GETTING STARTED GUIDE

applied biosystems® by life technologies™

AccuSEQ®Software v2.0

Mycoplasma SEQ Experiments

REAL-TIME PCR AND AUTOMATED MYCOPLASMA DETECTION CALLS

For use with:

Applied Biosystems® 7500 Fast Real-Time PCR System
PrepSEQ® Sample Preparation Kits
MycoSEQ™ *Mycoplasma* Detection Kits
resDNASEQ™ Quantitative CHO DNA Kit

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Revision C

life technologies™

For Research Use Only. Not for use in diagnostic procedures.

The information in this guide is subject to change without notice.

Contents

About This Guide

CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

IMPORTANT! Before using this product, read and understand the information the "Safety" appendix in this document.

Revision history

Revision	Date	Description
A	August 2009	First publication.
B	June 2010	Updated PCR patent statements.
C	January 2013	Updated to reflect AccuSEQ® Software v2.0.

Purpose

The *AccuSEQ*® Software v2.0 Mycoplasma SEQ Experiments Getting Started Guide provides reference information for the AccuSEQ® Software and describes how to prepare, maintain, and troubleshoot the system.

Prerequisites

This service guide is intended for service personnel who have been specifically trained by Life Technologies Corporation. The manufacturer is not liable for damage or injury that results from use of this manual by unauthorized or untrained parties.

This guide uses conventions and terminology that assume a working knowledge of the Microsoft® Windows® operating system, the Internet, and Internet-based browsers.

User attention words

Five user attention words may appear in this document. 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 or accurate chemistry kit use.

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.

Except for IMPORTANTs, the safety alert words in user documentation appear with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instrument. See the “Safety” appendix for descriptions of the symbols.

1 Get Started

This chapter covers:

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® v2.0 Software window, or by selecting **Help ▸ Contents and Index**.

About the MycoSEQ™ *Mycoplasma* detection system

Components of the MycoSEQ™ *Mycoplasma* Detection System include:

- **Software** – AccuSEQ® Software v2.0
- **Instrument** – Applied Biosystems 7500 Fast Real-Time PCR System
- **Reagents** –
  - PrepSEQ® Sample Preparation Kits
  - MycoSEQ™ *Mycoplasma* Detection Kits

About the AccuSEQ® Software

AccuSEQ® v2.0 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.

AccuSEQ® v2.0 Software features include:

- **Support for Applied Biosystems 7500 Fast Real-Time PCR Systems** – AccuSEQ® Software enables 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 PrepSEQ® Sample Preparation Kits or MycoSEQ™ *Mycoplasma* Detection Kits.
  - **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** – Enables the AccuSEQ® v2.0 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 PrepSEQ® Sample Preparation Kits.

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 review of failed results.
- Provides results reports that you can print, or save as pdf (Adobe® Acrobat® file type), html, ppt (Microsoft® PowerPoint® file type), or xls (Microsoft® Excel® file type).

About the MycoSEQ™ *Mycoplasma* Detection Kits

The MycoSEQ™ *Mycoplasma* Detection Kits are 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 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.
- (*Optional, but recommended*) **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 before PCR amplification to activate the AmpliTaq Gold® enzyme.
- **Cycling (Amplification)** – Part of the run method in which PCR produces amplification of the target.
- **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 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 pass, fail, present, absent, and review results, and displays results in plate view or table view.
- **Quality Summary** – Provides a description and troubleshooting information for samples requiring additional review.
- **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 *MycoSEQ® Mycoplasma Detection Kits User Guide*.
- 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®*Software v2.0 *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.
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 15.

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. 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

Workflow	Description	See...
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 and Support

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

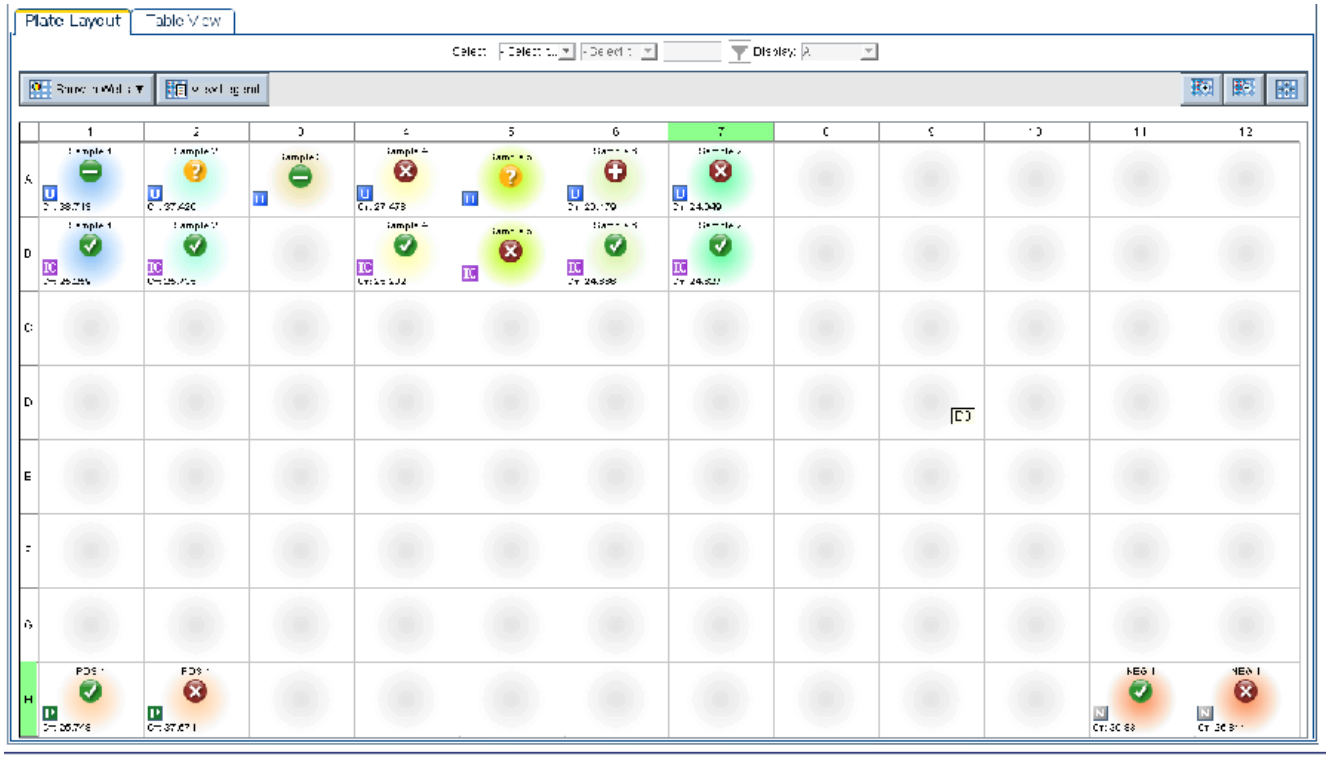
The objective of the example *Mycoplasma* SEQ experiment (*example experiment*) is to detect the presence of *Mycoplasma* species DNA in each test sample.

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:



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 Presence Absence Detection by MycoSEQ Example.eds, where <*drive*> is the computer hard drive on which the AccuSEQ® Software is installed.

The experiments folder of the AccuSEQ® Software contains additional example files for the Custom Experiments workflow that you can reference when analyzing your own data. The following example files install with the AccuSEQ® Software:

- CHO residual DNA Quantitation Example.eds
- HCP Quantitation by ProteinSEQ Example.eds
- Mycoplasma Presence Absence Detection by MycoSEQ Example.eds
- Presence Absence Example.eds
- Residual Protein A Quantitation by ProteinSEQ Example.eds

Note: Be sure to use the *Mycoplasma Presence Ab sence Detection by MycoSEQ 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.

Set up the experiment (Chapter 2)

- Create a new experiment.
- Define the experiment properties.
- Set up the samples and controls.
- (*Optional*) Review the run method.
- Print the experiment and reaction setup.
- Save the experiment.

Prepare the reactions (Chapter 3)

- Prepare the DNA template.
- Prepare the premix solution.
- Prepare the experiment plate.

Run the experiment (Chapter 4)

- Prepare for the run.
- (*Optional*) Enable the notification settings.
- Start the run.
- (*Optional*) Monitor the run.
- Unload the instrument.

Review the Results Summary (Chapter 5)

- Review the Results Summary.
- Export and print the data.

(Optional) Review the Results (Chapter 6)

- Review the Quality Summary.
- Review the Amplification Plot.
- Review the Melt Curve.
- Review the table data.
- Export and print the data.

(Optional) Investigate the Results (Chapter 7)

- Review the Multicomponent Plot.
- Review the Raw Data Plot.
- Review the analysis settings.
- Omit wells from the analysis.

2 Set up the experiment

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 14).

About predefined settings

For the example experiment, the SEQ workflow guides you through Life Technologies recommended best practices and demonstrates different result scenarios. You will enter design parameters to set up, run, and analyze Mycoplasma experiment samples prepared with the MycoSEQ™ 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, positive control, and negative control.

For more information

For more information on:

- Instrument – 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*®Software v2.0 *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.

Set up the experiment (Chapter 2)

▼

1. Create a new experiment.
2. Define the experiment properties.
3. Set up the samples and controls.
4. (*Optional*) Review the run method.
5. Print the experiment and reaction setup.

▼

Prepare the reactions (Chapter 3)

▼

Run the experiment (Chapter 4)

▼

Review the Results Summary (Chapter 5)

▼

(Optional) Review the Results (Chapter 6)

▼

(Optional) Investigate the Results (Chapter 7)

▼

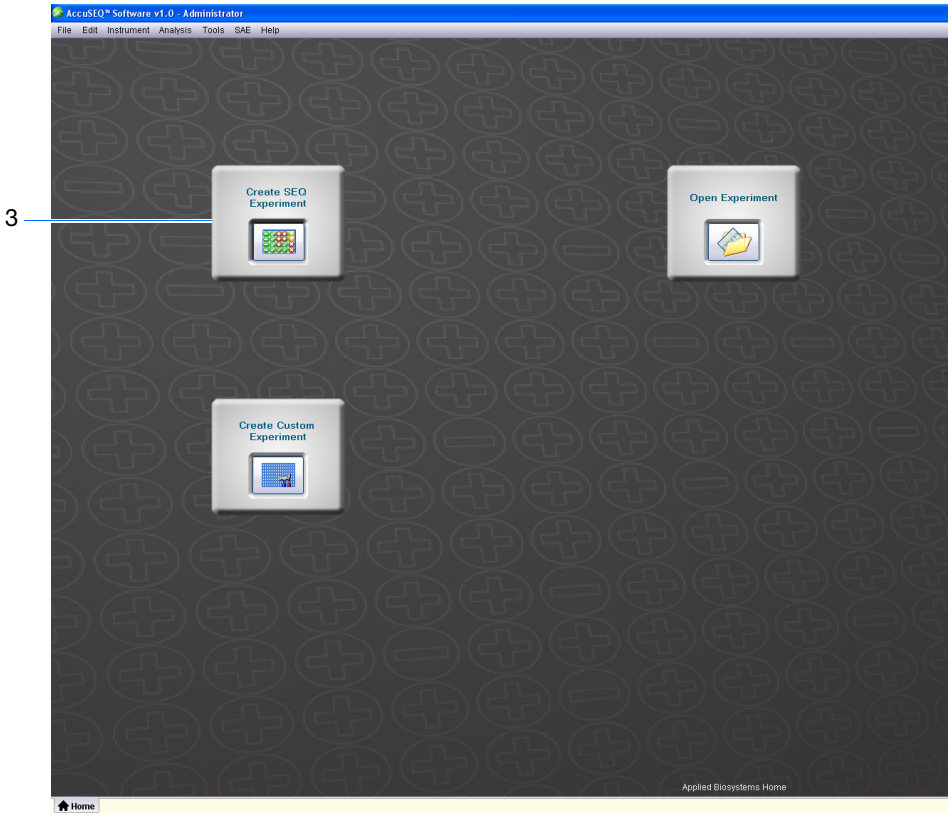
End experiment.

Create a new experiment

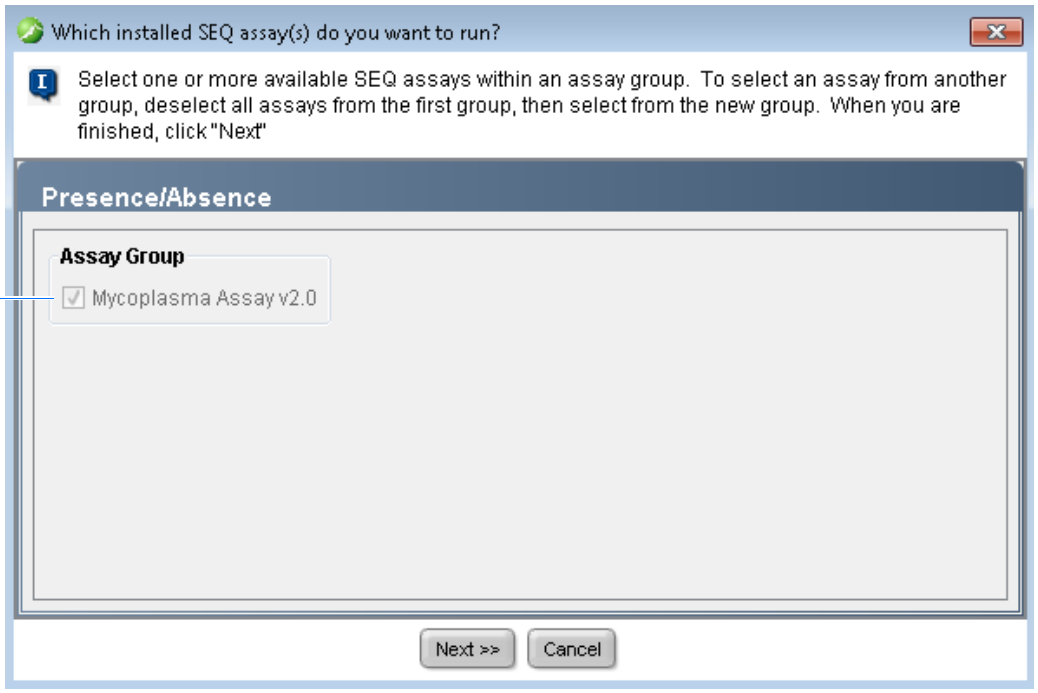
Create a new experiment using the SEQ Experiment workflow in the AccuSEQ® Software.

1. Double-click (**AccuSEQ® v2.0 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 v2.0** 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 (Mycoplasma Presence Absence Detection by MycoSEQ Example).
- The SEQ experiment type is presence/absence.
- The SEQ assay used is Mycoplasma Assay v2.0.

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 Presence Absence Detection by MycoSEQ Example Setup**.

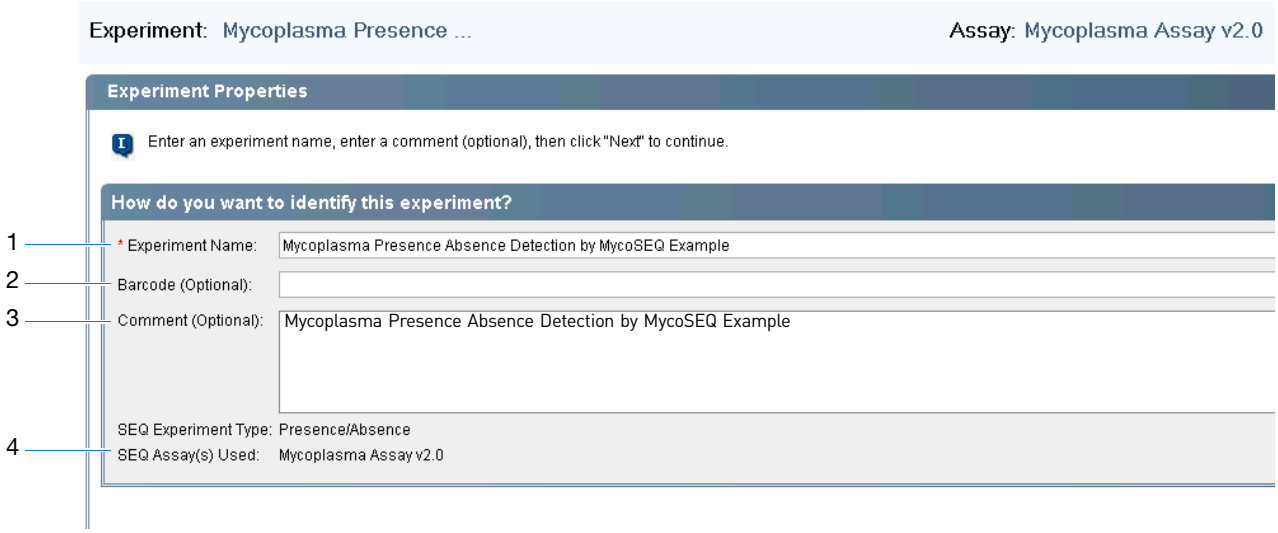
   Note: The experiment header updates with the experiment name you entered.

2. Leave the Barcode field empty.
3. Click the **Comment** field, then enter **Mycoplasma Presence Absence Detection by MycoSEQ 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 parameters for a SEQ presence/absence experiment are 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 v2.0**. The SEQ assay used indicates the organism detected by the assay.
5. Click **Next**.



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*® v2.0 Software *Help* by clicking or pressing **F1**.
- Mycoplasma experiments – Refer to the *MycoSEQ*® *Mycloplasma 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 28) and to calculate the required reaction component volumes for each sample type, based on the *MycoSEQ Mycoplasma* Detection Kits User Guide guidelines.

About the example experiment

The *Mycoplasma Presence Absence Detection by MycoSEQ Example* experiment uses:

- Seven samples, where the presence/absence of the target (a mycoplasma contaminant) is unknown.
- One Inhibition Control replicate for all samples except Sample 3. Each Inhibition Control replicate contains positive control template (Mycoplasma Real-Time PCR DNA Control) and sample template. Running an inhibition control is recommended for each unknown. Sample 3 demonstrates the new feature in AccuSEQ 2.0 that enables the user to choose not to run the recommended inhibitory control.
- 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

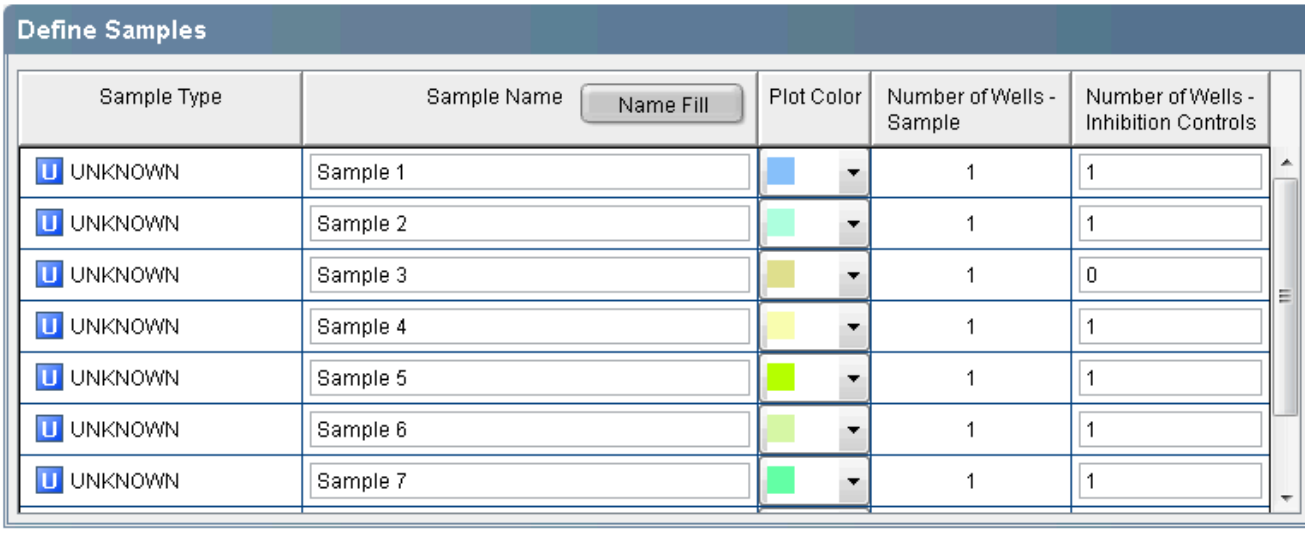
1. In the *Mycoplasma Assay v2.0* column of the *How many samples and controls do you want to run?* table, specify the number of samples and sample volume.
   a. Specify the number of samples and replicates:

For Number of...†	Enter...‡
Samples	7
Sample replicates	1
Inhibition control replicates for each sample§	1
Positive control replicates	2
Negative control replicates	2

† At least one positive control, one negative control are required.
‡ The number that you specify is listed below in the sample type list (see step 2).
§ A minimum value of zero is acceptable. One inhibitory control per reaction is recommended but not required.

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

2. Verify the contents of the *Define Samples for Assay: Mycoplasma Module v2.0* table:
   a. Sample Names for samples with Sample Type = UNKNOWN:
      - **Sample 1**
      - **Sample 2**
      - **...**
      - **Sample 7**



   b. Sample Names for samples with Sample Type = POS and NEG:
      - **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.
   d. Specify the number of of inhibition controls assigned to individual samples in the column *Number of Inhibition Controls*:
      - In this example, change the number of inhibitory controls for Sample 3 from 1 to 0.
      - In the table *How many samples and controls do you want to run?*, verify that the cell for *Number of inhibition control replicates for each sample* changes from the number 1 to the text VARIABLE, indicating a variable number of inhibitory controls assigned to each samples.

        Note: When you click a well, it becomes blank. If you did not mean to change the well, press **ESC** to restore VARIABLE.

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 26). 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:

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:
   - 7 Unknowns U
   - 6 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. *(Optional)* Review the run method (see page 29).

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

5. 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. One inhibition control per sample recommended but not required.

- 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 (Part no. 4358293) for your reactions, a maximum of 6 tube strips can be used in the 7500 Fast instrument (see page 43 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*® v2.0 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 24. You can review, but not edit, the pre-defined thermal profile in the Run Method screen.

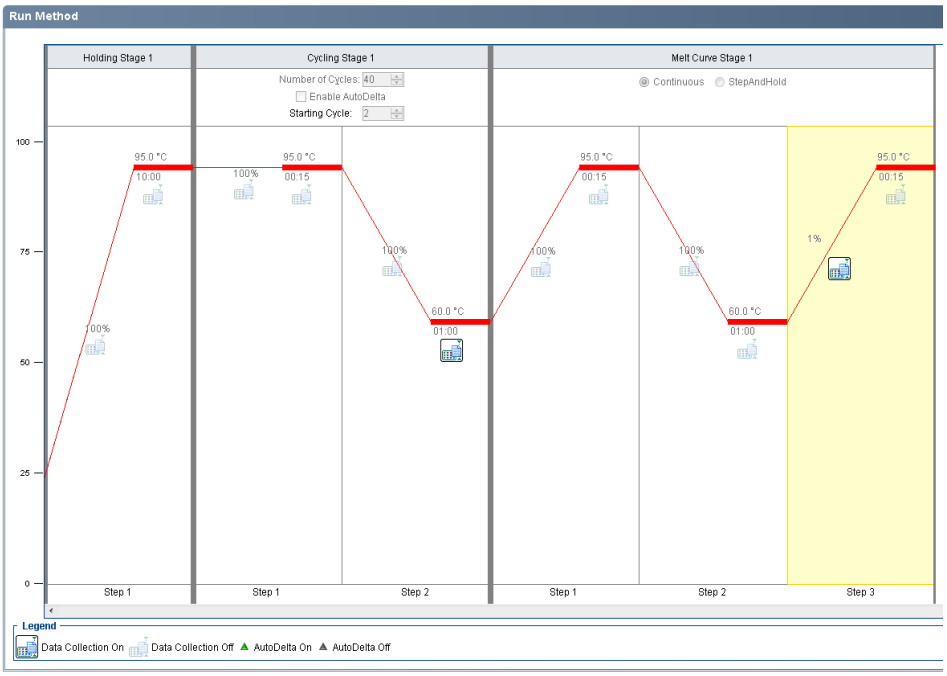
Note: The SEQ experiment workflow automatically specifies the setup information (experiment type, reagents, targets) and an optimized run method for the selected assay.

About the example experiment

For the *Mycoplasma Presence Absence Detection by MycoSEQ Example* experiment, the pre-defined run method is optimized for use with the Mycoplasma v2.0 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*® v2.0 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 26), the reaction setup instructions for the *Mycoplasma Presence Absence Detection by MycoSEQ Example* experiment includes the following information:

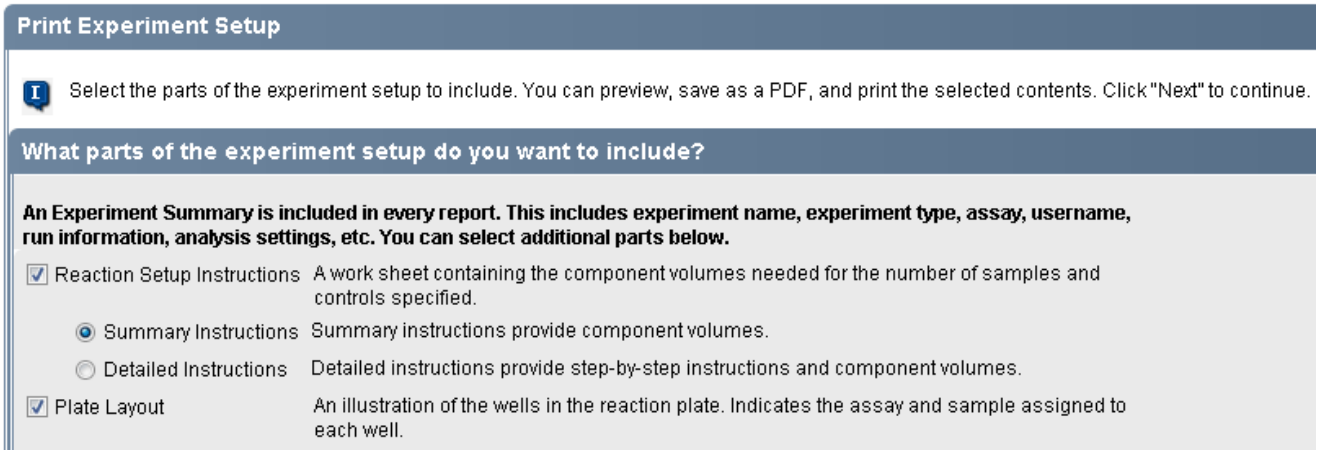
- The SEQ assay type is Mycoplasma Assay v2.0.
- The reaction volume per well is 30 µL with a 10% excess.
- The reaction components are:
  - *Power* SYBR® Green PCR Master Mix (2×)
  - *Mycoplasma* Real-Time PCR Primer Mix (10×)
  - *Mycoplasma* Real-Time PCR DNA Control (positive control)

  - 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:
   - Reaction Setup Instructions
   - Detailed Instructions
   - Plate Layout



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 87.
5. Set the printed reaction setup instructions aside until you prepare the reactions (see page 35).

For more information

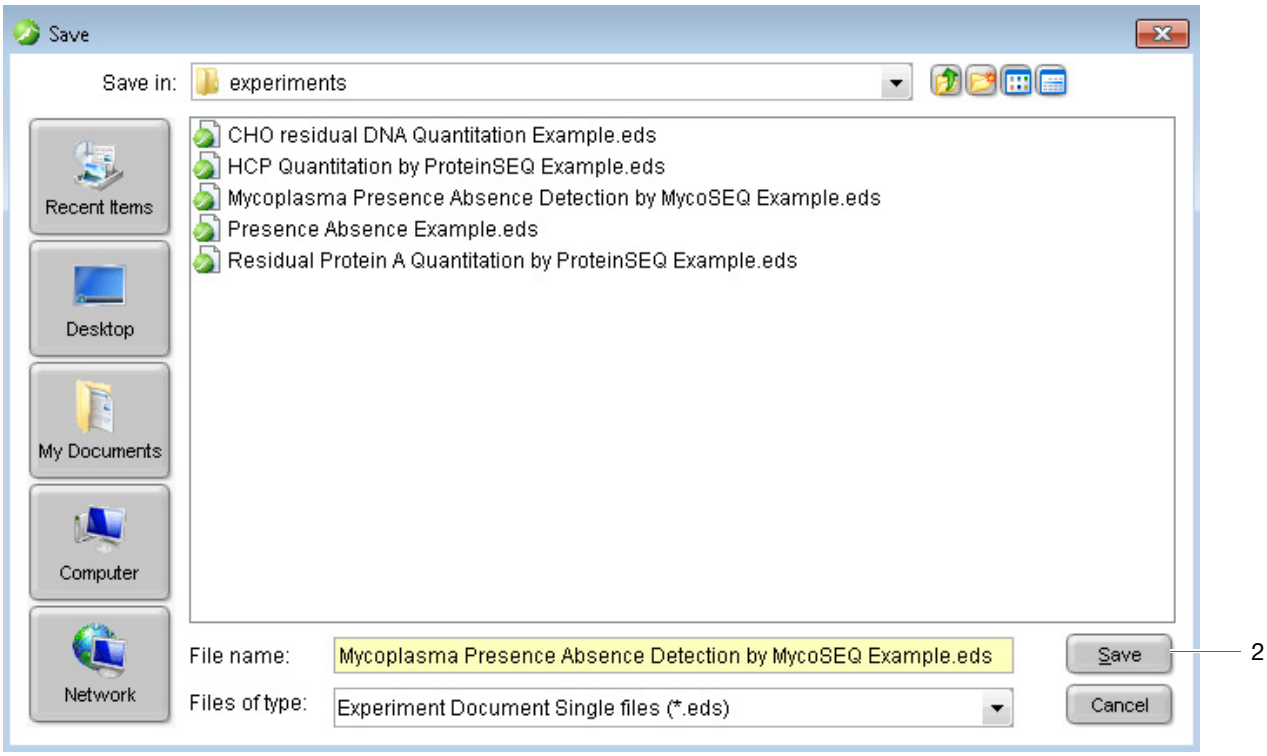
For more information on printing the plate layout and reaction setup instructions, access the *AccuSEQ*® v2.0 Software *Help* by clicking or pressing **F1**.

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 Presence Absence Detection by MycoSEQ Example.eds* 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 87.
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 6.
   - 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 open, edit, or manipulate the experiment files stored on the hard drive of the computer outside of AccuSEQ® Software v2.0. If you do so, you will corrupt the experiment file and you will not be able to open it in AccuSEQ® Software v2.0.

For more information

For more information on using SEQ templates, see "SEQ template workflow" on page 90.

3 Prepare the reactions

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 is shown on page 35:

Start experiment

▼

Set up the experiment (Chapter 2)

▼

Prepare the reactions (Chapter 3)

- Prepare the DNA template.
- Prepare the premix solution.
- Prepare the experiment plate.

▼

Run the experiment (Chapter 4)

▼

Review the Results Summary (Chapter 5)

▼

(Optional) Review the Results (Chapter 6)

▼

(Optional) Investigate the Results (Chapter 7)

▼

End experiment

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 7 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 premix solution

Prepare the premix solution using the components and volumes that were calculated by the AccuSEQ® Software (see page 30). 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 (without overage) are:

Component	Volume (µL) for 1 Reaction
Power SYBR® Green PCR Master Mix (2×)	15.0
Mycoplasma Real-Time PCR Primer Mix (10×)	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 91.

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 17 Reactions†
Power SYBR® Green PCR Master Mix (2X)	280.5
Mycoplasma Real-Time PCR Primer Mix (10X)	56.1
Total Premix Solution Volume	**336.6**

† 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.
7. Place the premix solution on ice until you prepare the reactions.

Prepare the experiment plate

In this section, prepare all reactions in a 96-well plate; use the volumes that were calculated by AccuSEQ® Software v2.0 (see page 30).

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 per well.
- The reaction plate for this example contains 7 Unknowns, 6 Inhibition Controls, 2 Positive Controls, and 2 Negative Controls.
- The total volume of each reaction is 30 µL.

Required materials

- MicroAmp® Fast Optical 96-Well Reaction Plate, also called a Fast reaction plate (Part no. 4346906)
- MicroAmp® Optical Adhesive Film (Part no. 4311971)
- Premix solution (see page 36)
- Unknown sample DNA
- Mycoplasma Real-Time PCR DNA Control (Positive Control)
- Negative Control (water)

- Microcentrifuge tubes
- Pipettors
- Pipette tips
- Vortexer
- Centrifuge

Prepare the reactions

1. Label one MicroAmp® Fast Optical 96-Well Reaction Plate with an appropriate name or barcode.
2. Add 18.0 µL of premix solution to each well
3. Add 12.0 µL of water to each Negative Control sample well.
4. Add 2 µL of water to each Unknown sample well.
5. Add 10 µL of water to each Positive Control well.

   Note: Do not add water to the Inhibition Controls.
6. Add 10 µL of sample to the appropriate Unknown sample and Inhibition Control wells.
7. Completely thaw the *Mycoplasma* Real-Time PCR DNA Control (Positive Control). Life Technologies recommends thawing the Positive Control at 37 °C for five minutes to ensure consistent results.
8. Vortex, then spin down the Positive Control.
9. Add 2.0 µL of Positive Control DNA to each Inhibition Control and Positive Control well.
10. Mix each sample by gently pipetting up and down.
11. Seal the plate with MicroAmp® Optical Adhesive Film.
12. Briefly centrifuge the reaction plate.

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 (2×) 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:
  - Thoroughly mix the master mix by briefly vortexing, then centrifuging the tube.
  - Resuspend the primer mix by vortexing, then briefly centrifuge the tube.

- Thaw the Positive Control DNA at 37 °C for 5 minutes, then vortex and centrifuge to ensure consistent results.
- Thaw frozen samples by placing them on ice. When thawed, resuspend the samples by vortexing, then briefly centrifuge the tubes.

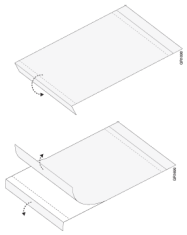
Experiment preparation guidelines

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** (Part no. 4346906) – Seal the reaction plate with optical adhesive film (Part no. 4311971)
  - **Fast reaction tube strips** (Part no. 4358293) – Cover the tubes with optical caps (Part no. 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 (Part no. N8010531).
  b. Load the reaction plate as desired.
  c. Remove a single optical adhesive film (film) from the box.

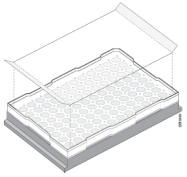
     Fold back one of the end-tabs. Hold the film with its backing side up.



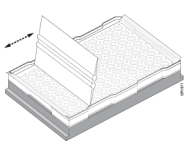
  d. In one continuous movement, peel back the white protective backing from the center sealing surface. Do not touch the center sealing surface.

     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.

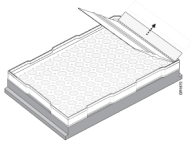
  e. While holding the film by the end-tabs, lower the film onto the reaction plate (adhesive side facing the reaction plate). Be sure the film completely covers all wells of the reaction plate.



  f. While applying firm pressure, move the applicator (Part no. 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*® v2.0 Software *Help* by clicking or pressing **F1**.

4 Run the experiment

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 is shown on page 42.

Start experiment

▼

Set up the experiment (Chapter 2)

▼

Prepare the reactions (Chapter 3)

▼

Run the experiment (Chapter 4)

▼

1. Prepare for the run.
2. (*Optional*) Enable the notification settings.
3. Start the run.
4. (*Optional*) Monitor the run.
5. Unload the instrument.

▼

Review the Results Summary (Chapter 5)

▼

(*Optional*) Review the Results (Chapter 6)

▼

(*Optional*) Investigate the Results (Chapter 7)

▼

End experiment

Prepare for the run

Before you run the experiment, calibrate the 7500 Fast instrument. Refer to the *Applied Biosystems 7500/7500 Fast Real-Time PCR System Installation Guide* (Pub. no. 4387788) or the *AccuSEQ*® Software *Help*.

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**® v2.0 Software).
2. Enter your user name and password, then click **OK**.

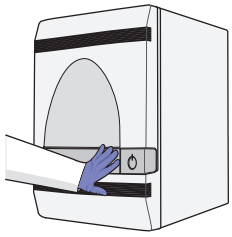
   Note: See your system administrator for user name and password.
3. In the Home screen, click **Open Experiment**.
4. In the Open dialog box, navigate to the **experiments** folder (default):
   <*drive*>:\Applied Biosystems\AccuSEQ\experiments
5. Double-click **Mycoplasma SEQ Example Setup.eds** to open the example experiment file you created in Chapter 2.

Load the reaction plate into the instrument

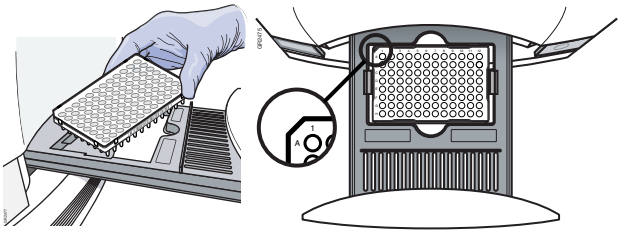
CAUTION! PHYSICAL INJURY HAZARD. During operation, the sample block can be heated to 100 °C. Before performing the following procedure, be sure to wait until the sample block reaches room temperature.

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

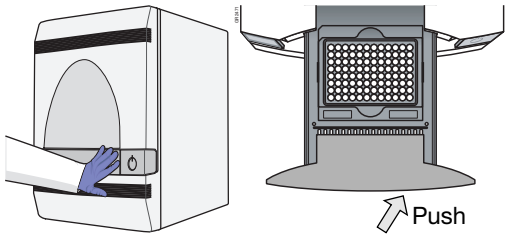
1. Push the tray door to open it.



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 (Part no. 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:

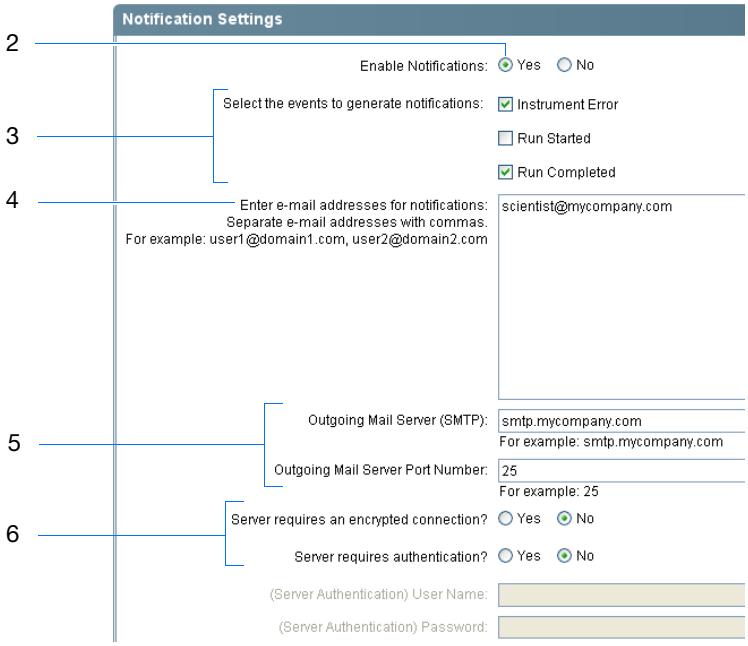
a. Enter the name of the Outgoing Mail Server (SMTP).
For example: **smtp.mycompany.com**.

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.



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 87.

(Optional) Monitor the run

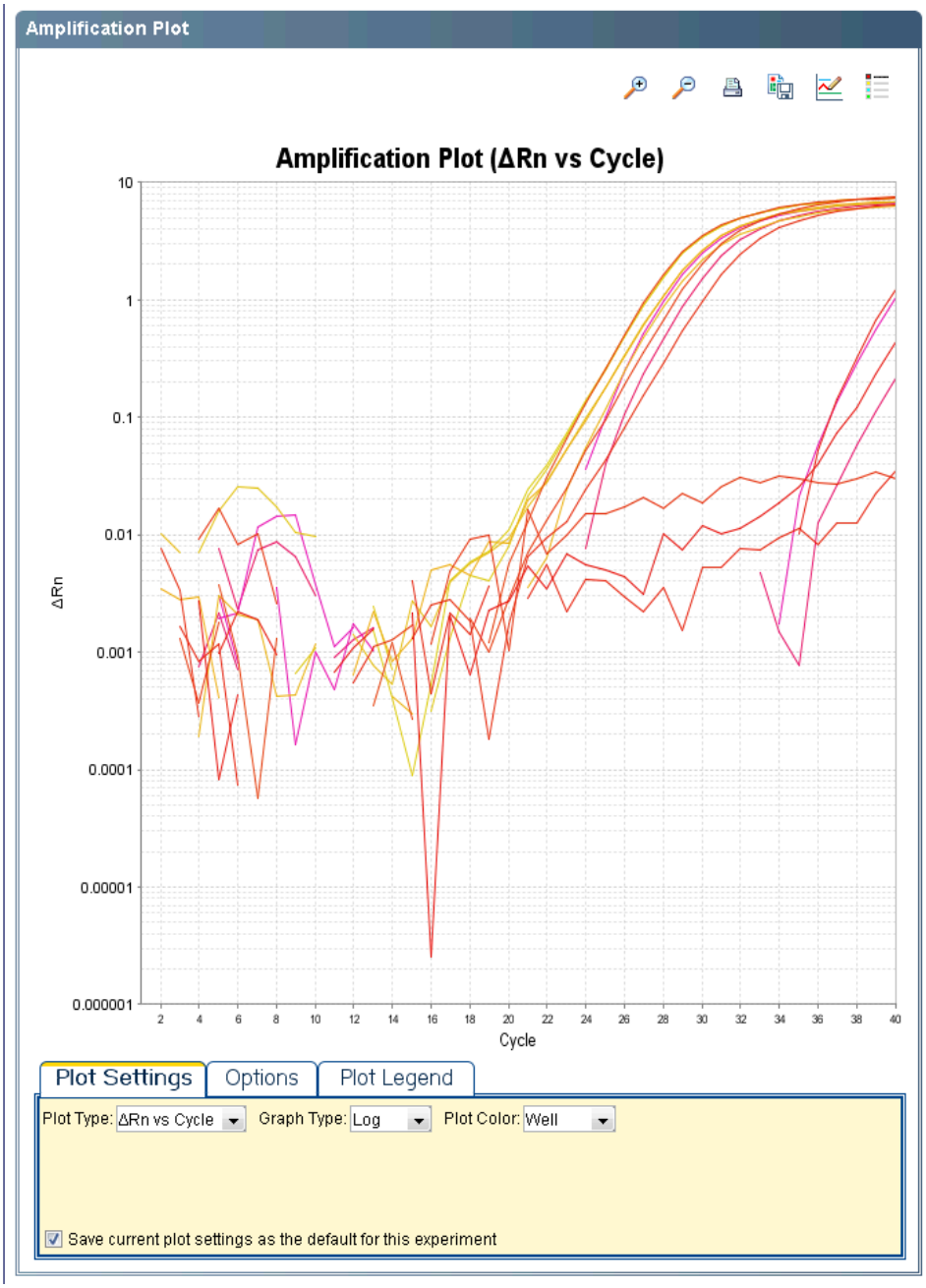
You can view the progress of the run in real time as described below. During the run, periodically view all three available plots (Amplification, Melt Curve, Temperature) from the AccuSEQ® Software for potential problems.

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 46.
View the melt curve in real time	Select **Melt Curve**. See "About the Melt Curve screen" on page 48.
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 49.
Enable/disable notifications in the Notification Settings screen	Select or deselect **Enable Notifications**. See "(Optional) Enable the notification settings" on page 44.

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 (ΔRn) and cycle number. The Amplification Plot screen as it appears during the example experiment is shown on page 47.



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

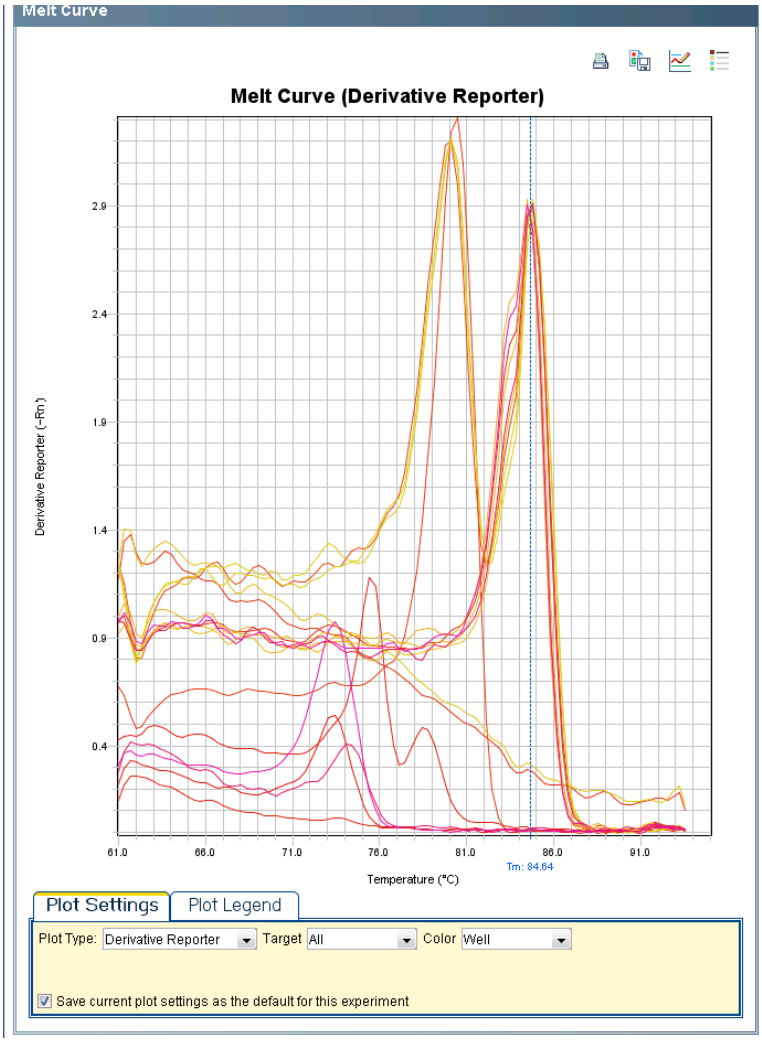
The Amplification Plot screen is useful for identifying and examining abnormal amplification. Abnormal amplification can include the following:

- 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*® v2.0 Software *Help* (click or press **F1**).

About the Melt Curve screen

The melt curve 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 reporter signal (Rn) and temperature (°C). The Melt Curve screen as it appears during the example experiment is shown below:



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® v2.0 Software Help* (click or press **F1**).

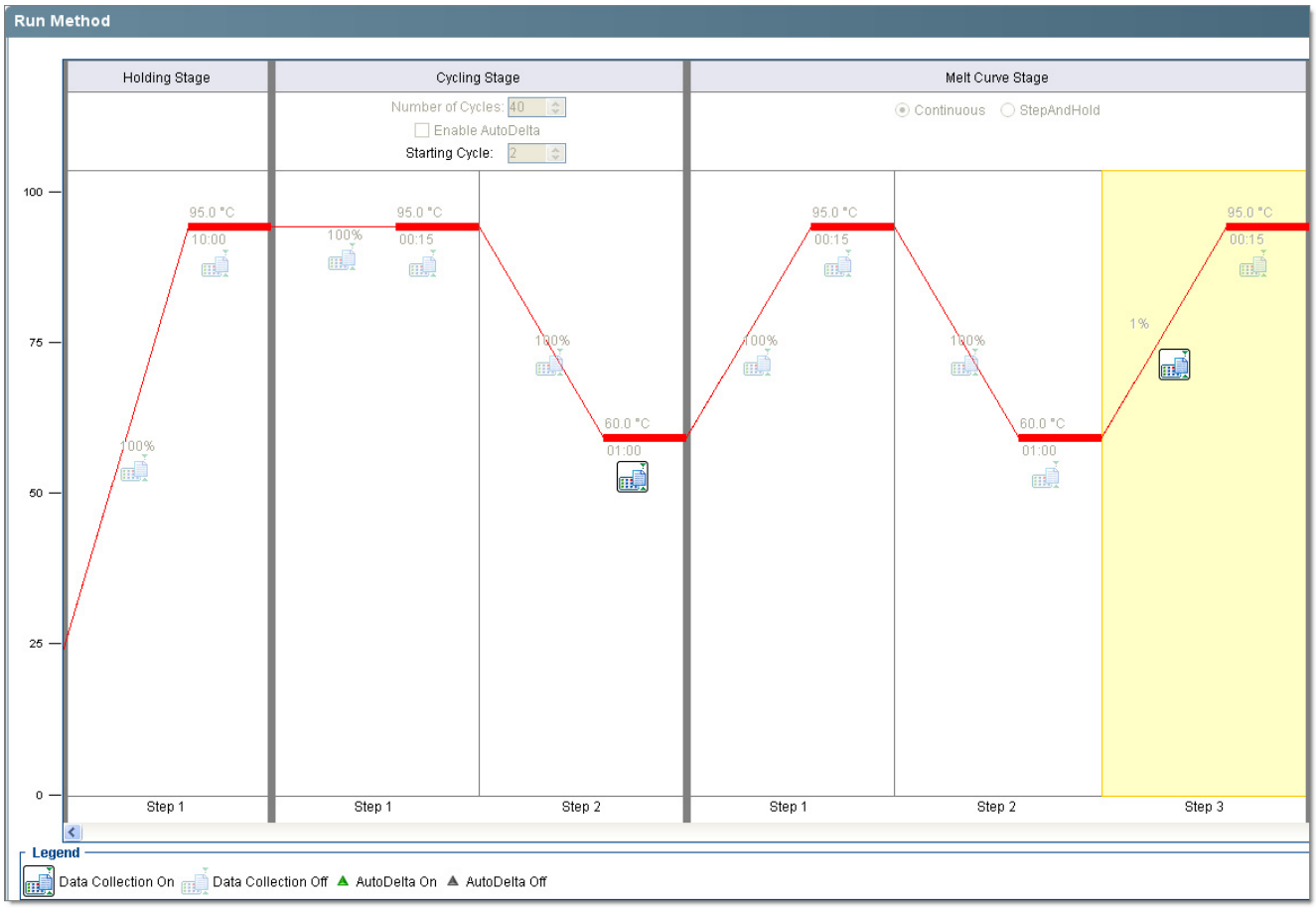
About the Run Method screen

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*® v2.0 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.

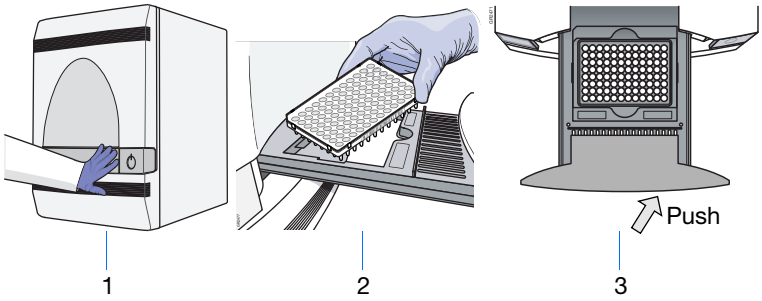
Note: When the instrument completes a run, the system saves the details of the run to the instrument log.

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 reaction plate as appropriate.

5 Review the Results Summary

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 89 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.

Start experiment

▼

Set up the experiment (Chapter 2)

▼

Prepare the reactions (Chapter 3)

▼

Run the experiment (Chapter 4)

▼

Review the Results Summary (Chapter 5)

1. Review the Results Summary.
2. Export and print the data.

▼

(Optional) Review the Results (Chapter 6)

▼

(Optional) Investigate the Results (Chapter 7)

▼

End experiment

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

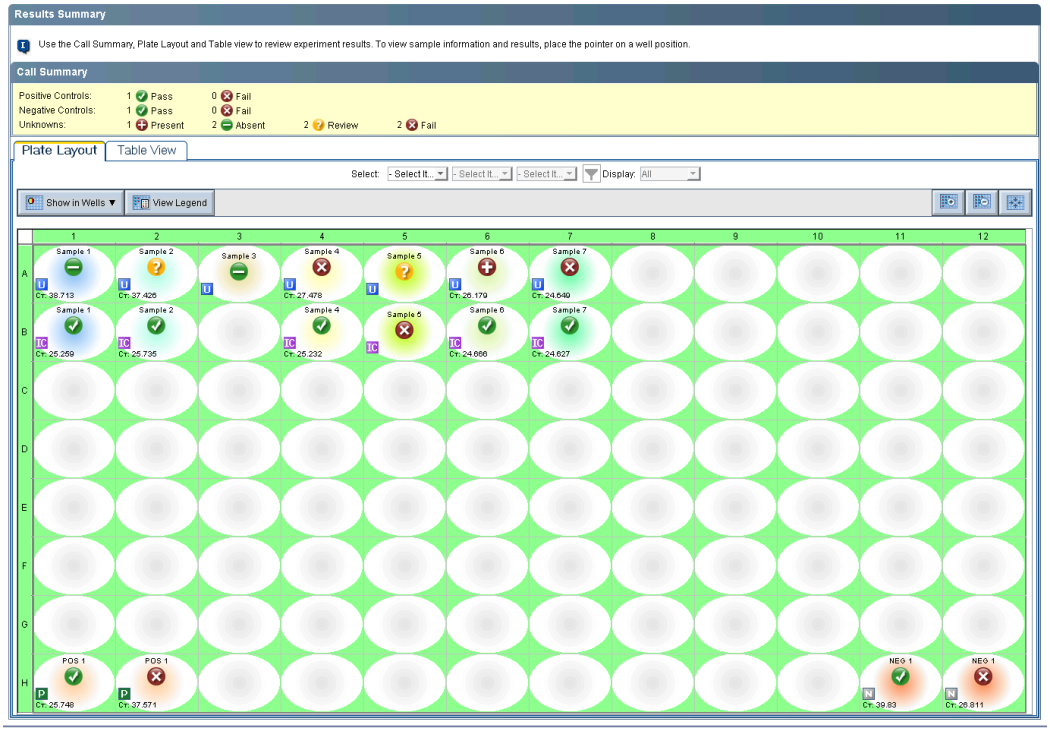
The AccuSEQ® Software automatically analyzes the example experiment data using the default analysis settings for the mycoplasma target. To view the example *Mycoplasma* SEQ experiment results, open the data file that installs with the AccuSEQ® Software. The data file was created with the same design 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® v2.0 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 Presence Absence Detection by MycoSEQ Example.eds** to open the example experiment data file.

   Note: The experiments folder contains several data files; be sure to select *Mycoplasma Presence Absence Detection by MycoSEQ 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*® v2.0 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:

- 7 Unknowns U – 1 Present, 2 Absent, 2 Review, 2 Fail
- 6 Inhibition Controls IC – 5 Pass, 1 Fail
- 2 Positive Controls P – 1 Pass, 1 Fail
- 2 Negative Controls N – 1 Pass, 1 Fail

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.

1. From the navigation pane, select **Results** ▸ **Results Summary**.
2. Review the Call Summary for results:

Sample Type	Call†	Description
Positive Control	Pass	Target detected
	Fail	No target detected (target should be detected)
Negative Control	Pass	No target detected
	Fail	Target detected (no target should be detected)

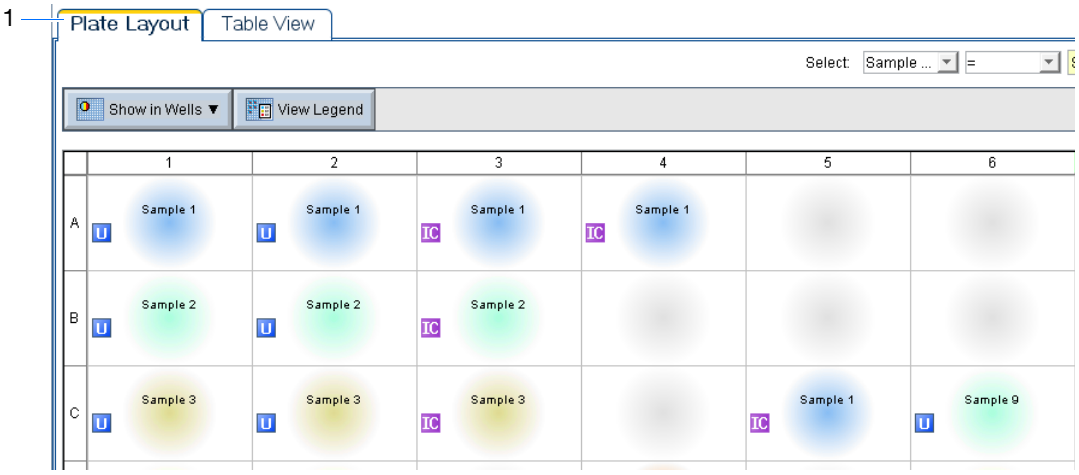
Sample Type	Call†	Description
Unknown	Present	Target signal is present and is within the temperature range, derivative range, and C_T range for the assay.
	Fail	No target signal detected.
	Absent	Target signal is not present within the temperature range, derivative range, and C_T range for the assay.
	Review	AccuSEQ® Software is unable to determine a result.

† Causes of a fail or review result are displayed in the Results Summary (see step 3 on page 55) and the Quality Summary (see page 59).

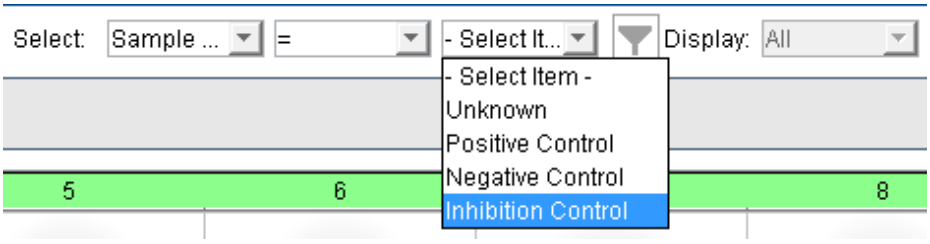
Review the results in the plate layout

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

1. 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).

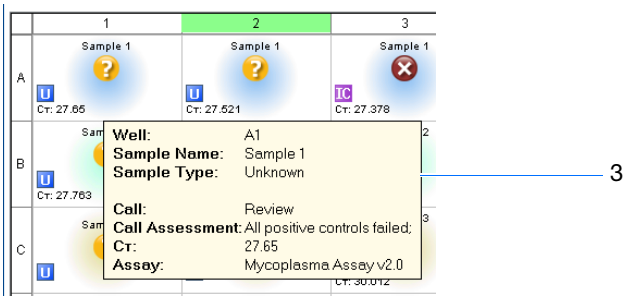


2. In the Select Wells With drop-down list, select **Sample Type**, **=**, then **Inhibition Control**. The AccuSEQ® Software selects the associated wells in the Plate Layout.



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

3. To display the call assessment for an fail or review result, place the mouse pointer on a well. For the example experiment, display wells: **A1**, **A2**, **B1** and **B2**. The call assessment for well A1 is shown below.



4. *(Optional)* To further review fail or review 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*® v2.0 Software *Help* by clicking ? or pressing **F1**.

6 Review the results

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 89 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.

Start experiment

▼

Set up the experiment (Chapter 2)

▼

Prepare the reactions (Chapter 3)

▼

Run the experiment (Chapter 4)

▼

Review the Results Summary (Chapter 5)

▼

(Optional) Review the Results (Chapter 6)

1. Review the Quality Summary.
2. Review the Amplification Plot.
3. Revew the Melt Curve.
4. Review the table data.
5. Export and print the data.

▼

(Optional) Investigate the Results (Chapter 7)

▼

End experiment

About the analysis results

About the results

The Quality Summary provides a call assessment description and troubleshooting information for any review or fail 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 *Mycoplasma Presence Absence Detection by MycoSEQ Example* experiment results, open the data file that installs with the AccuSEQ® Software (see page 17). For the example experiment, investigate the results for wells: **A2** and **A5**.

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 61).
- *(Optional)* 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
All negative controls failed	All negative controls yield fail result.
All positive controls failed	All positive controls yield fail result.
Bad passive reference signal	Passive reference signal is abnormal.
Baseline algorithm failed	The software cannot calculate the best fit baseline for the data.
C_T algorithm failed	The software cannot calculate C_T.
Exponential algorithm failed	The software cannot identify the exponential region of the amplification plot.
Failed IC prevents call	Values for C_T, DV, and T_m met requirements for negative call but detection of inhibition in IC prevents definitive absent call.
High background signal	The background fluorescence signal is higher than 1,300,000 fluorescent standard units (FSU).
Inhibition detected	Delta C_T and T_m did not meet specified values required for "Pass."
NC criteria failed	C_T, DV, or T_m did not meet specified values required for negative control to"Pass."
No signal in well	The well produced very low or no fluorescence.
Not supported by IC	C_T, DV, and T_m results support absent call, but inhibition control was not run. Absent call should be supported by additional data demonstrating a lack of matrix inhibition.
PC criteria failed	C_T, DV, or T_m did not meet specified values required for "Pass."
Positive control signal detected	Peak for discriminatory positive found in sample labeled as an unknown indicating potential error in sample preparation or plate setup.
Review C_T and DV values	Significant peak found within specified temperature range and within specified C_T threshold, but DV magnitude insufficient for positive call. *and/or* C_T and T_m results support absent call but DV magnitude too high for absent call.
Thresholding algorithm failed	Data did not fit thresholding model.

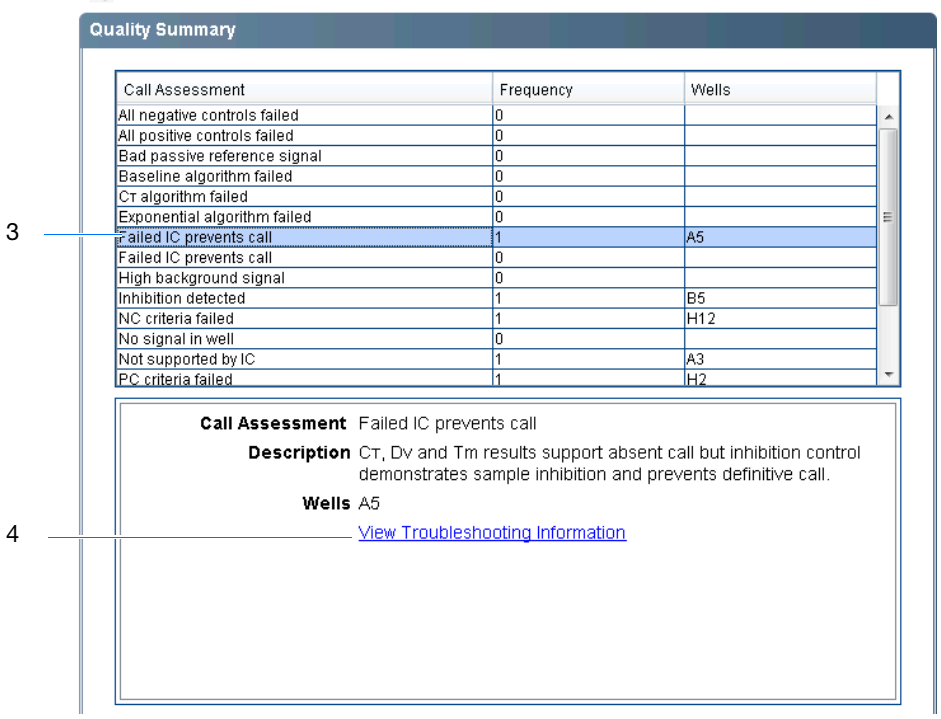
IMPORTANT! The parameters for a SEQ presence/absence experiment are customized for detection of specific organisms and species and may yield different results than a custom presence/absence experiment.

About the example experiment

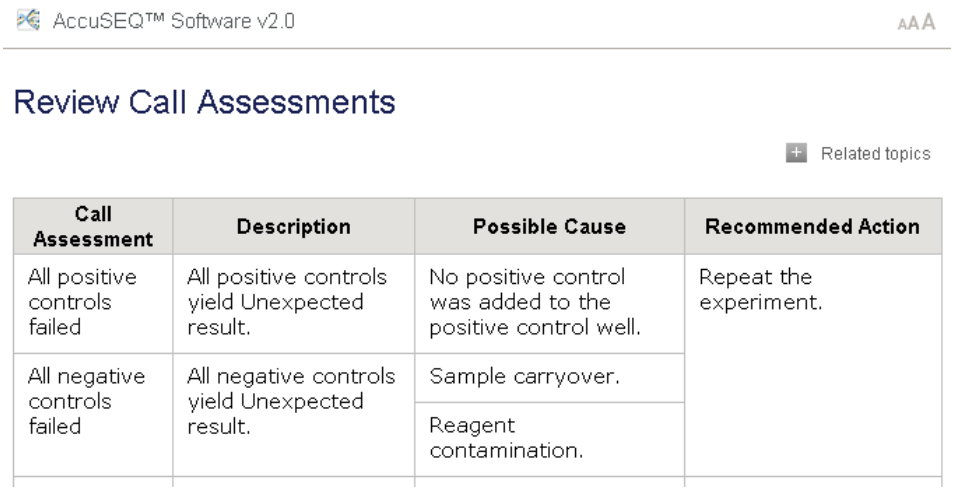
In the *Mycoplasma Presence Absence Detection by MycoSEQ Example* experiment, you review the Quality Summary for any call assessments generated by the experiment data. In the example experiment, call assessments are generated for multiple wells, including A1, A2, A4, and A5.

View the Quality Summary

1. Click **Results** in the navigation pane, then click **Quality Summary**.
2. In the Quality Summary table, look in the Frequency and Wells columns to determine which call assessments appear in the experiment.
3. Click a call assessment in the table to display more information about the assessment. The call assessment for multiple wells in the example experiment is shown below.



4. Click a troubleshooting link below the table to view information on correcting the call assessment. An example of troubleshooting information is shown below.


AccuSEQ™ Software v2.0

Review Call Assessments

Related topics

Call Assessment	Description	Possible Cause	Recommended Action
All positive controls failed	All positive controls yield Unexpected result.	No positive control was added to the positive control well.	Repeat the experiment.
All negative controls failed	All negative controls yield Unexpected result.	Sample carryover.	
		Reagent contamination.	

5. To investigate the data for results, review plots (see page 61).

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*® v2.0 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:

- **ΔRn vs Cycle** – ΔRn is Rn minus the baseline; ΔRn is the magnitude of the normalized fluorescence signal generated by the reporter at each cycle during the PCR amplification. This plot displays ΔRn 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.
- **Rn vs Cycle** – Rn is the fluorescence of the reporter dye divided by the fluorescence of a passive reference dye; that is, Rn is the reporter signal normalized to the fluorescence signal of the passive reference.
- **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).

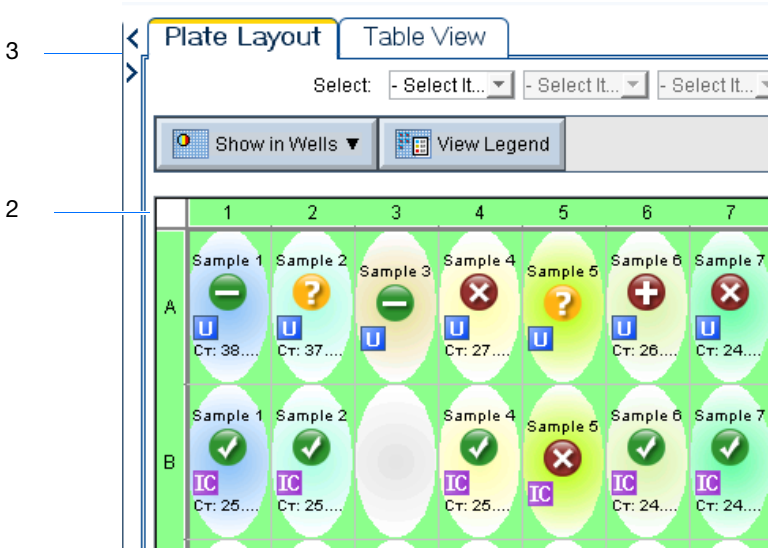
About the example experiment

You can examine the amplification plot to help with troubleshooting and quality control. In the *Mycoplasma Presence Absence Detection by MycoSEQ Example* experiment, you review the amplification plot for:

- C_T values
- Irregular amplification
- Outliers

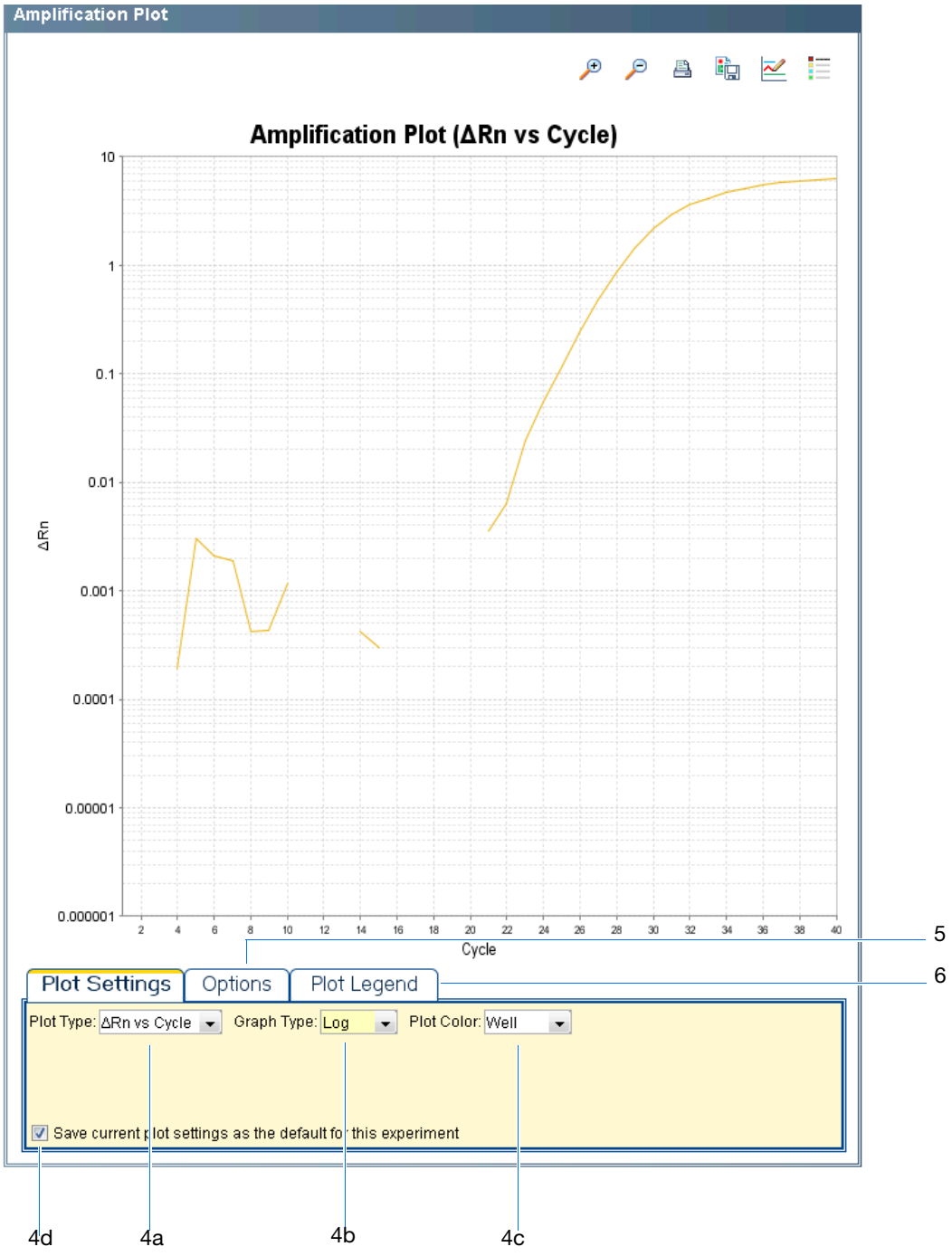
View the Amplification Plots

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.

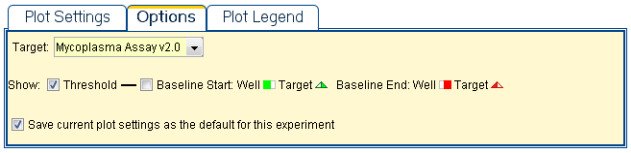


4. Select the **Plot Settings** tab, and adjust the plot display, as shown on page 63:
   a. From the Plot Type drop-down list, select **ΔRn vs Cycle**.
   b. From the Graph Type drop-down list, select **Log**.
   c. From the Plot Color drop-down list, select **Well**.

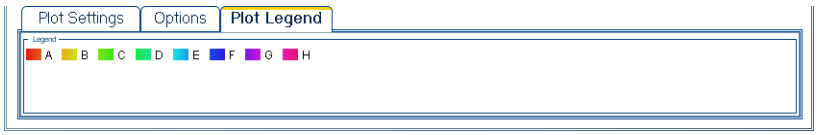
d. *(Optional)* Deselect the **Save current plot settings as the default for this experiment** checkbox.



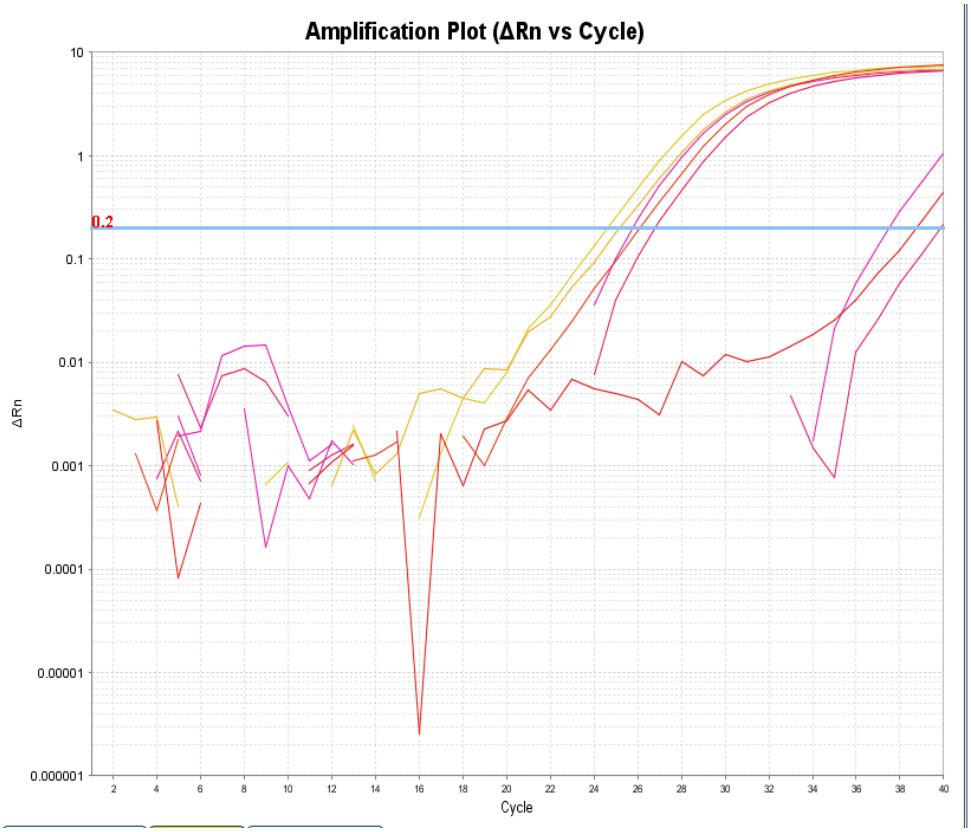
5. Select the **Options** tab, then select to show the **Threshold** (0.2).



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

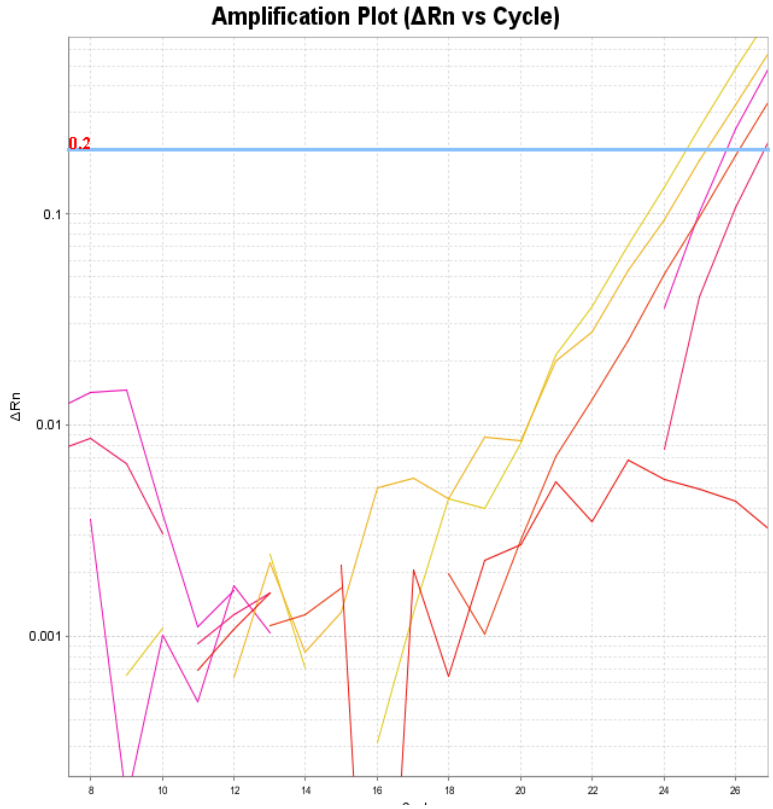


7. Select wells in the plate layout to view in the amplification plot (see page 89 for tips). For the example experiment, display the following replicate wells: **A1**, **B1** (Sample 1), **A6**, **B6** (Sample 6), **H1**, **H2** (POS) and **H11**, **H12** (NEG).



While not present in this view, 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. A quadrant in the plot to the left of the area you want to review.
   b. (Zoom in).



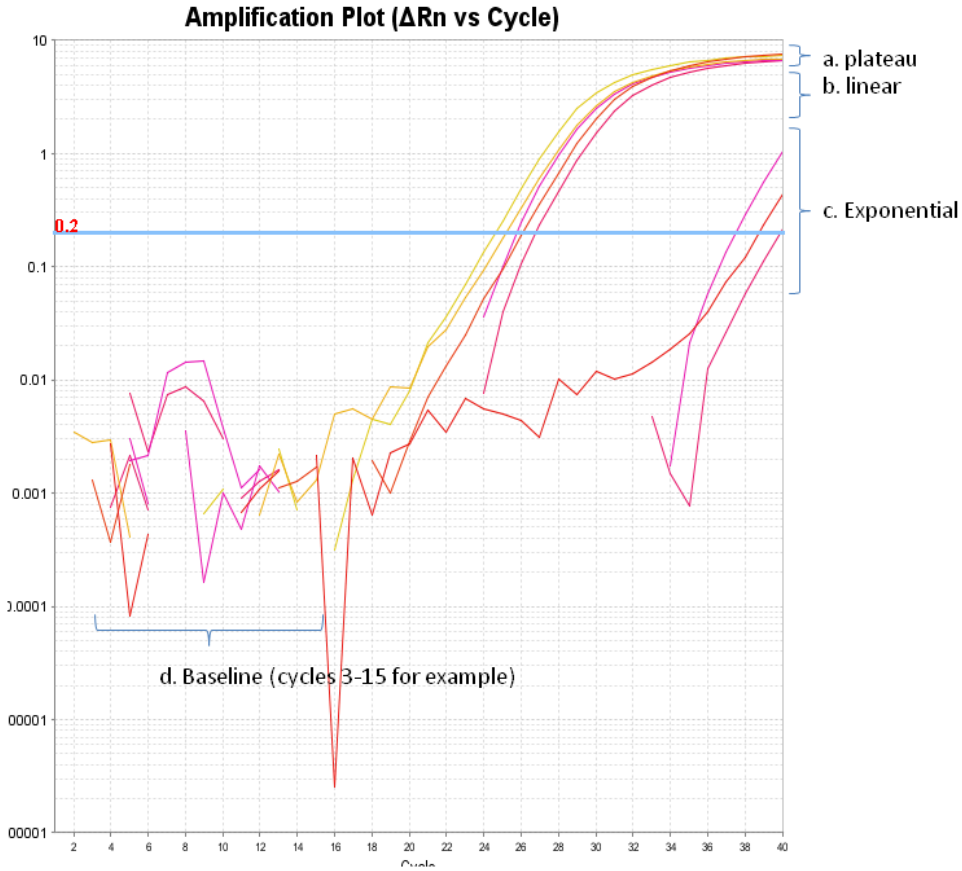
9. *(Optional)* Click (Hide the plot legend).

   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

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 84).

For more information

For more information on the Amplification Plot screen, access the *AccuSEQ*® v2.0 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 (DV) 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 *Mycoplasma Presence Absence Detection by MycoSEQ Example* experiment:

- For the 7 Unknown wells:
  - The mycoplasma target is present in Sample 6.
  - The mycoplasma target is absent in Samples 1 and 3 (although Sample 3 lacks an IC).
  - The Positive Control cross-contaminated Samples 4 and 7, leading to failed calls.
  - The AccuSEQ® Software v2.0 is unable to determine a result for Sample 2.
- One POS well failed because no peak in the POS control target range was detected.
- One NEG failed due to cross-contamination with the POS control DNA.

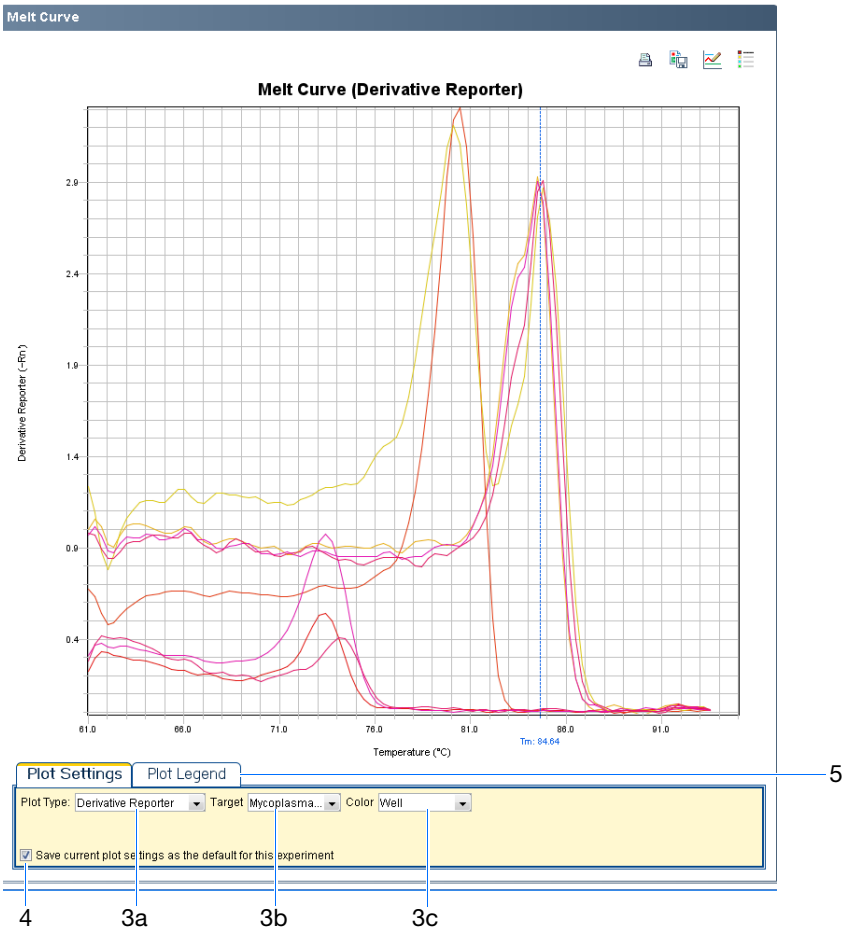
View the Melt Curve

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 Assay
   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.

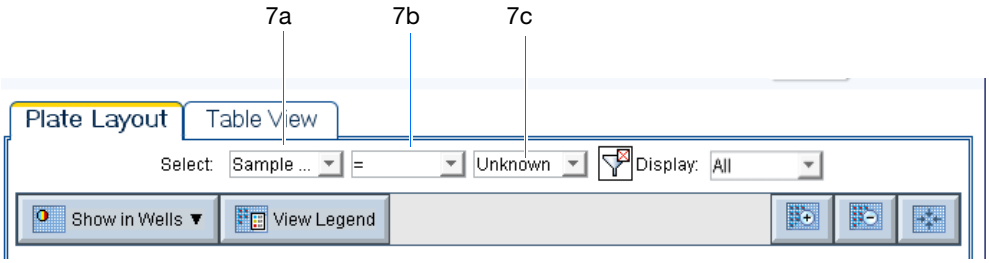


6. *(Optional)* Click (Hide the plot legend).

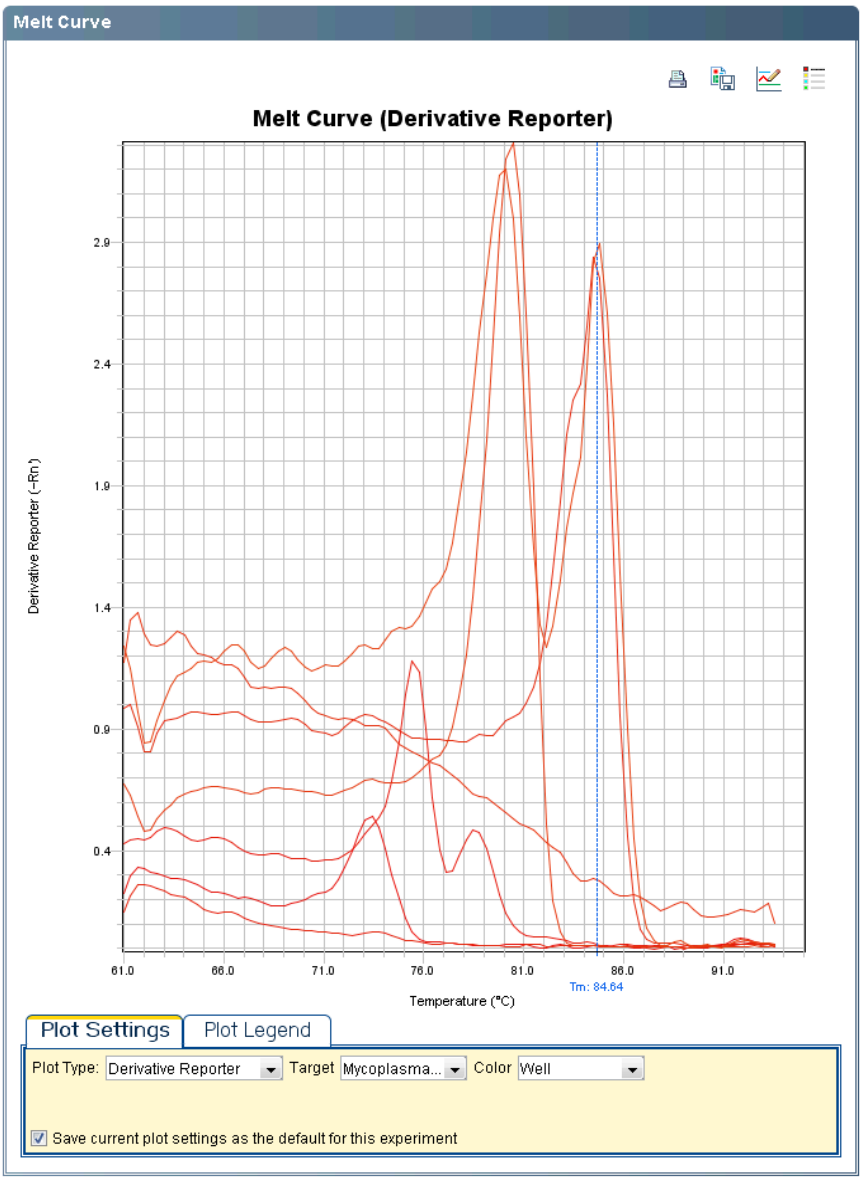
   Note: This is a toggle button. When the legend is hidden, the button changes to Show a legend for the plot.

7. Select wells in the plate layout to view in the melt curve plot:
   a. In the first Select Item drop-down list, select **Sample Type**.
   b. In the second Select Item drop-down list, select **=**.
   c. In the third Select Item drop-down list, select:
      - **Unknown** to view only UNKNOWN wells. The example experiment contains review results for all UNKNOWN wells.
      - **Positive Control** to view only POS wells. The example experiment contains fail results for all POS replicate wells.

- **Negative Control** to view only NEG wells. The example experiment contains pass results for both IC well.
- **Inhibition Control** to view only IC wells. The example experiment contains fail results for all of the IC 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 or may not be the 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.
- **Unknown Calls**:
  - Present
  - Absent
  - Review
  - Fail
- **Control Well Calls**:
  - Pass
  - Fail

For more information

For more information on the Melt Curve screen, access the *AccuSEQ*® v2.0 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, and call assessment
- Calculated values: C_T, target T_m, DV, and ΔRn

Note: The ΔRn value is only available for display in the Table View when it is accessed from the Plot Analysis screens.

About the example experiment

For the *Mycoplasma Presence Absence Detection by MycoSEQ Example* experiment, group the wells in the Table View by:

- Call
- Call assessment

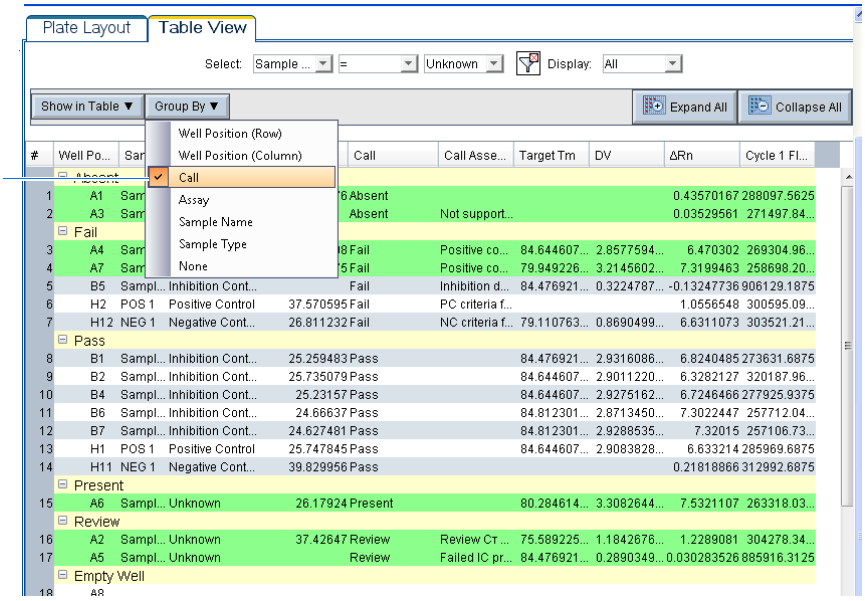
View the Table View

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.

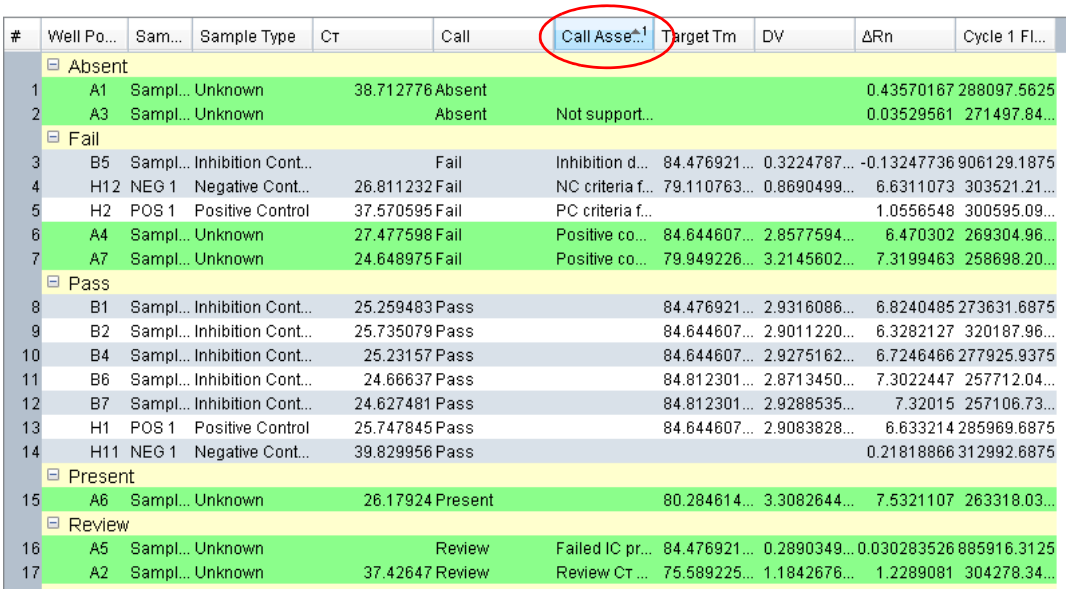
   For the example experiment:

a. From the Group By drop-down list, select **Call**. Wells are listed in the order:
   - Fail
   - Pass
   - Review
   - Empty Well (no call)



In the example experiment:
- **Review** – Unknown sample wells A2 and A5
- **Fail** – A4, B5, A7, H2 and H12

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 Po...	Sam...	Sample Type	Ct	Call	Call Asse...	Target Tm	DV	ΔRn	Cycle 1 Fl...
	Absent									
1	A1	Sampl...	Unknown	38.712776	Absent				0.43570167	288097.5625
2	A3	Sampl...	Unknown		Absent	Not support...			0.03529561	271497.84...
	Fail									
3	B5	Sampl...	Inhibition Cont...		Fail	Inhibition d...	84.476921...	0.3224787...	-0.13247736	906129.1875
4	H12	NEG 1	Negative Cont...	26.811232	Fail	NC criteria f...	79.110763...	0.8690499...	6.6311073	303521.21...
5	H2	POS 1	Positive Control	37.570595	Fail	PC criteria f...			1.0556548	300595.09...
6	A4	Sampl...	Unknown	27.477598	Fail	Positive co...	84.644607...	2.8577594...	6.470302	269304.96...
7	A7	Sampl...	Unknown	24.648975	Fail	Positive co...	79.949226...	3.2145602...	7.3199463	258698.20...
	Pass									
8	B1	Sampl...	Inhibition Cont...	25.259483	Pass		84.476921...	2.9316086...	6.8240485	273631.6875
9	B2	Sampl...	Inhibition Cont...	25.735079	Pass		84.644607...	2.9011220...	6.3282127	320187.96...
10	B4	Sampl...	Inhibition Cont...	25.23157	Pass		84.644607...	2.9275162...	6.7246466	277925.9375
11	B6	Sampl...	Inhibition Cont...	24.66637	Pass		84.812301...	2.8713450...	7.3022447	257712.04...
12	B7	Sampl...	Inhibition Cont...	24.627481	Pass		84.812301...	2.9288535...	7.32015	257106.73...
13	H1	POS 1	Positive Control	25.747845	Pass		84.644607...	2.9083828...	6.633214	285969.6875
14	H11	NEG 1	Negative Cont...	39.829956	Pass				0.21818866	312992.6875
	Present									
15	A6	Sampl...	Unknown	26.17924	Present		80.284614...	3.3082644...	7.5321107	263318.03...
	Review									
16	A5	Sampl...	Unknown		Review	Failed IC pr...	84.476921...	0.2890349...	0.030283526	885916.3125
17	A2	Sampl...	Unknown	37.42647	Review	Review Ct ...	75.589225...	1.1842676...	1.2289081	304278.34...

In the example experiment:

- **Inhibition detected** – Unknown sample B5
- **Failed IC prevents call** – Unknown sample A5

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 review or fail results:
  - Target T_m and DV
  - C_T
- *(Optional)* To further investigate questionable results, see Chapter 7.

For more information

For more information on the Table View, access the *AccuSEQ*® v2.0 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*® v2.0 Software *Help* by clicking or pressing **F1**.

7 (Optional) Investigate the results

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 89 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 on page 74.

Start experiment

▼

Set up the experiment (Chapter 2)

▼

Prepare the reactions (Chapter 3)

▼

Run the experiment (Chapter 4)

▼

Review the Results Summary (Chapter 5)

▼

(Optional) Review the Results (Chapter 6)

▼

(Optional) Investigate the Results (Chapter 7)

1. Review the Multicomponent Plot.
2. Review the Raw Data Plot.
3. Revew the Raw Data Plot.
4. Review the analysis settings.
5. Omit wells from the analysis.

▼

End experiment

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 *Mycoplasma Presence Absence Detection by MycoSEQ Example* experiment results, open the data file that installs with the AccuSEQ® Software (see page 17). For the example experiment, investigate wells: **A2**, **A4**, **A5**, **A7**, **B5**, **H2** and **H12**.

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 61).
- 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 84).

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.

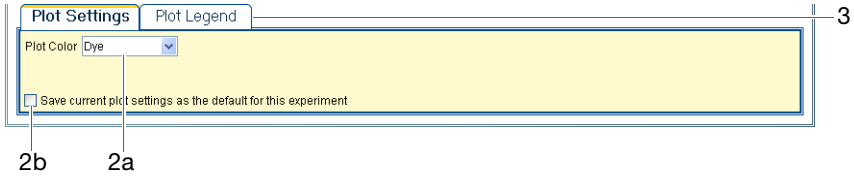
About the example experiment

In the *Mycoplasma Presence Absence Detection by MycoSEQ Example* 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.

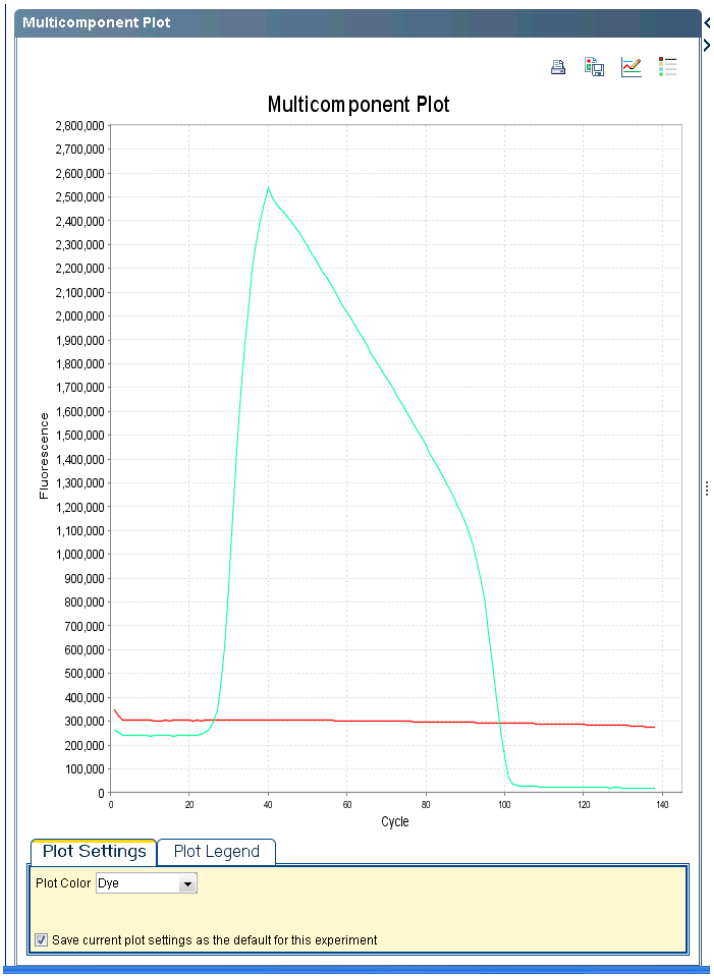


4. *(Optional)* Click  (Hide the plot legend).

   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:
   a. Click the **Plate Layout** tab.

b. Select one well in the plate layout; the well is shown in the Multicomponent Plot screen. The plot for well A6 (= review 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 significantly increase for NEG wells, if the call for an UNKNOWN well is Absent, or if the call for a POS well is Fail. The SYBR dye signal should increase for POS and IC 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 be no significant amplification in the Negative Control wells. Non-specific amplification may occur and result in a C_T value, but T_m will not be within target range.

For more information

For more information on the Multicomponent Plot screen, access the *AccuSEQ*® v2.0 Software *Help* by clicking or pressing **F1**.

Review the Raw Data Plot

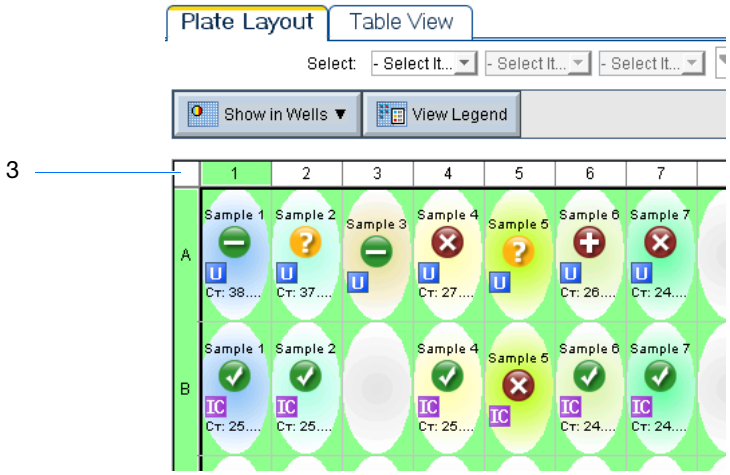
The Raw Data Plot screen displays the raw fluorescence (not normalized) for each optical filter for the selected wells during each cycle of the real-time PCR.

About the example experiment

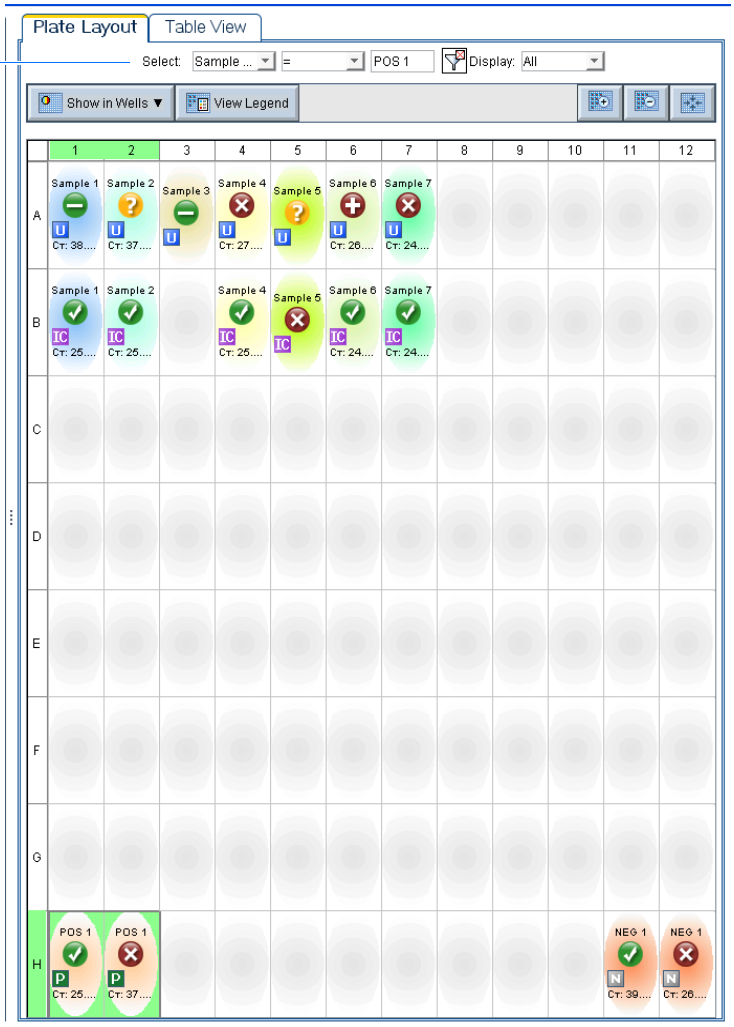
In the *Mycoplasma Presence Absence Detection by MycoSEQ Example* experiment, you review the Raw Data Plot screen for a stable increase in signal (no abrupt changes or dips) from the appropriate filter.

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) – In the Select section, select Sample Name in the first drop-down list. In the second drop-down list,. select =. In the data entry field, enter a sample name, for example, POS 1. Then click to display results:



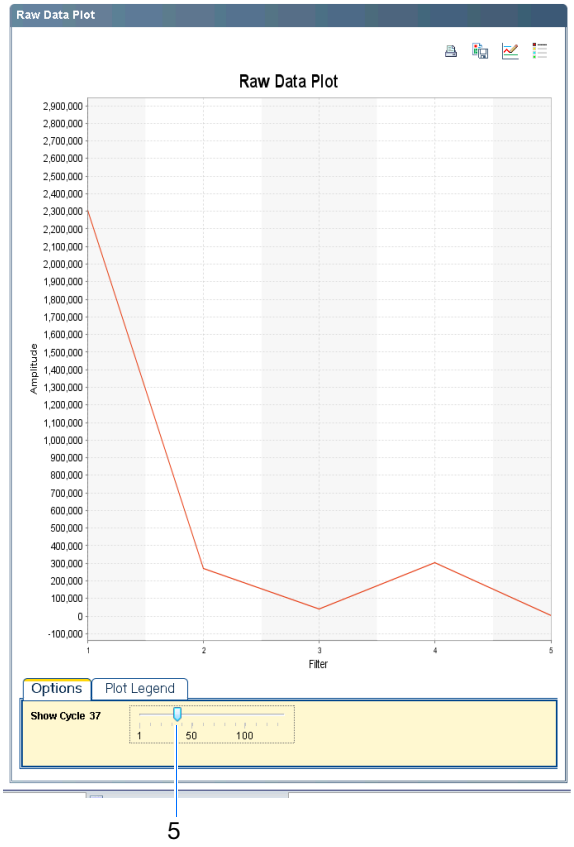
   Use filters to specify wells for selection. Your specification can be whole (for example, Sample Name=Sample 7) or partial (for example, Sample Name contains 7). You can narrow your specification to selected or unselected wells.

5. Click and drag the Show Cycle pointer from cycle 1 to cycle 138, 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 6 in the example experiment is 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*® v2.0 Software *Help* by clicking or pressing **F1**.

Review the analysis settings

Note: You must have administrator or scientist privileges to access the Analysis-Analysis Settings menu.

You can review and edit analysis settings for the threshold cycle (C_T) for *Mycoplasma* SEQ experiments, *Mycoplasma* Presence Absence calls, and quality check (QC) and peak noise.

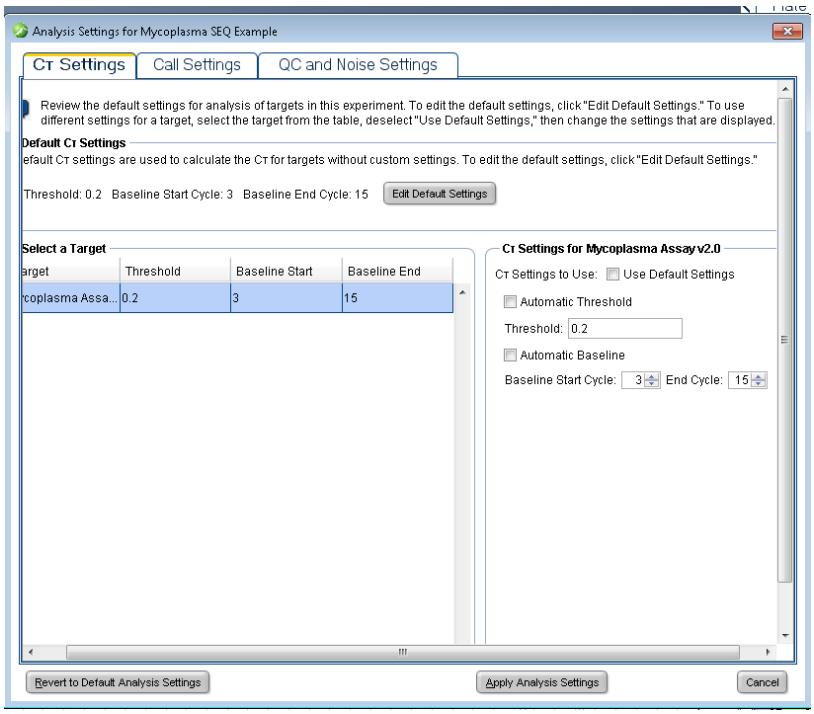
About the example experiment

In the *Mycoplasma Presence Absence Detection by MycoSEQ Example* 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:



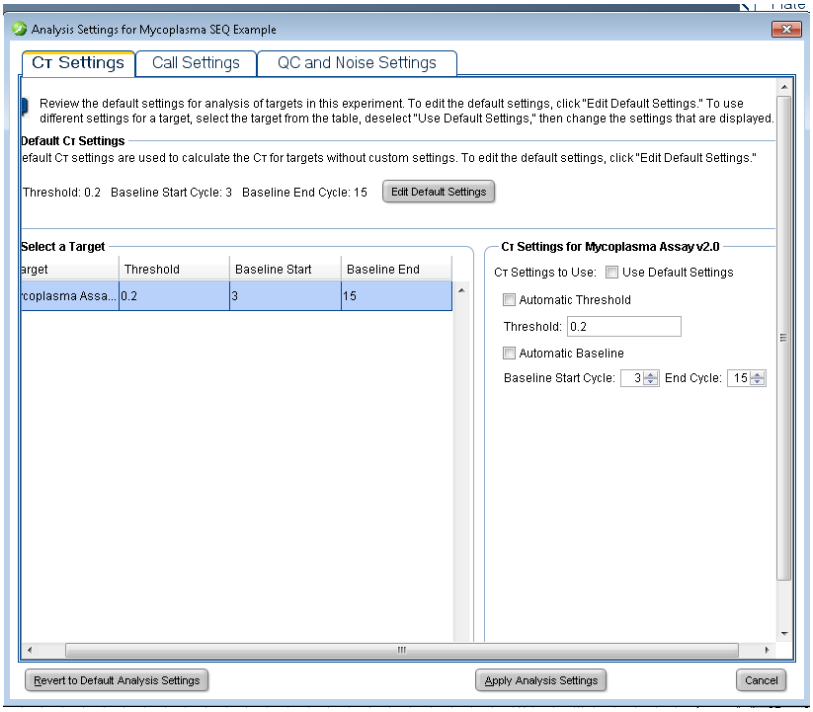
C_T Settings

The C_T Settings tab shows the pre-defined C_T analysis settings for *Mycoplasma* SEQ experiments, optimized in the AccuSEQ® Software for use with the PrepSEQ® Sample Preparation Kits.

Note: For details on the Mycoplasma assay and detection kit, refer to the *MycoSEQ Mycoplasma* Detection Kits User Guide.

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:



Call Settings

To review Mycoplasma and Presence Absence call settings for unknown samples, positive control and inhibition control, and negative control, click the **Call Settings** tab.

Edit the settings for thresholds and ranges by clicking and dragging a "thumb control" left or right. As you drag a thumb control, the settings in the table change.

Set C_T limits for calls

You can change the C_T limits for the following calls:

- **Unknowns:** C_T for Unknown samples
- **Positive Control and Inhibition Control:** DV Threshold for Postive Control and Delta C_T for Inhibition Control
- **Negative Control:** C_T for Negative Control and DV Threshold for Negative Control

To change threshold settings, click anywhere in a threshold, then drag the thumb control. For finer granularity, use keyboard arrows to move the thumb control.

Range settings

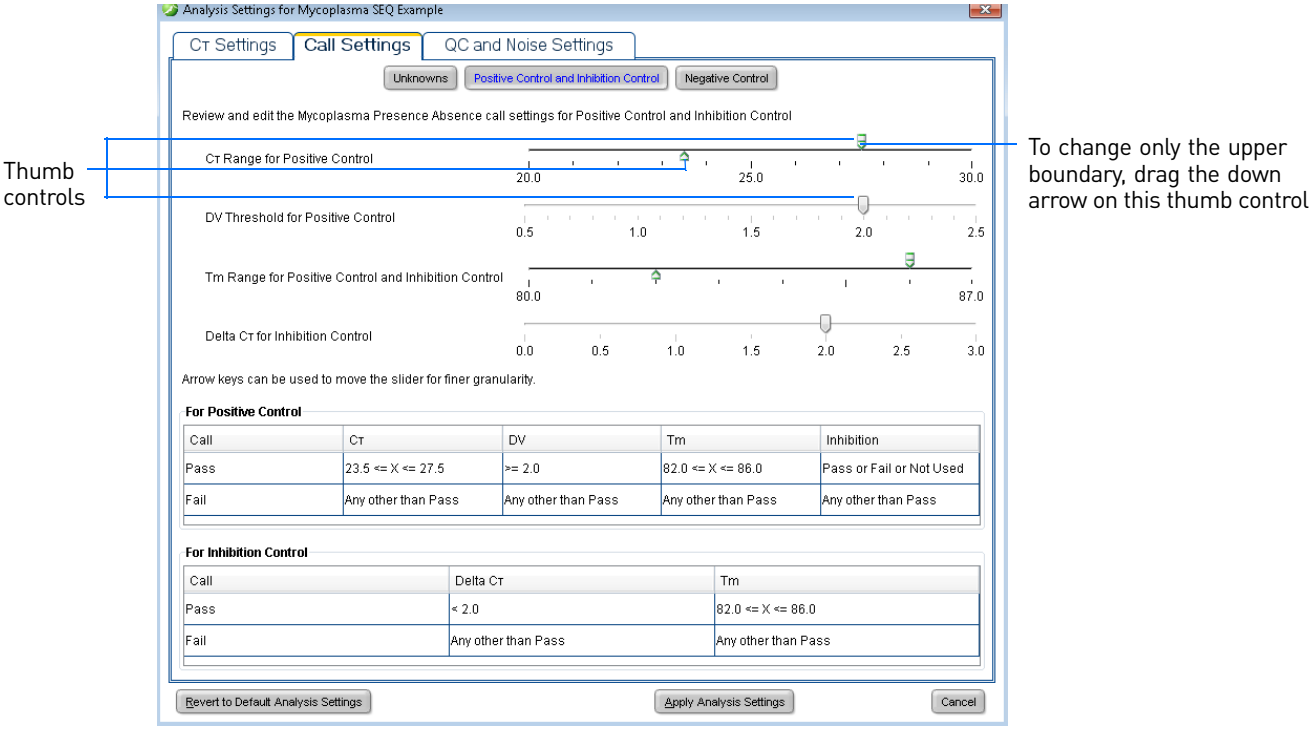
You can change the settings for the upper and lower boundaries of ranges on the following calls:

- **Unknowns:** DV Range for Unknown samples and T_m Range for Unknown samples
- **Positive Control and Inhibition Control:** C_T Range for Postive Control and T_m Range for Positive Control and Inhibition Control
- **Negative Control:** T_m Range for Negative Control

To change the setting for *only the upper boundary range*, drag the bottom, down arrow on the thumb control.

To change the setting for the lower boundary range, drag the thumb control.

To simultaneously change the upper and lower boundaries, place your cursor on the control. When the cursor changes to , drag the control.

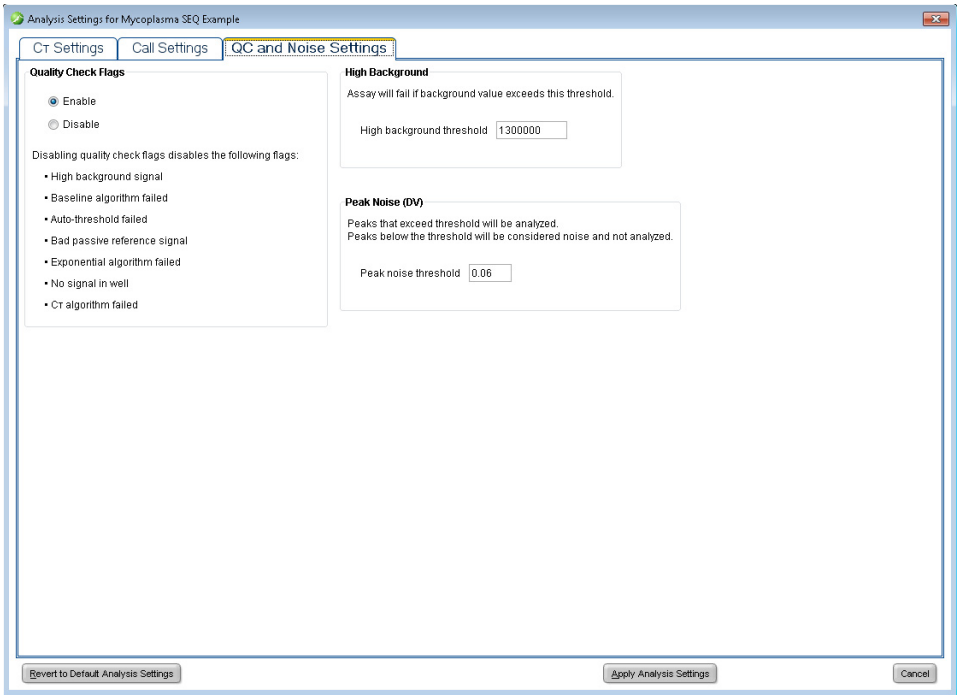


QC and Noise Settings

In Mycoplasma SEQ experiments, you can edit QC and noise settings, which control enabling or disabling quality check flags, and editing the high background threshold and the derivative value (DV) for the peak noise threshold.

To edit QC and noise settings:

1. Click **Analysis** in the top menu, then **Analysis Settings**.
2. Click the **QC and Noise Settings** tab.
3. To change the default Enable setting for quality check flags, click **Disable**.
4. To edit the high background threshold, enter a value in the data entry field.
5. To edit the peak noise threshold, enter a value in the data entry field.



Restore default analysis settings

To restore the default call settings, click **Revert to Default Analysis Settings**.

Save edited analysis settings

To use the edited call settings, click **Apply Analysis Settings**. If the run status for the experiment is complete, the data are reanalyzed.

Guidelines for your experiment

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*® v2.0 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.

About the example experiment

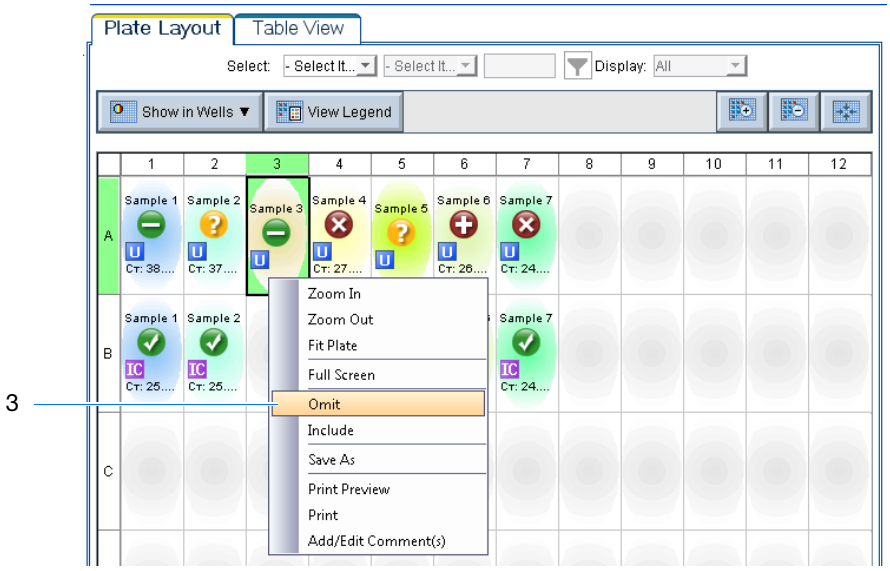
In the *Mycoplasma Presence Absence Detection by MycoSEQ Example* experiment, there are no outliers; no wells need to be removed from analysis.

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 89 for tips).

   Note: Alternatively, you can omit wells from the Table View. See the *AccuSEQ*® v2.0 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 87.

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 87.

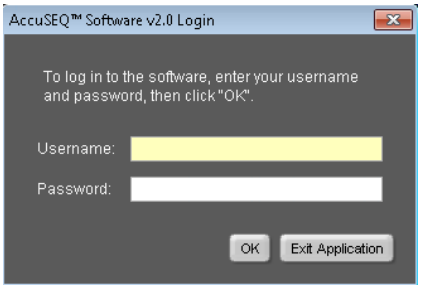
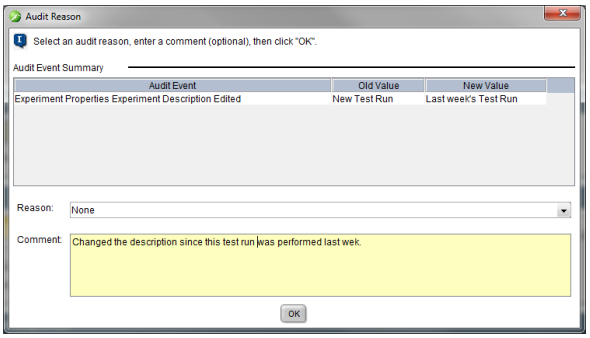
For more information

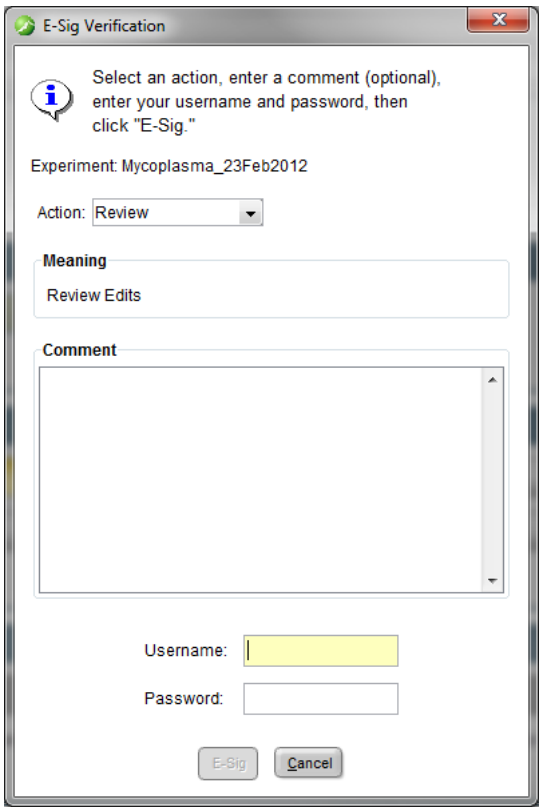
For more information on omitting wells from the analysis, access the *AccuSEQ*® v2.0 Software *Help* by clicking or pressing **F1**.

A Reference Information

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

The AccuSEQ® v2.0 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® v2.0 Software Help*.

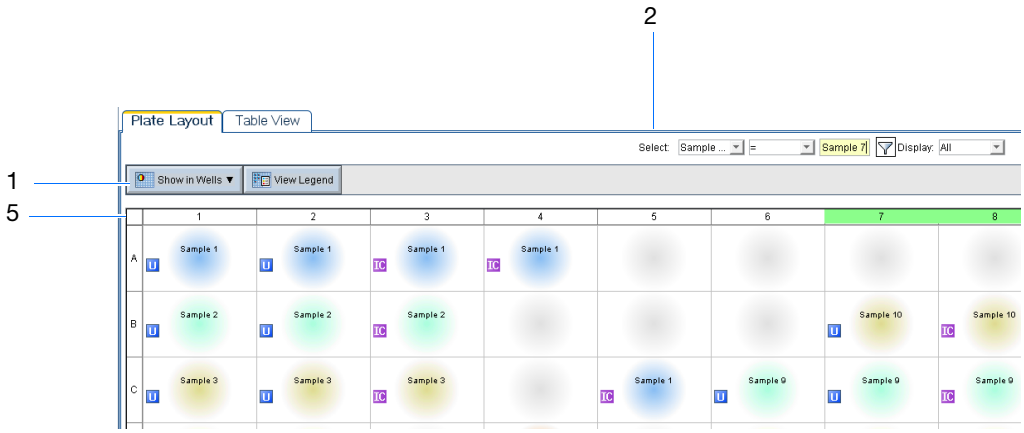
Navigation tips

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:

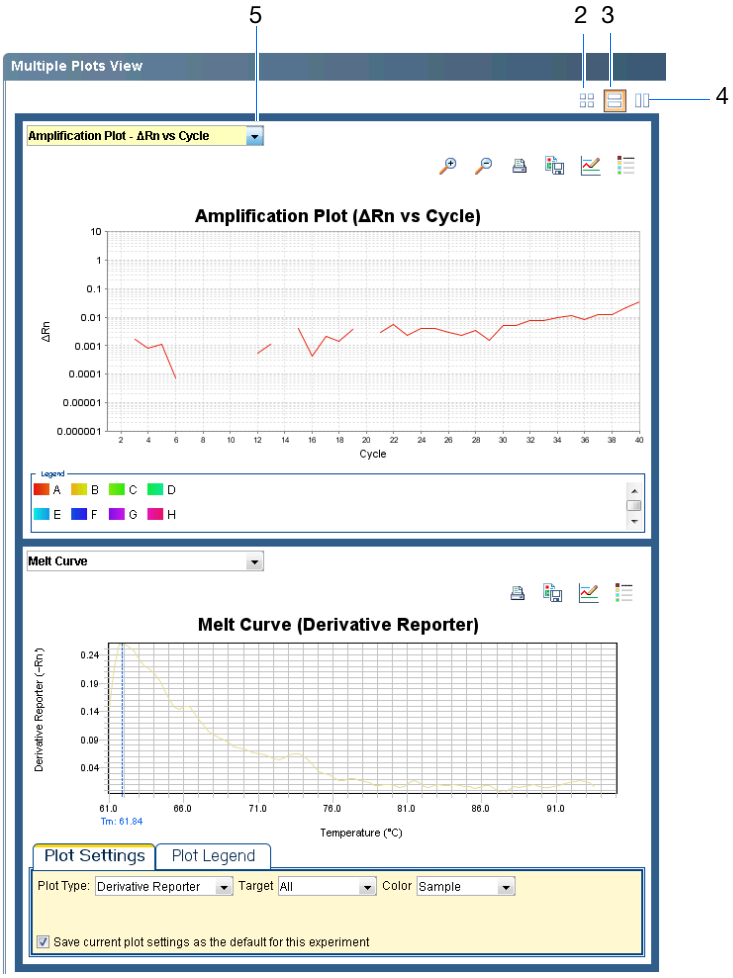
1. To change the data displayed in the Plate Layout, click **Show in Wells**, then select (or deselect) the data to display in the plate layout.
2. To select wells of a specific type, use the Select drop-down lists. Select a well position; omit a well; or select a sample name, sample type, assay, C_T, call, call assessment, target T_m, or DV. Use filters to specify your selection, for example, Sample Name=Sample 12.
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 42), or create a new SEQ experiment (see page 23).
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 87.
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 experiement, 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 87.
7. Run the experiment, as needed.

Glossary

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 "pass" 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 (ΔRn) vs. cycle
- Normalized reporter (Rn) 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 (ΔRn)	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 ΔRn vs. Cycle amplification plot, ΔRn is calculated at each cycle as: ΔRn (cycle) = Rn (cycle) – Rn (baseline), where Rn = normalized reporter. See also normalized reporter (Rn).
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 Rn (ΔRn)	See baseline-corrected normalized reporter (ΔRn).
derivative reporter (–Rn')	The negative first-derivative of the normalized fluorescence generated by the reporter during PCR amplification. In the derivative reporter (–Rn') 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.
pass 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 (;).
fail 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 a fail result are displayed in the Results Summary and the Quality Summary.
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.
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). Monitors for inhibitors in the unknown sample (inhibition in the presence of a positive target). A minimum of one inhibition control is recommended 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 *dissociation curve*.
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.
Mycoplasma 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 *NEG* or *no template control* (*NTC*).
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 "pass" 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 *premix solution*.
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.
review result	In *Mycoplasma* SEQ experiments, a sample result indicating that the AccuSEQ® Software is unable to determine a result. Causes of a review result are displayed in the Results Summary and the Quality Summary.
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: • U Unknown • P POS (positive control) • N NEG (negative control) • ***Mycoplasma*** SEQ experiment**s:** If you specify inhibition controls for samples, each sample is assigned inhibition control wells IC.
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:

- CY®3 dye
- CY®5 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.

unknown

A DNA sample from media, cell culture, or other source that you are testing for the presence of mycoplasmas.

Documentation and Support

Related documentation

For information on new assays and updated product documentation, go to **www.lifetechnologies.com**.

The following related documents are shipped with the system:

Document	Publication number	Description
Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System Absolute Quantitation Using Standard Curve Getting Started Guide	4347825	Provides brief, step-by-step procedures for absolute quantitation using a standard curve. It is designed to help you quickly learn to use the Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System.
Applied Biosystems 7500/7500 Fast Real-Time PCR Systems Maintenance Guide	4387777	Explains how to maintain the 7500/7500 Fast system. Intended for laboratory staff responsible for the maintenance of the 7500/7500 Fast system.
MycoSEQ™ Mycoplasma Detection Kits *Quick Reference*	4393471	Provides brief, concise instructions on using the MycoSEQ™ *Mycoplasma* Detection Kits.
AccuSEQ® Software v2.0, *Custom Experiments, Quick Reference*	4425585	Provides abbreviated procedures for creating, analyzing, viewing, and interpreting custom standard curve experiments in the AccuSEQ software, using resDNASEQ CHO DNA kit data as an example.
AccuSEQ® Software v2.0, *Mycoplasma SEQ Experiments, Quick Reference*	4425586	Provides abbreviated procedures for creating, analyzing, viewing, and interpreting Mycoplasma SEQ experiments using AccuSEQ® Software.
MycoSEQ® Mycoplasma Detection Kits User Guide	4465874	Describes the MycoSEQ™ *Mycoplasma* Detection Kits and provides information on preparing, running, and troubleshooting Mycoplasma detection.
PrepSEQ® Sample Preparation Kits Quick Reference	4465875	Provides brief, concise instructions on using the PrepSEQ® Sample Preparation Kits.
MycoSEQ™ Mycoplasma Detection Kits *Quick Reference*	4465876	Provides brief, concise instructions on using the MycoSEQ™ *Mycoplasma* Detection Kits.
PrepSEQ® Sample Preparation Kits User Guide	4465957	Describes the PrepSEQ® Sample Preparation Kits and provides information on preparing, running, and troubleshooting sample preparation.
MycoSEQ™ Mycoplasma Real-Time PCR Detection Kit Product Insert	4482248	Describes the MycoSEQ™ *Mycoplasma* Real-Time PCR Detection Kit.

Note: To open the user documentation included on the AccuSEQ®Software v2.0 CD, use the Adobe® Reader® software available from **www.adobe.com**

Note: For additional documentation, see "Obtaining support" on page 102.

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 ▸ Contents and Index**.
- 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

Obtaining support

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

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

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Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
For support visit www.lifetechnologies.com/support or email techsupport@lifetech.com

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