

Note: You must have administrator or scientist privileges to access this function.

About the example experiment

In the example Mycoplasma SEQ experiment, there are no outliers; no wells need to be removed from analysis.

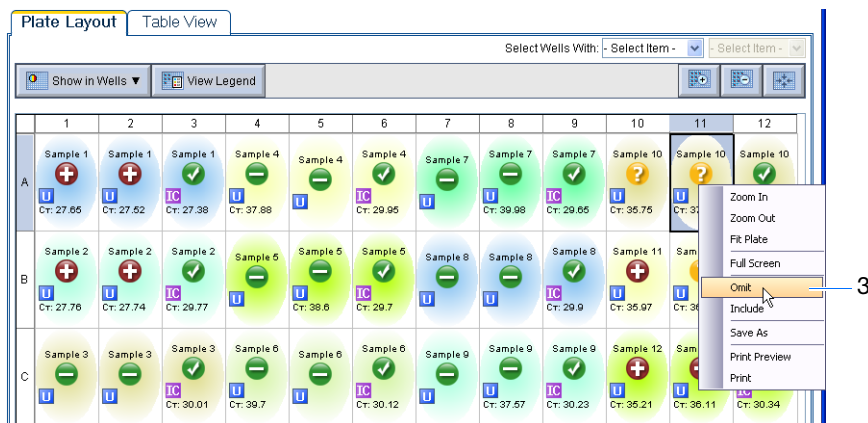
Omit wells

To omit wells in your own Mycoplasma SEQ experiment:

1. Click **Plot Analysis** in the navigation pane. The software displays the Amplification Plot screen by default.
2. In the Plate Layout tab, select one or more wells to omit from analysis (see [page 86](#) for tips).

Note: Alternatively, you can omit wells from the Table View. See the *AccuSEQ Software Help* for more information.

3. Right-click the well(s), then select **Omit**. For example:



Results for the well are removed (indicated by ☒).

4. Click **Analyze** to re-analyze the data without the omitted wells.
5. Specify a reason for change, if prompted. For more information, see [“Security, Audit and E-Signature \(SAE\) for users”](#) on page 84.

Guidelines for your experiment

When you review your own Mycoplasma SEQ experiment, carefully consider which wells to omit from analysis. If needed, remove outliers manually using the Table View.

1. From the navigation pane, select **Plot Analysis** ▶ **Amplification Plot**.
2. In the Amplification Plot screen, select **C_T vs Well** from the Plot Type drop-down list.
3. Select the **Table View** tab, then review the table data:
 - a. Look for any outliers.
 - b. Select the **Omit** check box next to the outlying well(s).
4. Click **Analyze** to re-analyze the experiment data with the outlying well(s) removed from the analysis.
5. Specify a reason for change, if prompted. For more information, see [“Security, Audit and E-Signature \(SAE\) for users”](#) on page 84.

For more information

For more information on omitting wells from the analysis, access the *AccuSEQ Software Help* by clicking or pressing **F1**.



Reference Information

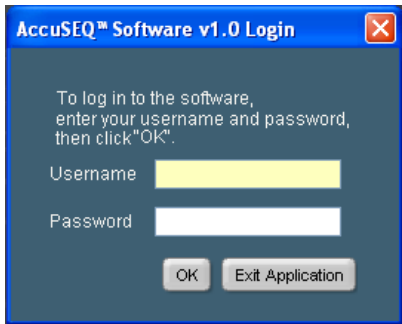
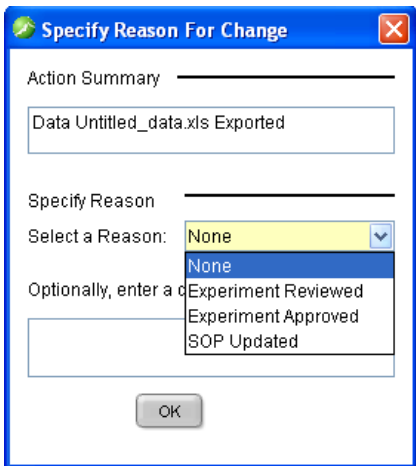
This appendix covers:

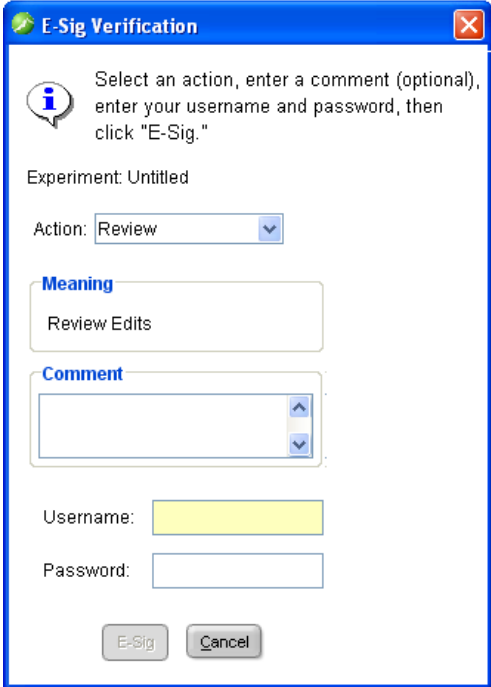
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Note: For more information about any of the topics discussed in this guide, access the Help system by pressing **F1**, by clicking  in the toolbar of the AccuSEQ Software window, or by selecting **Help ▶ Contents and Index**.

Security, Audit and E-Signature (SAE) for users

The AccuSEQ Software includes security, audit, and electronic signatures features. Depending on the way that your administrator configures these features, you may see the following dialog boxes and prompts when you use the software:

Feature	Dialog Boxes and Prompts	Description
Security 		To access the software, you provide a user name and password. If your system is configured for periodic password expiration, you will periodically be prompted to change your password. If your system is configured to monitor consecutive failed log in attempts, you will be locked out of the software if you incorrectly enter your user name or password for a specified number of times.
Audit  		If your system is configured for auditing, you may be prompted to specify a reason when you make certain changes in the software. Additionally, you can generate and print an experiment audit report that includes all audit records for an experiment.

Feature	Dialog Boxes and Prompts	Description
Electronic Signature  		If your system is configured for electronic signature, you may be prompted to provide your user name and password when you perform certain actions in the software. You can also sign an item on-demand (for example, you can sign results after viewing them). Note: The electronic signature function is available only after an experiment contains data; the E-Sig button is dimmed for new experiments. If an item is set to require two signatures, the signers are not required to sign at the same time. When the first signer signs, the E-Sig status is set to Partially Signed. When the second signer signs, the E-Sig status is set to Signed. Additionally, you can generate and print an electronic signature report that all electronic signature records for an experiment.

For more information on the SAE features available in the AccuSEQ software, refer to the *AccuSEQ Software Help*.

Navigation tips

Use the following procedures to navigate within the Results and Plot Analysis screens.

How to select wells in the plate layout

To display specific wells in the analysis results screens, select the wells in the Plate Layout tab as follows:

1. To change the data displayed in the Plate Layout, click  **Show in Wells**, then select (or deselect) the data to display in the plate layout.
2. To select wells of a specific type, use the Select Wells With drop-down lists: Select **Sample Name**, **Sample Type**, or **Assay**, then select a sample name, sample category, or assay name.
3. To select one well, click the well in the plate layout.
4. To select multiple wells, click and drag over the desired wells, or press **Ctrl-click** or **Shift-click** in the plate layout.
5. To select all 96 wells, click the top left corner of the plate layout.



How to display multiple plots

Use the Multiple Plots view to display up to four plots simultaneously. To navigate within the Multiple Plots view:

1. From the navigation pane, select  **Plot Analysis** ▶  **Multiple Plots View**.
2. To display four plots, click  **Show plots in a 2 X 2 matrix**.

3. To display two plots in rows (default view), click  **Show plots in two rows**.
4. To display two plots in columns, click  **Show plots in two columns**.
5. To display a specific plot, select the plot from the drop-down list above each plot display.



SEQ template workflow

You can use a SEQ template (*.edt file type) to create a new SEQ experiment (*.eds file type). Templates allow you to create many experiments with the same setup information.

Create a template

1. Double-click  (AccuSEQ Software).
2. Enter your user name and password, then click **OK**.

Note: See your system administrator for user name and password.

3. Open an existing SEQ experiment (see [page 39](#)), or create a new SEQ experiment (see [page 12](#)).
4. Select **File ▶ Save As Template**.
5. Enter a file name, select a location for the template, then click **Save**.
Click  **Close**.

Save a template as an experiment

1. In the Home screen, click  **Open Experiment**.
2. From the Files of Type list, select **Experiment Document Template files (*.edt)**.
3. Locate and select the template to use, then click **Open**.
4. *(Optional)* Modify the setup information from the template, as needed:
 - Experiment properties
 - Sample setup
 - Plate layout
5. Select **File ▶ Save As** to save the template as an experiment (*.eds file type).
6. Enter a file name, select a location to save the experiment. then click **Save**.
7. Specify a reason for change, if prompted. For more information, see [“Security, Audit and E-Signature \(SAE\) for users” on page 84](#).
8. Run the experiment, as needed.

Create an experiment with a template

1. Select **File ▶ New Experiment ▶ From Template**.
2. Locate and select the template to use (*.edt file type), then click **Open**. A new experiment (*.eds file type) is created using the setup information from the template:
 - Experiment properties
 - Sample setup
 - Plate layout
3. *(Optional)* Modify the setup information from the template, as needed.
4. Click  **Save** to save the experiment.
5. Enter a file name and select a location for the experiment, then click **Save**.
6. Specify a reason for change, if prompted. For more information, see [“Security, Audit and E-Signature \(SAE\) for users” on page 84](#).
7. Run the experiment, as needed.



Safety

This appendix covers:

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Instrumentation safety

Symbols on instruments

Electrical symbols on instruments

The following table describes the electrical symbols that may be displayed on Applied Biosystems instruments.

Symbol	Description
	Indicates the On position of the main power switch.
	Indicates the Off position of the main power switch.
	Indicates a standby switch by which the instrument is switched on to the Standby condition. Hazardous voltage may be present if this switch is on standby.
	Indicates the On/Off position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety symbols

The following table describes the safety symbols that may be displayed on Applied Biosystems instruments. Each symbol may appear by itself or with text that explains the relevant hazard (see [“Locations of warnings” on page 91](#)). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.

Symbol	Description
	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.

Environmental symbols on instruments

The following symbol applies to all Applied Biosystems electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	Do not dispose of this product as unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). European Union customers: Call your local Applied Biosystems Customer Service office for equipment pick-up and recycling. See www.appliedbiosystems.com for a list of customer service offices in the European Union.

Locations of warnings

The Applied Biosystems 7500 Fast Real-Time PCR System contains warnings at the locations shown below.





General instrument safety



WARNING! PHYSICAL INJURY HAZARD. Use this product only as specified in this document. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.



WARNING! PHYSICAL INJURY HAZARD. Using the instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

Moving and lifting the instrument



CAUTION! PHYSICAL INJURY HAZARD. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and lifting stand-alone computers and monitors



WARNING! Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Material Safety Data Sheets (MSDSs). See [“About MSDSs” on page 97](#).



**Cleaning or
decontaminating
the instrument**

CAUTION! Before using a cleaning or decontamination method other than those recommended by the manufacturer, verify with the manufacturer that the proposed method will not damage the equipment.

Physical hazard safety**Moving parts**

WARNING! PHYSICAL INJURY HAZARD. Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

Electrical safety

WARNING! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the Applied Biosystems 7500 Fast Real-Time PCR System without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

WARNING! FIRE HAZARD. Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.



WARNING! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

WARNING! ELECTRICAL HAZARD. Grounding circuit continuity is required for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.



WARNING! ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.



WARNING! ELECTRICAL HAZARD. Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage rating

The Applied Biosystems 7500 Fast Real-Time PCR System system has an installation (overvoltage) category of II, and is classified as portable equipment.

Workstation safety

Correct ergonomic configuration of your workstation can reduce or prevent effects such as fatigue, pain, and strain. Minimize or eliminate these effects by configuring your workstation to promote neutral or relaxed working positions.



CAUTION! MUSCULOSKELETAL AND REPETITIVE MOTION

HAZARD. These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

To minimize musculoskeletal and repetitive motion risks:

- Use equipment that comfortably supports you in neutral working positions and allows adequate accessibility to the keyboard, monitor, and mouse.
- Position the keyboard, mouse, and monitor to promote relaxed body and head postures.

Safety and electromagnetic compatibility (EMC) standards

This section provides information on:

- [U.S. and Canadian safety standards](#)
- [Canadian EMC standard](#)
- [European safety and EMC standards](#)
- [Australian EMC Standard](#)

U.S. and Canadian safety standards



This instrument has been tested to and complies with standards:

- UL 3101-1/CSA 1010.1, “Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements.”
- UL 61010-1/CSA C22.2 No. 61010-1, “Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements.”
- UL 61010-2-010, “Particular Requirements for Laboratory Equipment for the Heating of Materials.”

Canadian EMC standard

This instrument has been tested to and complies with ICES-001, Issue 3: “Industrial, Scientific, and Medical Radio Frequency Generators.”

European safety and EMC standards



Safety

This instrument meets European requirements for safety (Low Voltage Directive 73/23/EEC). This instrument has been tested to and complies with standards:

- EN 61010-1:2001, “Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements.”



- EN 61010-2-010, “Particular Requirements for Laboratory Equipment for the Heating of Materials.”
- EN 61010-2-081, “Particular Requirements for Automatic and Semi-Automatic Laboratory Equipment for Analysis and Other Purposes.”

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 89/336/EEC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), “Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements.”

Australian EMC Standard



This instrument has been tested to and complies with standard AS/NZS 2064, “Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment.”

Chemical safety

General chemical safety

Chemical hazard warning



WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.



WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.



WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- 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. (See [“About MSDSs” on page 97.](#))
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, 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 (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

MSDSs

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.



Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to www.appliedbiosystems.com, click **Support**, then select **MSDS**.
2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
 - **Open** – To view the document
 - **Print Target** – To print the document
 - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards



CAUTION! HAZARDOUS WASTE. Refer to Material Safety Data Sheets and local regulations for handling and disposal.



WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.



WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- 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.

- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, 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 (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; bmbi.od.nih.gov)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html).



- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

Safety alerts

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see “[Safety alert words](#)” on page vii.

Specific chemical alerts



WARNING! CHEMICAL HAZARD. Power SYBR[®] Green PCR Master Mix causes eye, skin, and respiratory tract irritation. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin.

Specific instrumentation alerts



CAUTION! PHYSICAL INJURY HAZARD. During operation, the sample block can be heated to 100 °C. Before performing the procedure, be sure to wait until the sample block reaches room temperature.





absent result	In Mycoplasma SEQ experiments, a sample result indicating that: • Target signal is not present within the temperature range, derivative range, and C_T range for the assay. • Passive reference is found, noise is below threshold, controls have "expected" results.
amplification	The process of making copies of and thereby increasing the amount of a specific DNA sequence.
amplification plot	Display of data collected during the cycling stage of PCR amplification. Can be viewed as: • Baseline-corrected normalized reporter (ΔR_n) vs. cycle • Normalized reporter (R_n) vs. cycle • Threshold cycle (C_T) vs. well
amplification stage	Part of the instrument run in which PCR produces amplification of the target. The amplification stage, called a cycling stage in the thermal profile, consists of denaturing, primer annealing, and polymerization steps that are repeated. For Mycoplasma SEQ experiments, fluorescence data collected during the amplification stage are displayed in an amplification plot, and the data can be used for troubleshooting.
assay	In the AccuSEQ software, a predefined experiment designed to detect an organism or group of organisms.
Audit	Administrator-configurable function that tracks the user, date, and time associated with certain actions or changes made in the software. Can be set to prompt users to enter reasons for change. Audit reports can be viewed in the SAE Manager (administrators only) and the Experiment window.
AutoDelta	In a custom experiment run method, a setting to increase or decrease the temperature and/or time for a step with each subsequent cycle in a cycling stage. In SEQ experiments, AutoDelta is set to On and cannot be modified. In custom experiments, AutoDelta can be turned On or Off. When AutoDelta is enabled for a cycling stage, the settings are indicated by an icon in the thermal profile: • AutoDelta on: ▲ • AutoDelta off: ▲

automatic baseline	An analysis setting in which the software calculates the baseline start and end values for the amplification plot. You can apply the automatic baseline setting to specific wells in the reaction plate. See also baseline .
automatic C_T	An analysis setting in which the software calculates the baseline start and end values and the threshold in the amplification plot. The software uses the baseline and threshold to calculate the threshold cycle (C_T). See also threshold cycle (C_T) .
baseline	In the amplification plot, a line fit to the fluorescence levels during the initial stages of PCR, when there is little change in fluorescence signal.
baseline-corrected normalized reporter (ΔR_n)	The magnitude of normalized fluorescence generated by the reporter. In experiments that contain data from real-time PCR, the magnitude of normalized fluorescence generated by the reporter at each cycle during the PCR amplification. In the ΔR_n vs. Cycle amplification plot, ΔR_n is calculated at each cycle as: $\Delta R_n(\text{cycle}) = R_n(\text{cycle}) - R_n(\text{baseline})$, where R_n = normalized reporter. See also normalized reporter (R_n).
C_T	See threshold cycle (C_T) .
chemistry	See reagents .
custom experiment workflow	Workflow that allows you to design and run your own standard curve, melt curve, or presence/absence experiment, including selecting experiment type, designating tasks and targets, setting thermal cycling conditions, and performing data analysis. Note: An experiment you create in the custom experiment workflow cannot be accessed from the SEQ experiment workflow.
cycle threshold	See threshold cycle (C_T) .
cycling stage	See amplification stage .
data collection	A process during the instrument run in which an instrument component detects fluorescence data from each well of the reaction plate. The instrument transforms the signal to electronic data, and the data are saved in the experiment file. In the AccuSEQ software, a data collection point is indicated by an icon in the thermal profile: • Data collection on:  • Data collection off: 
delta R_n (ΔR_n)	See baseline-corrected normalized reporter (ΔR_n) .
derivative reporter ($-R_n'$)	The negative first-derivative of the normalized fluorescence generated by the reporter during PCR amplification. In the derivative reporter ($-R_n'$) vs. temperature melt curve, the derivative reporter signal is displayed in the y-axis.
dissociation curve	See melt curve .

electronic signature (E-Sig)	Administrator-configurable electronic signature function. Signatures (user name and password) can be applied on demand. The software can also be configured to require signatures to save, print, and/or export experiment data. Can be turned on or off. E-Sig reports can be viewed in the SAE Manager (administrators only) and the Experiment window.
expected result	In Mycoplasma SEQ experiments, a control result indicating: • Positive control – Target detected • Negative control – No target detected • Inhibition control (Mycoplasma assay) – Target detected
experiment	Refers to the entire process of performing a run using the 7500 Fast instrument, including setup, run, and analysis.
experiment name	Entered during experiment setup, the name that is used to identify the experiment. Experiment names cannot exceed 100 characters and cannot include any of the following characters: forward slash (/), backslash (\), greater than sign (>), less than sign (<), asterisk (*), question mark (?), quotation mark ("), vertical line (), colon (:), or semicolon (;).
holding stage	In the thermal profile, a stage that includes one or more steps and is used to activate enzymes, to inactivate enzymes, or to incubate a reaction. In SEQ assay experiments, the holding stage is not editable.
inconclusive result	In Mycoplasma SEQ experiments, a sample result indicating that the AccuSEQ software is unable to determine a result. Causes of an Inconclusive result are displayed in the Results Summary and the Quality Summary.
inhibition control	A reaction solution that includes the <i>Power</i> SYBR® Green PCR master mix, the unknown sample, and the positive control (<i>Mycoplasma</i> Real-Time PCR DNA control). Monitors for inhibitors in the unknown sample (inhibition in the presence of a positive target). A minimum of one inhibition control is required per sample.
manual baseline	An analysis setting in which you enter the baseline start and end values for the amplification plot. You can apply the manual baseline setting to specific wells in the reaction plate.
manual C_T	An analysis setting in which you enter the threshold value and select whether to use automatic baseline or manual baseline values. The software uses the baseline and the threshold values to calculate the threshold cycle (C_T).

melt curve	A plot of the derivative of the fluorescence versus temperature. Peaks in the melt curve can indicate the melting temperature (T_m) of the target (Target T_m) or can identify nonspecific PCR amplification (No Target T_m). You can view the melt curve as normalized reporter (Rn) vs. temperature or as derivative reporter ($-Rn'$) vs. temperature. Also called <i>dissociation curve</i> .
melt curve stage	In the thermal profile, a stage with a temperature increment to generate a melt curve.
melting temperature (T_m)	In Mycoplasma SEQ and custom melt curve experiments, the temperature at which 50% of the DNA is double-stranded and 50% of the DNA is dissociated into single-stranded DNA. The T_m is displayed in the melt curve.
multicomponent plot	A plot of the complete spectral contribution of each dye for the selected well(s) over the duration of the PCR run.
<i>Mycoplasma</i> Real-Time PCR DNA Control	In Mycoplasma SEQ experiments: A specially designed plasmid DNA used as the positive control whose amplification mimics the expected amplification of a mycoplasma target. Target signal that is not detected in a positive-control well indicates a pipetting error, possible inhibition, or a problem with amplification. A minimum of one positive control is required per run.
negative control (NC)	A reaction solution that contains water or buffer instead of sample template. Monitors for contamination (unexpected amplification in the absence of a target) and reagent integrity. A minimum of one negative control is required per run. Also called <i>no template control (NTC)</i> .
no template control (NTC)	See negative control (NC) .
no target T_m	In Mycoplasma SEQ experiments: A T_m in the no-target range can indicate nonspecific PCR amplification.
normalized reporter (Rn)	Fluorescence signal from the reporter dye normalized to the fluorescence signal of the passive reference.
omit well	An action that you perform before reanalysis to omit one or more wells from analysis. Because no algorithms are applied to omitted wells, omitted wells contain no results.
passive reference	A dye that produces fluorescence. Because the passive reference signal should be consistent across all wells, it is used to normalize the reporter dye signal to account for non-PCR related fluorescence fluctuations caused by minor well-to-well differences in concentrations or volume. Normalization to the passive reference signal allows for high data precision.
plate layout	An illustration of the grid of wells and assigned content in the reaction plate. In the 7500 Fast system, the grid contains 8 rows and 12 columns.

	In the AccuSEQ software, you can use the plate layout as a selection tool to assign well contents, to view well assignments, and to view results. The plate layout can be printed, included in a report, exported, and saved as a slide for a presentation.
plot color	In Mycoplasma SEQ experiments, a color assigned to a sample in Sample Setup to identify the sample in the plate layout and analysis plots.
Polymerase Chain Reaction (PCR)	Technology used to increase the amount of a DNA sequence.
positive control	See Mycoplasma Real-Time PCR DNA Control.
Power SYBR® Green PCR Master Mix	The master mix used to prepare the premix solution. It contains the DNA polymerase enzyme that initiates PCR in the presence of the necessary primers and DNA sample. It also contains SYBR® Green I dye, which binds to double-stranded (ds) DNA, thus providing a fluorescence signal that indicates the amount of dsDNA product generated during PCR.
present result	In Mycoplasma SEQ experiments, a sample result indicating that target signal is present and meets the following conditions: • Within the temperature range, derivative range, and C_T range for the assay. • Passive reference is found, noise is below threshold, positive and negative controls have "expected" results.
premix solution	See reaction mix.
primer	A segment of DNA that is complementary to the target DNA sequence and is needed to start amplification.
primer mix	PCR reaction component that contains the forward primer and reverse primer designed to amplify the target.
ramp	The rate at which the temperature changes during the instrument run. Except for the melt curve step, the ramp is defined as a percentage. For the melt curve step, the ramp is defined as a temperature increment. In the graphical view of the thermal profile, the ramp is indicated by a diagonal line.
raw data plot	A plot of raw fluorescence signal (not normalized) for each optical filter.
reaction mix	A solution that contains all components to run the PCR reaction, except for the template (sample, standard, or control). Also called <i>premix solution</i>.
reagents	The PCR reaction components you are using to amplify the target and to detect amplification.
real-time PCR	Process of collecting fluorescence data during PCR.

region of interest (ROI) calibration	Type of 7500 Fast system calibration in which the system maps the positions of the wells in the sample block. ROI calibration data are used so that the software can associate increases in fluorescence during a run with specific wells in the reaction plate.
replicates	Total number of identical reactions containing identical samples, components, and volumes.
reporter	A fluorescent dye used to detect amplification. For SYBR® Green reagents, the reporter dye is SYBR® Green dye.
Rn	See normalized reporter (Rn) .
ROX™ dye	A dye supplied by Applied Biosystems and calibrated on the 7500 Fast systems. ROX dye is used as the passive reference.
run method	Definition of the reaction volume and the thermal profile for the 7500 Fast instrument run.
sample	The template that you are testing.
SAE Manager	Controls Security, Audit, and E-Sig functions and reporting.
sample type	In SEQ experiments, the identifier you assign to a well in Sample Setup. Sample Type options are: •  Unknown •  POS (positive control) •  NTC (negative control) • Mycoplasma SEQ experiments: If you specify inhibition controls for samples, each sample is assigned inhibition control wells .
Security	Administrator-configurable function that controls user access to the software, manages user accounts, and determines security policies.
SEQ experiment workflow	Step-by-step workflow for a specific Applied Biosystems assay. The SEQ experiment workflow calculates reagent volumes, determines optimum plate setup, guides you through experiment set up, uses optimized thermal cycling conditions, and performs data analysis. Note: An experiment you create in the SEQ experiment workflow cannot be accessed from the custom experiment workflow.
SEQ template	An experiment document template file (*.edt) that includes setup information (experiment properties, sample setup, run method, and plate layout) for a specific Applied Biosystems assay.

stage	In the thermal profile, a group of one or more steps. There are three types of stages: holding stage, cycling stage (also called amplification stage), and melt curve stage.
SYBR® Green reagents	PCR reaction components designed to amplify the target and SYBR® Green dye to detect double-stranded DNA.
system dye	Dye supplied by Applied Biosystems and calibrated on the 7500 Fast instrument. Before you use system dyes in your experiments, make sure the system dye calibration is current in the Instrument Maintenance Manager. System dyes of the 7500 Fast instrument include: • CY3 dye • CY5 dye • FAM™ dye • JOE™ dye • NED™ dye • ROX™ dye • SYBR® Green dye • TAMRA™ dye • TEXAS RED® dye • VIC® dye
target	The nucleic acid sequence that you want to amplify and detect.
target T_m	In Mycoplasma SEQ experiments: The temperature at which 50% of the DNA is double-stranded and 50% of the DNA is dissociated into single-stranded DNA. The T_m displayed in the melt curve is the T_m associated with the largest peak, which may be the Target T_m or the No Target T_m .
template	The type of nucleic acid to add to the PCR reaction. The recommended template varies according to experiment type. For Mycoplasma SEQ experiments, Applied Biosystems recommends adding DNA templates to the PCR reactions.
thermal profile	Part of the run method that specifies the temperature, time, ramp, and data collection points for all steps and stages of the 7500 Fast instrument run.
threshold	1. In amplification plots, the level of fluorescence above the baseline and within the exponential growth region. The threshold can be determined automatically (see automatic C_T) or can be set manually (see manual C_T). 2. In presence/absence experiments, the level of fluorescence above which the software assigns a presence call.
threshold cycle (C_T)	The PCR cycle number at which the fluorescence meets the threshold in the amplification plot.
T_m	See melting temperature (T_m) , no target T_m , target T_m .

unexpected result	In Mycoplasma SEQ experiments, a control result indicating: • Positive control – No target detected (target should be detected) • Negative control – Target detected (no target should be detected) • Inhibition control (Mycoplasma assay) – No target detected (target should be detected) Causes of an unexpected result are displayed in the Results Summary and the Quality Summary.
unknown	A DNA sample from media, cell culture, or other source that you are testing for the presence of mycoplasmas.

About the System documentation

The documents listed below are shipped with the Applied Biosystems 7500 Fast Real-Time PCR System (7500 Fast system) using the AccuSEQ Real-Time PCR Detection Software (AccuSEQ software).

Guide	Purpose and Audience	PN
<i>Applied Biosystems AccuSEQ™ Real-Time PCR Detection Software Mycoplasma SEQ Experiments Getting Started Guide</i>	Explains how to perform Mycoplasma SEQ experiments on the 7500 Fast system. The documents function as both a: • Tutorial, using example experiment data provided with the AccuSEQ software. • Guide for your own experiments. Intended for laboratory staff and principal investigators who perform Mycoplasma SEQ experiments using the 7500 Fast system.	4425587
<i>Applied Biosystems AccuSEQ™ Real-Time PCR Detection Software Mycoplasma SEQ Experiments Quick Reference Card</i>		4425586
<i>Applied Biosystems AccuSEQ™ Real-Time PCR Detection Software Custom Experiments Quick Reference Card</i>	Explains how to perform custom experiments on the 7500 Fast system. The Quick Reference Card functions as both a: • Tutorial, using example experiment data provided with the AccuSEQ software. • Guide for your own experiments. Intended for laboratory staff and principal investigators who perform custom experiments using the 7500 Fast system.	4425585
<i>Applied Biosystems 7500/7500 Fast Real-Time PCR System Maintenance Guide</i>	Explains how to maintain the 7500 Fast system. Intended for laboratory staff responsible for the maintenance of the 7500 Fast system.	4412844
<i>Applied Biosystems 7500/7500 Fast Real-Time PCR System Computer Setup Guide</i>		4387778
<i>Applied Biosystems Real-Time PCR Systems Reagent Guide</i>	Provides information about the reagents you can use on Applied Biosystems Real-Time PCR Systems, including: • An introduction to TaqMan® and SYBR® Green reagents • Descriptions and design guidelines for the following custom experiment types: – Quantitation (standard curve) experiments – Presence/absence experiments Intended for laboratory staff and principal investigators who perform custom experiments using the 7500 Fast system.	4387787
<i>Applied Biosystems 7500/7500 Fast Real-Time PCR System Site Preparation Guide</i>	Explains how to prepare your site to receive and install the 7500 Fast system. Intended for personnel who schedule, manage, and perform the tasks required to prepare your site for installation of the 7500 Fast system.	4412843

Guide	Purpose and Audience	PN
<i>Applied Biosystems AccuSEQ™ Software v1.0 Help</i>	Explains how to use the AccuSEQ software to: - Set up and manage the security, audit, and electronic signature settings on the 7500 Fast system. - Set up, run, and analyze SEQ and custom experiments using the 7500 Fast system. - Calibrate a 7500 Fast instrument. - Verify that the performance of a 7500 Fast instrument with an RNase P run. Intended for: - Principal investigators who are responsible for controlling access to the 7500 Fast system. - Laboratory staff and principal investigators who perform experiments using the 7500 Fast system. - Laboratory staff responsible for the installation and maintenance of the 7500 Fast system.	NA

Related documentation

Table 1 Documents Related to Mycoplasma SEQ Experiments

Document	PN
<i>PrepSEQ™ Mycoplasma Nucleic Acid Extraction Kit Protocol</i>	4401253
<i>PrepSEQ™ Mycoplasma Nucleic Acid Extraction Kit Quick Reference Card</i>	4406304
<i>MicroSEQ® Mycoplasma Real-Time PCR Detection Kit Protocol</i>	4393111
<i>MicroSEQ® Mycoplasma Real-Time PCR Detection Kit Quick Reference Card</i>	4393471

Table 2 Documents Related to Custom Standard Curve Experiments

Document	PN
<i>PrepSEQ™ Residual DNA Sample Preparation Kit Protocol</i>	4415259
<i>resDNASEQ™ Quantitative CHO DNA Kit Protocol</i>	4415260

Table 3 Documents Related to the Reagent Guide

Document	PN
<i>Introduction to Taqman® and SYBR® Green Chemistries for Real-Time PCR Protocol</i>	4407003
<i>Applied Biosystems High-Capacity cDNA Reverse Transcription Kits Protocol</i>	4375575
<i>Custom TaqMan® Gene Expression Assays Protocol</i>	4334429
<i>Custom TaqMan® Genomic Assays Protocol: Submission Guidelines</i>	4367671
<i>Power SYBR® Green PCR Master Mix and RT-PCR Protocol</i>	4367218
<i>Primer Express® Software Version 3.0 Getting Started Guide</i>	4362460
<i>SYBR® Green PCR and RT-PCR Reagents Protocol</i>	4304965
<i>SYBR® Green PCR Master Mix and RT-PCR Reagents Protocol</i>	4310251

Note: For additional documentation, see [“How to obtain support” on page ix](#).

Obtaining information from the Help system

The AccuSEQ software has a Help system that describes how to use each feature of the user interface. Access the Help system by doing one of the following:

- Click  in the toolbar of the AccuSEQ software window.
- Select **Help ▶ AccuSEQ Software Help**.
- Press **F1**.

You can use the Help system to find topics of interest by:

- Reviewing the table of contents
- Searching for a specific topic
- Searching an alphabetized index

Send us your comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

IMPORTANT! The e-mail address above is for submitting comments and suggestions relating *only* to documentation. To order documents, download PDF files, or for help with a technical question, see [“How to obtain support” on page ix](#).

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Part Number 4425587 Rev. B 06/2010



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