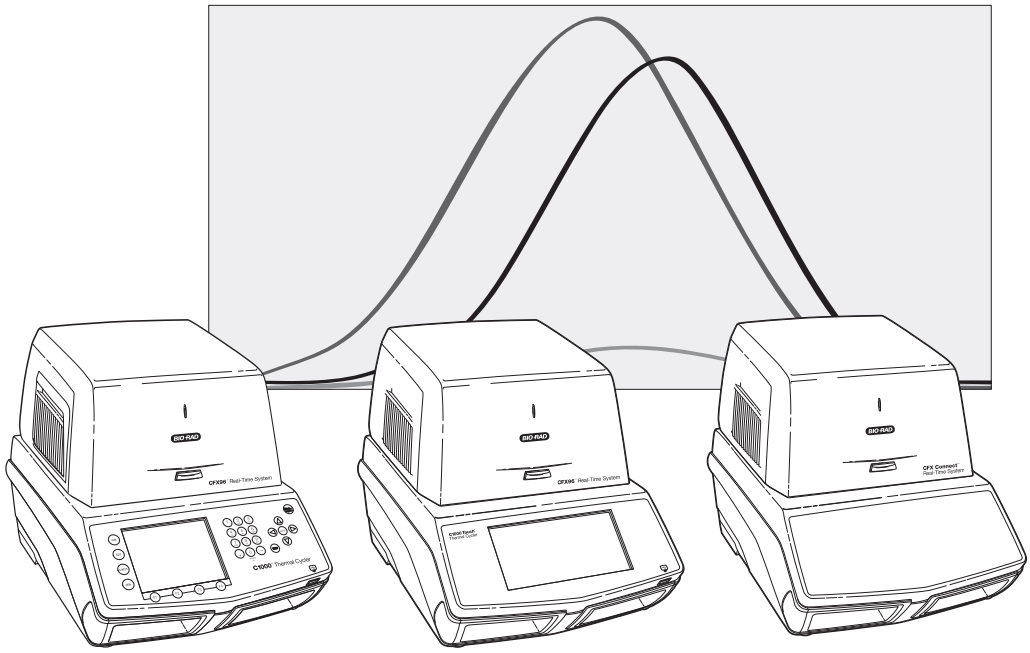
Precision Melt Analysis™ Software

User Guide



BIO-RAD

Precision Melt Analysis™ Software

User Guide

Version 1.0

BIO-RAD

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Table of Contents

Chapter 1 High Resolution Melt Analysis

Precision Melt Analysis™ software imports data files generated from all CFX real-time PCR detection systems and performs high resolution melt (HRM) analysis to genotype samples based on the thermal denaturation properties of double-stranded DNA. This chapter introduces high resolution melt (HRM) analysis.

Introduction to High Resolution Melt Analysis

Real-time PCR assays using nonspecific DNA binding dyes such as SYBR® Green generally include a post-PCR melt curve either to confirm that a single PCR product has been amplified or to detect the possible presence of primer dimers (PD) or other unwanted PCR products.

For melt curve analysis the temperature is gradually increased and fluorescence is monitored as a function of the temperature. As the temperature rises the fluorophore is released from the denaturing dsDNA and fluorescence decreases. There is a noticeable change in slope at the melting temperature (T_m) of the dsDNA, the theoretical temperature at which half the DNA is double stranded and half the DNA is single stranded. The rate of change is determined by plotting the negative first regression of relative fluorescence (RFU) against temperature (-d(RFU)/dT), yielding visible peaks that represent the T_m of the double-stranded DNA complexes. Primer-dimers typically melt at lower temperatures due to their smaller size, enabling primer-dimers or other nonspecific products to be discontinued from the amplified DNA product.

HRM analysis can be considered the next generation of the melt curve technique. HRM analysis generates DNA melt curve profiles that are both specific enough and sensitive enough to distinguish nucleic acid species based on small nucleic acid differences, which enables mutation scanning, methylation analysis, and genotyping.

HRM analysis can be used to characterize samples based on sequence length, GC content, and DNA sequence complementarity. For example, HRM analysis can be used to detect single base sequence variations, such as single nucleotide polymorphisms (SNPs), or to discover unknown genetic mutations. It can also be used to quantitatively detect a small proportion of variant DNA in a background of wild-type sequence at sensitivities approaching 5%. This approach can be used, for example, to study somatically acquired mutations or changes in the methylation state of CpG islands.

SNP Genotyping

Representative of the smallest genetic change, the detection and genotyping of SNPs underlines the sensitivity of HRM analysis. Unknown mutations are often a single nucleotide change, but they may also comprise multiple base changes, insertions and/or deletions. In general, the more base changes in the DNA, the easier they are to detect by HRM.

Venter et al (2001) divided SNPs into four classes, as summarized in Table 1. The most difficult to genotype are the class 4 (A>T conversions).

Table 1. SNP classes as defined by Venter et al (2001)

SNP Class	Base Change	Typical T_m Melt Curve Shift	Rarity (in the Human Genome)
1	C/T and G/A	Large >0.5°C	64%
2	C/A and G/T	Large >0.5°C	20%
3	C/G	Large >0.5°C	9%
4	A/T	Very small <0.2°C	7%

While the shift in T_m is important, what enables even small changes in T_m to be analyzed using HRM is the magnitude of change in the fluorescence intensity (y-axis). This change can be accentuated by minimizing the amplicon size.

For SNP analysis, homozygous allelic variants are characterized by a temperature (x-axis) shift in a HRM melt curve, whereas heterozygotes are characterized by a change in melt curve shape. The change in curve shape is a result of destabilized heteroduplex annealing between some of the wild type and variant strands. The heterozygote melting curve is thus a composite of both heteroduplex and homoduplex components, and because it dissociates more readily it shifts to a lower temperature.

Dye Compatibility

Third generation intercalating dyes, such as EvaGreen, LCGreen and SYTO 9, have been used successfully for high resolution melt analysis. These dyes have low toxicity and can be used at higher concentrations in real-time PCR reactions. These dyes are used at higher concentration for greater saturation of dsDNA and less dynamic dye redistribution to non-denatured regions of the nucleic strand during melting. The high fidelity of these third generation dyes provides greater sensitivity and higher resolution melt profiles.

Risk of danger! SsoFast™ EvaGreen® supermix cannot be used with bisulfite-converted DNA for methylation studies. For this purpose, use Precision Melt supermix.

Finding Out More

After installing Precision Melt Analysis software, you can access this guide from the Help menu in any view.

For information about setting up PCR protocols and plates, see the CFX Maestro™ Software User Guide, which is available on the CFX Maestro Software installation USB drive.

Tip: Click the Bio-Rad logo in the upper right corner of any Precision Melt Analysis software window to launch Bio-Rad's website. This site includes links to technical notes, manuals, product information, and technical support. This site also provides many technical resources on a wide variety of methods and applications related to PCR, real-time PCR, and HRM analysis.

Chapter 2 Installing Precision Melt Analysis Software

This chapter explains how to install Precision Melt Analysis™ software and enable it for use at your site. For information about setting up Bio-Rad's supported thermal cyclers, see the appropriate guide.

Instrument Compatibility

You can use any of the following real-time PCR detection systems in combination with Precision Melt Analysis software to perform HRM analysis and characterize samples based on sequence length, GC content, and DNA sequence complementarity.

- CFX96™
- CFX96 Touch™
- CFX96 Touch Deep Well
- CFX Connect™
- CFX384™
- CFX384 Touch™

Precision Melt Analysis software can open only data files generated from an experiment performed on one of these instruments and analyzed using CFX Maestro™ software. Real-time PCR data files (.pcrd) generated by CFX Maestro software are converted to melt files (.melt) when Precision Melt Analysis software displays the data.

Note: When performing high resolution melt analysis using a CFX system, use the SYBR/FAM-only scan mode with SYBR selected as the fluorophore.

Tip: For optimal high resolution melt results, use a 0.2°C temperature increment between steps and a hold time minimum of 10 seconds in the melt curve protocol.

Precision Melt Analysis Software Components

The Precision Melt Analysis software package includes the components listed below. If any items are missing or damaged, contact your local Bio-Rad office.

- Precision Melt Analysis software CD, which contains this guide (Precision Melt Analysis Software User Guide)
- Two hardware protection (HASP) keys
- Precision Melt Analysis software quick guide
- Melt calibration kit (# 1845020), which contains the following:
  - Melt Calibration DNA standard
  - Melt Calibration primers
  - Precision melt supermix
  - Melt Calibration instructions for Precision Melt Analysis software

System Requirements

Table 2 lists the minimum and recommended system requirements for the computer running Precision Melt Analysis software.

Table 2. Computer requirements for Precision Melt Analysis software

System	Minimum	Recommended
Operating system	Microsoft Windows 7 SP1 Pro	Any of the following: ■ Microsoft Windows 7 SP2 Pro (32- and 64-bit) ■ Microsoft Windows 10 Pro (64-bit only) ■ Microsoft Windows 10 Enterprise (64-bit only)
	Important: Secure Boot must be disabled on both Microsoft Windows 10 Pro and Enterprise.	
Ports	2 USB 2.0 High-speed ports	2 USB 2.0 High-speed ports
Hard disk space	128 GB	128 GB

Table 2. Computer requirements for Precision Melt Analysis software, continued

System	Minimum	Recommended
Processor speed	2.4 GHz, Dual Core	2.4 GHz, Quad Core
RAM	4 GB RAM	8 GB RAM
Screen resolution	1024 x 768 with true-color mode	1280 x 1024 with true-color mode
PDF Reader	Internet Explorer	Adobe PDF Reader or Windows PDF Reader from one of the supported Microsoft Office Suites: ■ 2007 ■ 2010 ■ 2013
Bio-Rad software	Precision Melt Analysis software is compatible with the following Bio-Rad applications: ■ CFX Maestro software 1.0 ■ CFX Manager™ software 2.1 and later	

Tip: Precision Melt Analysis software and CFX Maestro software are compatible and can be installed on the same computer system.

Installing Precision Melt Analysis Software

Important: If you are installing Precision Melt Analysis software on either version of Windows 10, ensure that Secure Boot is disabled before beginning the installation procedure.

Note: The software must be installed on the computer by a user with administrative privileges. After the installation completes, you must restart the computer before launching the software.

To install Precision Melt Analysis software

1. Log in to the Precision Melt Analysis computer with administrative privileges.
2. Insert the Precision Melt Analysis software CD into the computer's CD drive.
3. In Windows Explorer, navigate to and open the Precision Melt Analysis software CD drive.

   The Precision Melt Analysis CD contains the following files:

   - Precision Melt Analysis software installer (Setup.exe)
   - Precision Melt Analysis Software Readme.rtf
   - Precision Melt Analysis Software User Guide (this document)

4. Double-click Setup.exe to install Precision Melt Analysis software.
5. Follow the on-screen installation instructions.
6. After the installation completes, safely remove the installation CD and restart the computer.

   After restarting the computer, the Bio-Rad Precision Melt Analysis icon appears on the desktop.
7. To use the software, you must first insert the hardware protection key into a USB port on the computer. See Starting Precision Melt Analysis Software that follows for more information.

Starting Precision Melt Analysis Software

Note: If you lose your HASP dongles, contact your local Bio-Rad office.

Bio-Rad provides two hardware protection keys (HASP dongles)in the Precision Melt Analysis software package. The key is required to run Precision Melt Analysis software.

Starting Precision Melt Analysis software

▶ Insert one of the dongles into a USB port on your computer before starting the application.

Software File Types

Table 3 lists the Precision Melt Analysis software file types.

Table 3. File types in Precision Melt Analysis software

File Type	Extension	Details
Data	.pcrd	Contains the results of a melt run and PCR analysis
Melt	.melt	Contains the results of precision melt analysis
Melt study	.mlts	Contains results of multiple melt runs and precision melt analyses
Calibration	.mcal	Contains the results of a melt calibration **Note:** These files cannot be viewed.

Chapter 3 The Home Window

Precision Melt Analysis™ software presents two primary workspaces:

- The Home window
- The Data Analysis window

Precision Melt Analysis software opens to the Home window and displays the Startup Wizard, from which you can open or create a melt file or melt study file. From the Home window you can also set up melt analysis options or import a melt calibration file.

This chapter introduces Precision Melt Analysis software and describes the features accessible from the Home window.

For information about the Data Analysis window, see Chapter 5, Data Analysis Overview.

The Home Window

Precision Melt Analysis software opens to the Home window, which displays the Startup Wizard. Using the Startup Wizard you can

- Open a melt file
- Create a new melt file
- Open a melt study
- Create a new melt study
- Open the Analysis Options Manager, from which you can set preferences for analyzing melt files

Tip: Click the Bio-Rad logo in the upper right corner of any Precision Melt Analysis software window to launch the Bio-Rad website. Check the website often for updates to Precision Melt Analysis software and documentation.

File Menu Commands

New — opens a dialog box from which you can choose to create a new melt file or melt study file.

Open — opens a dialog box from which you can choose to navigate to and open an existing melt file or melt study file.

Recent — displays a list of the most recently opened melt files and melt study files.

Exit — closes Precision Melt Analysis software.

User Menu Command

Bio-Rad Service Login — for Bio-Rad service personnel use only.

Tools Menu Commands

Analysis Options Manager — opens the Analysis Options Manager dialog box, in which you can set parameters for analyzing melt data files.

Import Melt Calibration — opens the Import Precision Melt Calibration File dialog box, from which you can navigate to and open a melt calibration file (.pcrd) generated on a CFX system to create a melt calibration file (.mcal).

Help Menu Commands

Tip: The Help menu is available on the menu bar in all Precision Melt Analysis software software windows.

Open User Guide — displays the Precision Melt Analysis Software User Guide in PDF format.

About — displays Precision Melt Analysis software copyright and version information.

Home Window Toolbar

The toolbar in the Home window provides quick access to common software functions.

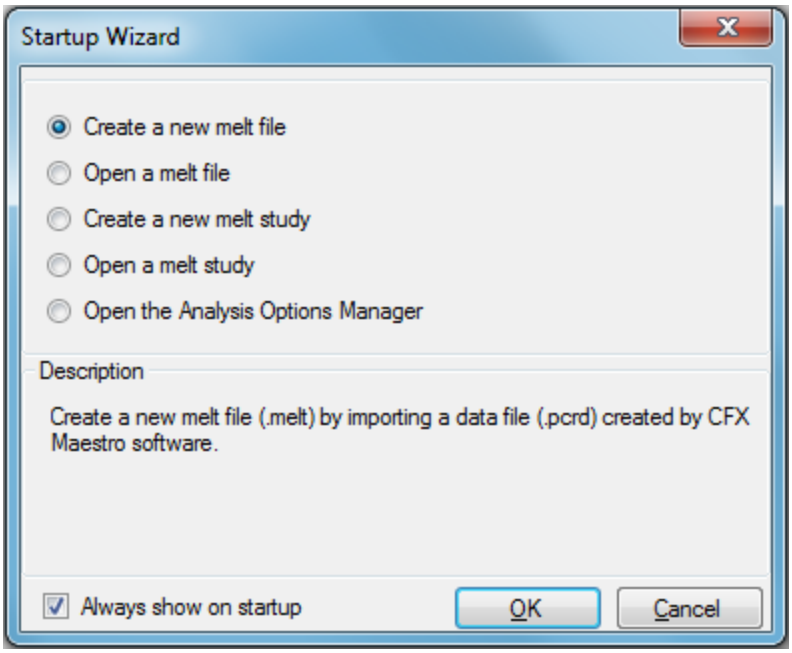
Table 4 lists the function each buttons in the toolbar.

Table 4. Toolbar in the Data Analysis window

Button	Name	Function
	Open a Melt File	Opens the Open Melt Data File dialog box, in which you can locate a melt file and open it in the Data Analysis window.
	Open a Melt Study File	Opens the Open Precision Melt Study Data File dialog box, in which you can locate a melt study file and open it in the Melt Study window.
	New Melt File	Opens the Import PCRD Data File dialog box, in which you can locate and open a .pcrd file from which to create a new melt file.
	New Melt Study File	Opens the Precision Melt Study window, in which you can create a new melt study.
	Startup Wizard	Opens the Startup Wizard, from which you can open a melt file or melt study file, or create a new melt file or melt study file.
	Analysis Options Manager	Opens the Analysis Options Manager dialog box, in which you can set or change analysis options.
	Help	Opens the Precision Melt Analysis Software User Guide in PDF format (this guide).

Startup Wizard

Precision Melt Analysis software opens to the Home window and displays the Startup Wizard. If it does not appear, click Startup Wizard on the main software toolbar.



Use the Startup Wizard to:

- Create a new melt file by importing a data file (.pcrd) generated in CFX Maestro™ software.
- Open a melt file to analyze.
- Create a new melt study to analyze the results of multiple melt files.
- Open a melt study to analyze.
- Open the Analysis Options Manager to view or modify the default analysis settings.

  See Analysis Options Manager on page 19 for more information.

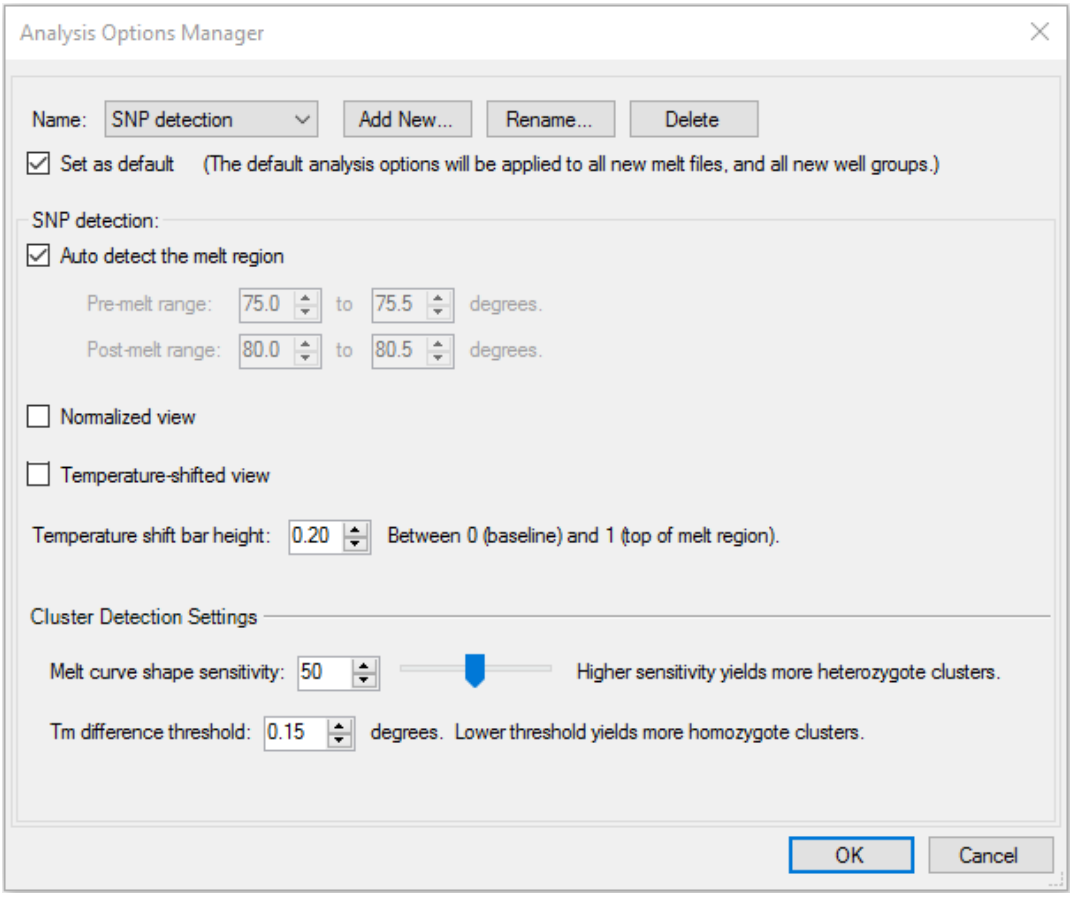
Analysis Options Manager

Precision Melt Analysis software tracks preferences for analyzing melt files. Use the Analysis Options Manager to customize the analysis options and apply them to different melt files.

To open the Analysis Options Manager

▶ Do one of the following:

- Click Analysis Options Manager in the toolbar.
- Select Tools > Analysis Options Manager on the Home window.



For information about using the Analysis Options Manager, see Melt Analysis Profiles on page 44.

Chapter 4 Performing a Melt Calibration

To create a melt file you perform the following tasks:

1. Perform a melt calibration on the CFX real-time PCR system using CFX Maestro™ software.

   Note: You must perform a melt calibration on the CFX real-time PCR system before Precision Melt Analysis™ software can analyze the generated data. A melt calibration is required regardless of the intercalating dye that the experiments use, including SYBR® Green.

   Tip: You need to perform the melt calibration only one time for each instrument. You can import the same file into multiple instances of Precision Melt Analysis software.

2. Import the melt calibration file into Precision Melt Analysis software.
3. Perform a melt experiment on the CFX real-time PCR system in CFX Maestro software.
4. Open the melt .pcrd file in Precision Melt Analysis software to analyze the date.

This chapter explains how to set up and perform a melt calibration.

For detailed information about setting up experiment protocols and plates, see the CFX Maestro Software User Guide.

Preparing a Melt Calibration Plate

Table 5 lists the materials that are required to create a melt calibration plate. Table 6 on page 22 lists the required volume for each component depending on the type of system.

Table 5. Required melt calibration materials

Description	Catalog #
Melt Calibration kit, including	**1845020**
■ Melt Calibration DNA Standard	10016289
■ Melt Calibration primers	10016273
■ Precision Melt supermix	1725110

Table 5. Required melt calibration materials, continued

Description	Catalog #
WARNING! Precision Melt supermix is stable for six months when stored in a constant temperature freezer at –20°C, protected from light. For convenience, it can be stored at 2–8°C for up to three months. Repeatedly freezing and thawing the supermix is not recommended.	
■ Microseal® 'B' adhesive seals, optically clear	MSB1001
■ PCR-grade tubes and nuclease-free water	
For CFX384™ systems ■ Hard-Shell® thin-wall 384-well skirted PCR plates with clear and white wells	HSP3805
For CFX96™ or CFX Connect™ systems ■ Hard-Shell thin-wall 96-well skirted PCR plates with white shell and clear wells	HSP9601
■ Hard-Shell thin-wall 96-well skirted PCR plates with white shell and white wells	HSP9655
Tip: Choose white or clear wells depending on the plate type you plan to use in your experiments.	

Table 6. Reaction volumes for melt calibration plates

Component	Volume for 96-well systems, µl	Volume for 384-well systems, µl
Precision Melt supermix	1,200	2,250
Melt calibration DNA standard	120	450
Melt calibration primers	14.4	27
PCR-grade water	1,065.5	1,733
Total	**2,400**	**4,500**

To prepare the melt calibration plate

1. Add the required volumes of each component to an appropriately sized tube.

   See Table 6 on page 22 for the volume to use.

2. Cap the tube and mix the reaction components gently by vortexing.
3. Briefly centrifuge the tube to remove air bubbles and collect contents at the bottom of the tube.
4. Add the appropriate volume of the mixture into each well of a reaction plate:
   - For a 96-well system, add 20 µl to each well of a 96-well plate.
   - For a 384-well system, add 10 µl to each well of the 384-well plate.
5. Seal the reaction plate with Microseal 'B' adhesive film.
6. Centrifuge the melt calibration plate to move all the reaction components to the bottom of the well.

Creating a Melt Calibration Protocol

Note: You create the melt calibration protocol using CFX Maestro software. For more information, see the CFX Maestro Software User Guide.

Parameters for a Melt Calibration Protocol

Table 7 lists the parameters required for a melt calibration protocol.

Table 7. Parameters for a melt calibration protocol

Cycling Step	Temperature	Time	# of Cycles
Enzyme activation	98°C	2 min	1
Denaturation	98°C	5 sec	35
Annealing/Extension	55°C	10 sec	35
	95°C	1 min	1
	55°C	1 min	1
Melt curve	70–95°C (in 0.2°C increments)	10 sec/step	1

To create a melt calibration protocol

1. Start CFX Maestro software.
2. In the Home window, open the Startup Wizard if it is not already in view.

3. If necessary, select the instrument type from the dropdown list.
4. Click User-defined as the run type.

   The Run Setup dialog box opens to the Protocol tab and displays the default protocol file.
5. In the Protocol tab, select Create New to open the Protocol Editor.
6. In the Protocol Editor, create a melt calibration protocol.

   Use the parameters indicated in Table 7 on page 23.
7. Click OK to save the protocol and return to the Experiment Setup window.

Running a Melt Calibration Protocol

You can run the melt calibration protocol on a CFX96 Touch™, CFX Connect™, or CFX384 Touch™ PCR system. The melt calibration protocol generates a melt calibration data file (.pcrd). You then import this file into Precision Melt Analysis software to create the melt calibration file (.mcal).

To run a melt calibration protocol

1. Turn on the real-time PCR system if it is not already running.
2. In the Startup Wizard, click User-defined and select the Protocol tab in the Run Setup dialog box.
3. On the Protocol tab, click Select Existing.

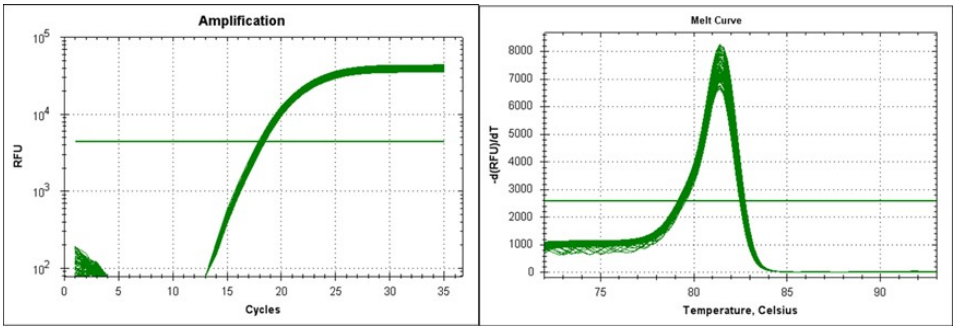
   The Select Protocol dialog box appears.
4. In the Select Protocol dialog box, do one of the following:
   - Navigate to and open the folder in which you saved your user-defined melt calibration protocol.
   - Navigate to and open Sample Files > MeltCalibration.

     In the MeltCalibration folder, select the appropriate protocol name based on your instrument:
     - For a 96-well system, select Melt Calibration Protocol_96.prcl.
     - For a 384-well system, select Melt Calibration Protocol_384.prcl.
5. Click Open to open the protocol and close the Select Protocol dialog box.
6. In the Run Setup dialog box, click the Plate tab.
7. On the Plate tab, click Select Existing.

The Select Plate dialog box appears.

8. In the Select Plate dialog box, navigate to and open Sample Files > MeltCalibration.
9. In the MeltCalibration folder, select the plate name for the instrument on which you plan to run the protocol:
   - For a 96-well system, select Melt Calibration Plate_96 wells_Clear or Melt Calibration Plate_96 wells_White.
   - For a 384-well system, select Melt Calibration Plate_384 wells_White.
10. Click Open to open the plate file and close the Select Plate dialog box.
11. In the Run Setup dialog box, click the Start Run tab.
12. In the Start Run on Selected Blocks section, select the checkbox for the instrument on which you plan to run the protocol.
13. Click Open Lid, and load the melt calibration plate into the instrument.
14. Click Close Lid.
15. Click Start Run to run the experiment.
16. At the prompt, save the melt calibration data file.

    When the melt calibration run is complete, CFX Maestro software automatically displays the data file. Review the data file to ensure all wells display a tight amplification and a single melt peak, for example:



Importing the Melt Calibration File into Precision Melt Analysis Software

Before you can create a precision melt data file, you must import into Precision Melt Analysis software the melt calibration file for the CFX instrument on which you ran the melt experiment. Precision Melt Analysis software detects whether the melt calibration file for the CFX has already been imported. If you have not already imported the file, Precision Melt Analysis software prompts you to do so when you open a .pcrd file.

Tip: You only need to import the melt calibration file once for each instrument.

To import the melt calibration file

1. Start Precision Melt Analysis software.
2. On the Home window, click Tools > Import Melt Calibration.
3. Select the melt calibration experiment data file (.pcrd extension) and click Open.

   A confirmation window appears indicating that the melt calibration was successful.
4. Click OK to proceed and use the Precision Melt Analysis software.

Chapter 5 Data Analysis Overview

Precision Melt Analysis™ software processes melt data automatically and displays the data in the Data Analysis window.

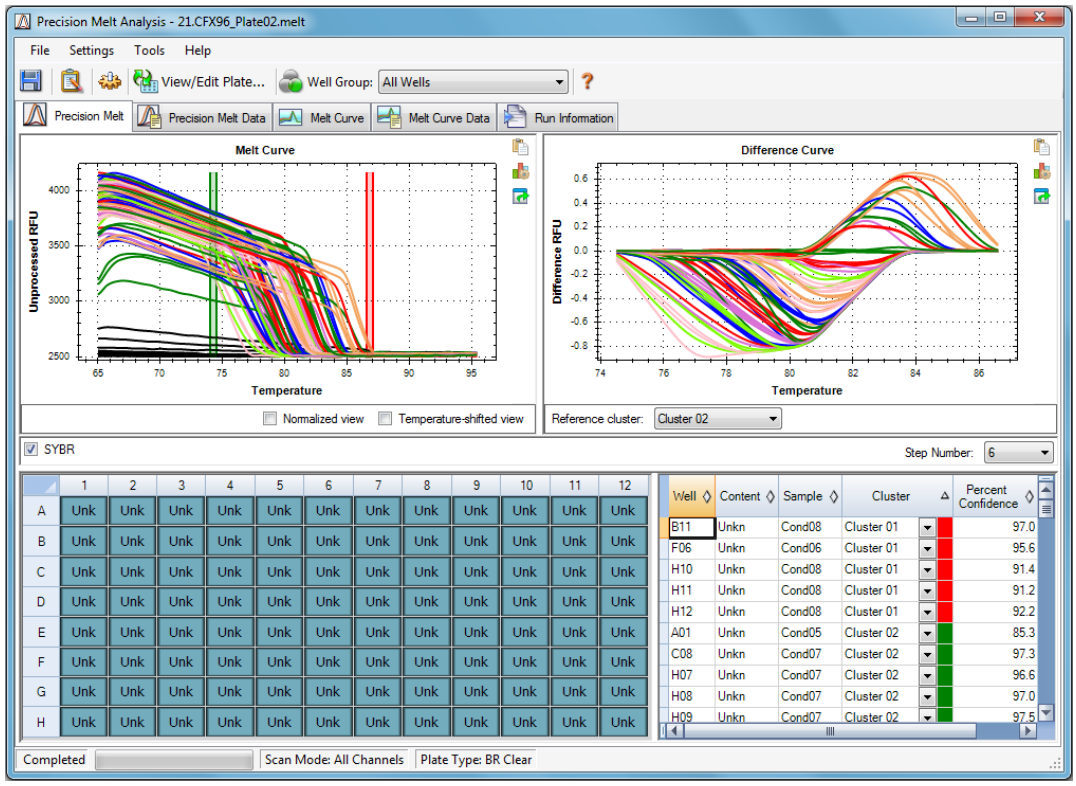
Precision Melt Analysis software provides several methods to open and view melt data files. You can:

- Drag a melt file (.melt extension) onto the Home window and release it.
- Select File > Open Melt File in the Home window and browse to the target .melt file.
- Select File > New Melt File in Home window to create a new melt file.

Data Analysis Window

During data analysis, changing the way the data are displayed never changes the fluorescence data that are collected from each well during the run. You cannot delete the data, but you can choose to remove them from view and analysis.

The Data Analysis window displays data in charts and spreadsheets for a specific analysis method in one of five tabs.



To open a melt data file in the Data Analysis window

▶ Do any of the following:

- Drag a melt file (.melt extension) into the Home window.
- Select File > New > Melt File in the Home and select a CFX Maestro™ software .pcrd file in the Windows browser.

  Tip: Opening the file in Precision Melt Analysis software converts it to a melt file.
- Select File > Open > Melt File in the Home window to select a melt file in the Windows browser.
- Select File > Recent Files to select from a list of the most recently opened melt data files.

Data Analysis Menu Commands

Table 8 lists the menu commands in the Data Analysis window.

Table 8. Data Analysis window menu commands

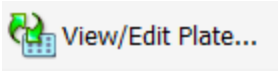
Menu Item	Command	Function
File		
	Save	Saves the melt file.
	Save As	Saves the melt file with a new name.
	Close	Closes the Data Analysis window.
	View/Edit Plate	Opens the Plate Editor to view and edit the plate for the current melt file. **Tip:** For detailed information about editing a plate, see the CFX Maestro Software User Guide.
Settings		
	Mouse Highlighting	Turns on or off the simultaneous highlighting of data with the mouse pointer. **Tip:** If Mouse Highlighting is turned off, press the Control key to temporarily turn on highlighting.
	Restore Default Plate Layout	Reverts any changes to the plate.
Tools		
	Analysis Options	Opens the Analysis Options dialog box, which displays the options profile for the current melt file.
	Reports	Opens the Report dialog box for the current melt file.
	Replace Plate	Opens a browser window, from which you can select a plate to replace the current plate in the melt analysis.

Data Analysis Toolbar

The toolbar in the Data Analysis window provides quick access to common software functions.

Table 9 lists the function of each button in the toolbar.

Table 9. Toolbar in the Data Analysis window

Button	Name	Function
	Save	Saves the current melt file.
	Report	Opens the Reports dialog box for the current melt file.
	Analysis Options Manager	Opens the Analysis Options Manager dialog box, in which you can set or change analysis options.
	View/Edit Plate	Opens the Plate Editor to view and edit the plate for the current melt file. **Tip:** For detailed information about editing a plate, see the CFX Maestro Software User Guide.
	Well Group	Selects an existing well group name from the dropdown menu. The default selection is All Wells. This button appears only when well groups are created.
	Help	Opens the Precision Melt Analysis Software User Guide (this guide).

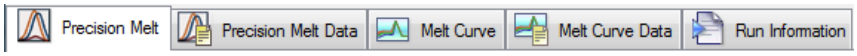
Data Analysis Tabs

The Data Analysis window comprises five tabs. Each tab displays data in charts and spreadsheets for a specific analysis method with a well selector to select the data you want to show. The Data Analysis window opens displaying the contents of the Precision Melt tab.

Tip: Right-click any chart, spreadsheet, or well selector for more options.

Tip: Click View/Edit Plate to open the Plate Editor in CFX Maestro software and change the contents of the wells.

Note: The software links the data in the panes of each data analysis tab. For example, when you position the mouse pointer over a well in the well selector to highlight it, the data in all the other panes is also highlighted.



The tabs display melt curve data from one experiment (a protocol and plate file run on one instrument).

- **Precision Melt** — displays the melt data in four views:
  - Melt Curve chart
  - Difference Curve chart
  - Well selector
  - Data spreadsheet

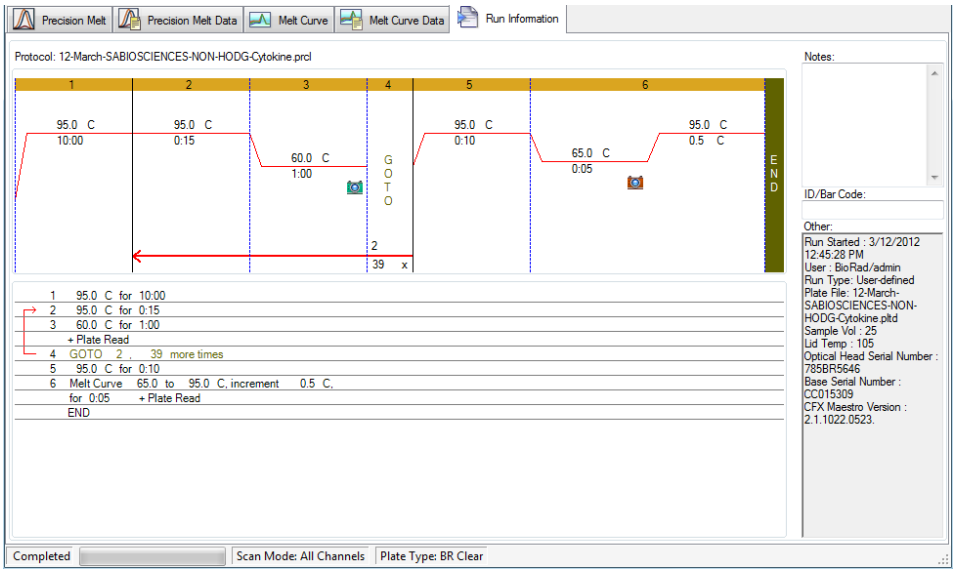
  Use the data in this tab to set the data analysis conditions, including normalization and temperature shift.
- **Precision Melt Data** — displays a spreadsheet view of the data in different formats:
  - Results
  - Charts
  - Plate View
  - Raw RFU
  - Normalized RFU
  - Difference RFU
- **Melt Curve** — displays the melt curve data for each well in four views:
  - Melt curve chart

- Melt peak chart
- Well selector
- Data spreadsheet

Use the data in this tab to measure the melting temperature (T_m) of PCR products.

- **Melt Curve Data** — displays a spreadsheet view of the data in different formats:
  - Melt Peaks
  - Plate
  - Amplification
  - RFU
  - -d(RFU)/dT
- **Run Information** — displays information about the experiment, including the protocol, optional notes, optional ID, and run log. You can edit the data ID for the run by typing in the ID box.

  The Other pane displays events such as error messages that might have occurred during the run, for example:



Tip: Right-click any chart, spreadsheet, or well selector for more options.

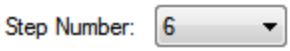
Tip: Click View/Edit Plate to open the Plate Editor and change the contents of the wells.

Note: The software links the data in the panes of each data analysis tab. For example, when you position the mouse pointer over a well in the well selector to highlight it, the data in all the other panes is also highlighted.

Step Number Selector

The CFX96 Touch™, CFX96 Touch Deep Well, CFX Connect™, and CFX384 Touch™ systems can acquire fluorescence data at multiple protocol steps; the software maintains the data acquired at each step independently. The software displays the Step Number selector below the Difference Curve chart on the Precision Melt and Melt Curve tabs. When a protocol contains at least one data collection step, Precision Melt Analysis software displays the data from the first collection step.

If the protocol contains more than one collection step, you can select another the step from the dropdown list, for example:



When you select a step, the software applies that selection to all the data that are shown in the Data Analysis window.

Creating Well Groups

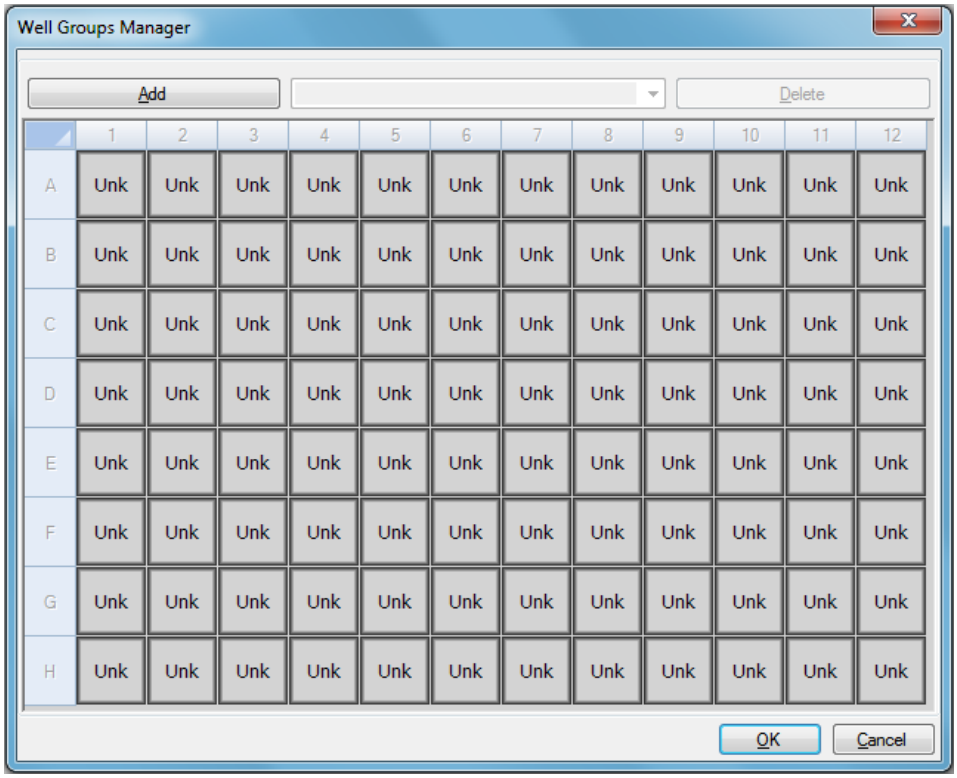
Well groups divide a single plate into subsets of wells that can be analyzed independently in the Data Analysis window. Once well groups are set up, select one in the Data Analysis window to analyze the data as an independent group. For example, set up well groups to analyze multiple experiments run in one plate or to analyze each well group with a different standard curve.

Note: The default well group is All Wells.

To create well groups

1. In the Data Analysis window, click View/Edit Plate to open the Plate Editor.
2. In the Plate Editor, click Well Groups in the toolbar.

   The Well Groups Manager dialog box appears.



3. Click Add to create a new group. The dropdown menu displays the group name as Group 1 for the first group.

4. Select the wells for the well group in the plate view by clicking and dragging across the group of wells. Selected wells appear blue in the Manager.
5. (Optional) To change the name of the group, select its name in the dropdown menu and type a new name.
6. (Optional) To delete a well group, select its name in the dropdown list and click the Delete.
7. Click OK to finish and close the window, or click Cancel to close the window without making changes.

Right-Click Menu Items for the Well Groups Manager Dialog Box

Table 10 lists the menu items available in the Well Groups Manager dialog box when you right-click on any well.

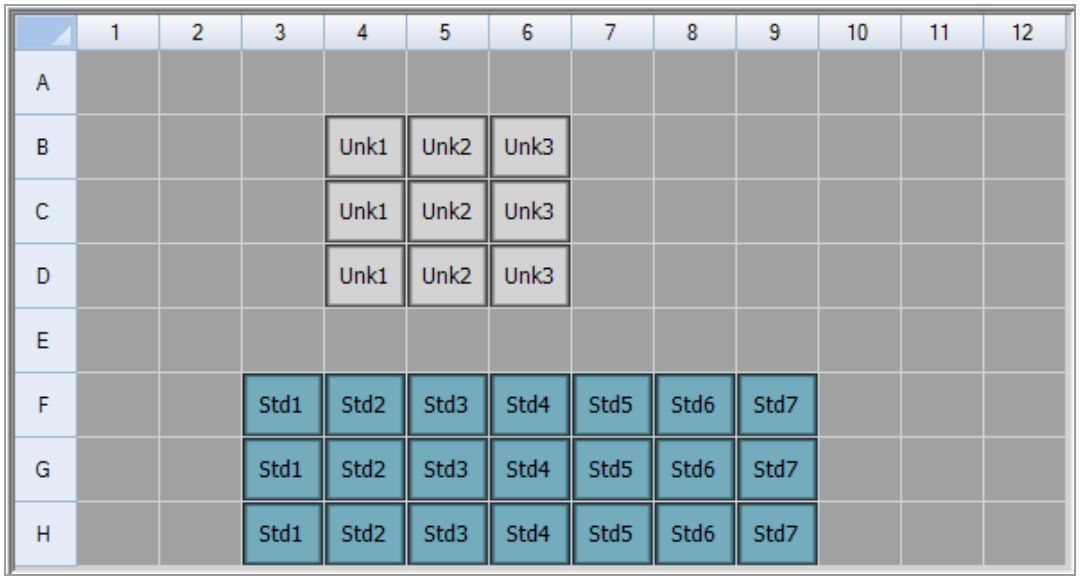
Table 10. Right-click menu items in the Plate Editor Well Selector dialog box

Item	Function
Copy	Copies the well contents, which can then be pasted into another well or wells.
Copy as Image	Copies the well selector view as an image.
Print	Prints the well selector view.
Print Selection	Prints only the selected cells.
Export to Excel	Exports the data to an Excel spreadsheet.
Export to Csv	Exports the data as a comma-separated document.
Export to Xml	Exports the data as an .xml document.
Export to Html	Exports the data as an .html document.

Well Selector

Use the Well Selector to display or hide the well data in the charts or spreadsheets throughout the Data Analysis window. Only wells loaded with sample can be selected in the well selector. The software colors the wells in the Well Selector:

- **Blue** — indicates selected wells. The data from selected wells appear in the Data Analysis window.
- **Light gray** — indicates unselected wells. The data from unselected wells do not appear in the Data Analysis window.
- **Dark gray** — indicates empty wells.



To display or hide well data

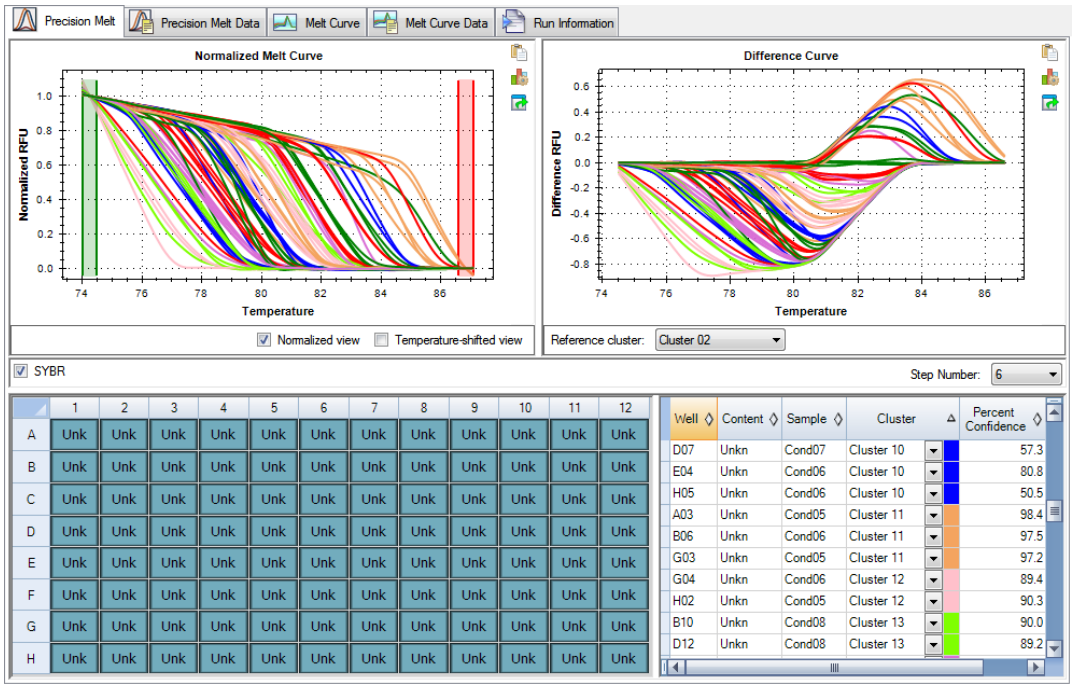
▶ In the well selector, do any of the following:

- To hide one well, click the individual well. To display that well, click the well again.
- To hide multiple wells, drag across the wells you want to select. To display those wells, drag across the wells again.
- Click the top left corner of the plate to hide all the wells. Click the top left corner again to display all wells.
- Click the start of a column or row to hide those wells. Click the column or row again to display the wells.

Viewing Well Groups in Data Analysis

Wells in the plate can be grouped into subsets for independent analysis using well groups. When you create well groups, their group names appear in the Data Analysis window Well Groups dropdown list on the toolbar.

If you created well groups, the software displays the default well group All Wells when you open the Data Analysis window, displaying the data in all wells with content in the charts and spreadsheets. Only the wells in that well group loaded with content appear in the well selector, and only data for those wells are included in the data analysis calculations.



Tip: To edit or delete well groups, click View/Edit Plate in the toolbar to open the Plate Editor. In the Plate Editor, click Well Groups in the toolbar to open the Well Groups Manager dialog box.

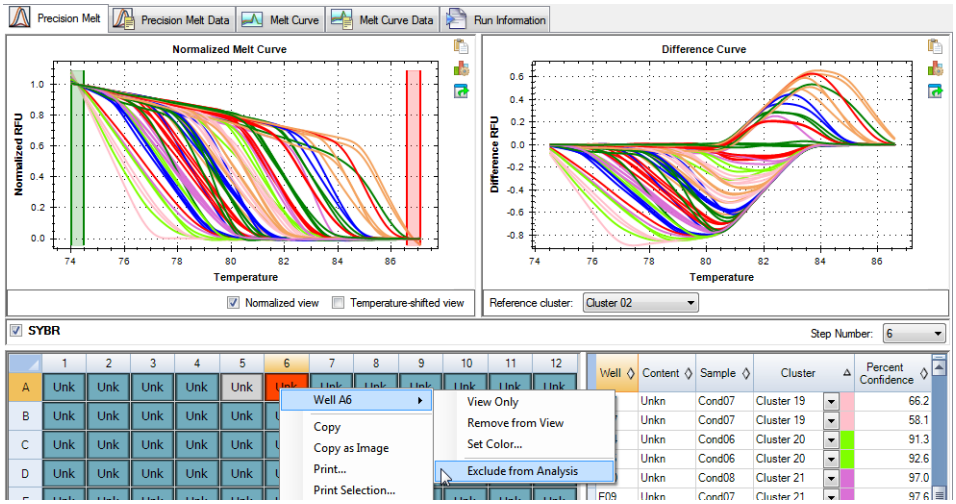
Note: If you did not create well groups, the Well Groups dropdown list does not appear in the toolbar.

Temporarily Excluding Wells from Analysis

Note: You can permanently remove wells from analysis by clearing their contents in the plate using the Plate Editor.

To exclude wells from data analysis temporarily

1. In either the Precision Melt or Melt Curve tab, right-click the well in the well selector.
2. From the right-click menu, choose Well #> Exclude from Analysis.



To include an excluded well

▶ Right-click the appropriate well in the well selector and select Well # > Include in Analysis.

Permanently Removing Wells from Analysis

To permanently remove wells from analysis

1. Click View/Edit Plate to open the Plate Editor.
2. Select the well or wells to permanently remove from analysis.
3. Click Clear Wells in the right pane.
4. Click OK and then Yes to apply changes and close the Plate Editor.

Important: You must reenter any well content that is cleared.

Well Selector Right-Click Menu Items

Table 11 lists the right-click options available in the well selector view.

Table 11. Right-click menu items in the well selectors

Item	Function
Well XX	Displays only this well, removes this well from view, set color for this well, or excludes this well from analysis.
Selected Wells (right-click and drag)	Displays only these wells, removes these wells from view, sets color for these wells, or excludse these wells from analysis.
Copy	Copies the content of the well to a clipboard, including Sample Type and optional Replicate #.
Copy as Image	Copies the well selector view as an image.
Print	Prints the well selector view.
Print Selection	Prints the current selection.
Export to Excel	Exports the data to an Excel spreadsheet.
Export to Csv	Exports the data as a text document.
Export to Xml	Exports the data as an .xml document.
Well Labels	Changes the well labels to Sample Type, Target Name, or Sample Name.

Charts

Each chart in the Data Analysis window displays the data in a different graph and includes options for adjusting the data.

Common Right-Click Menu Items for Charts

Table 12 lists the right-click menu items that are available on charts. Some of the available items are present for all charts, and these items can be used to change how the data are displayed or to easily export the data from a chart.

Table 12. Right-click menu items for charts

Item	Function
Copy	Copies the chart into the clipboard.
Save Image As	Saves the image at a specified size, resolution, and file type. The image formats available are PNG (default), JPG, and BMP.
Page Setup	Preview and select page setup for printing.
Print	Prints the chart.
Set Scale to Default	Returns the chart to its default view after magnifying the chart.
Chart Options	Opens the Chart Options window to change the chart, including changing the title, selecting limits for the x and y axes, showing grid lines, and showing minor ticks in the axes.

Spreadsheets

The spreadsheets shown in Data Analysis include options for sorting and transferring the data. Sort the columns by one of these methods:

- Click and drag a column to a new location in the selected table.
- Click the column header to sort the data in ascending or descending order.

To sort up to three columns of data in the Sort window

1. Right-click in the spreadsheet and select Sort.
2. In the Sort dialog box, select the first column title to sort. Sort the data in ascending or descending order.
3. Select a second or third column to sort and choose Ascending or Descending.
4. Click OK to sort the data or click Cancel to stop sorting.

Highlight the data on the associated charts and well selector by holding the mouse pointer over a cell. Click in a cell to copy and paste its contents into another software program.

Common Right-Click Menu Items for Spreadsheets

Table 13 lists the right-click menu items available on any spreadsheet view.

Table 13. Right-click menu items for spreadsheets

Item	Function
Copy	Copies the contents of the selected wells to a clipboard, then paste the contents into a spreadsheet such as Excel.
Copy as Image	Copies the spreadsheet view as an image file and paste it into a file that accepts an image file, such as text, image, or spreadsheet files.
Print	Prints the current view.
Print Selection	Prints the current selection.
Export to Excel	Exports the data to an Excel spreadsheet.
Export to CSV	Exports the data to a comma-separated file.
Export to Xml	Exports the data to an Xml file.

Table 13. Right-click menu items for spreadsheets, continued

Item	Function
Export to Html	Exports the data to an Html file.
Find	Searches for text.
Sort	Sorts the data in up to three columns.
Select Columns	Selects the columns that will be displayed in the spreadsheet.

Chapter 6 Analyzing Melt Data

The Data Analysis window comprises multiple tabs from which to view data. This chapter explains these tabs in detail.

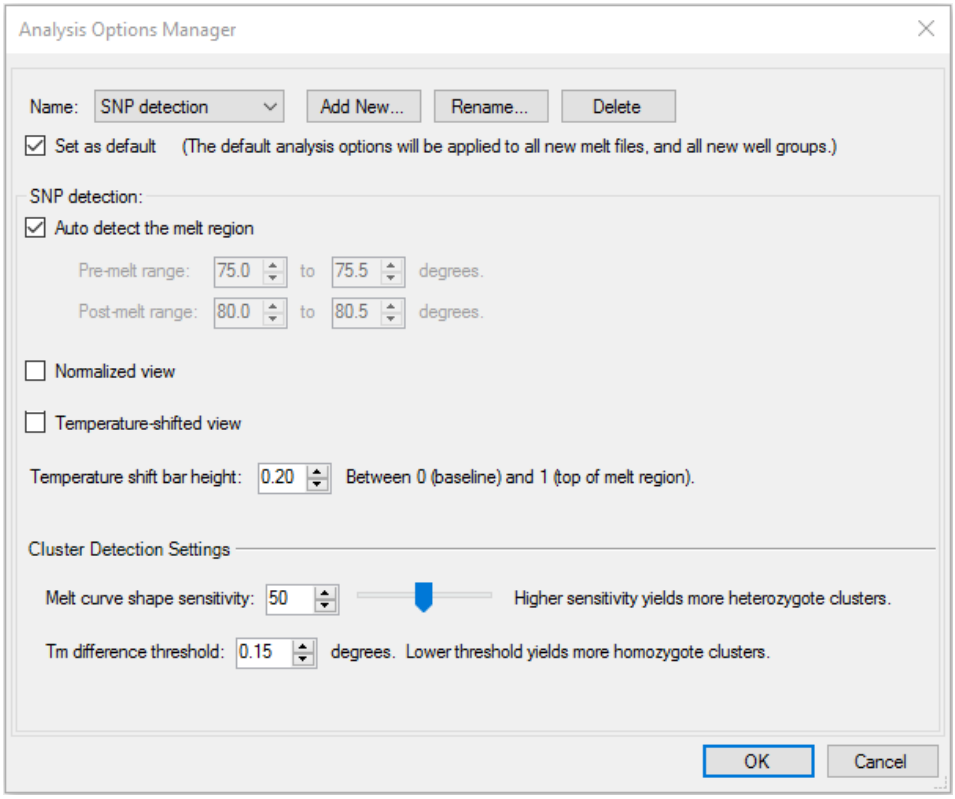
Processing Melt Data

Precision Melt Analysis™ software plots the relative fluorescence unit (RFU) data collected during a melt curve as a function of temperature. The software automatically starts with the raw melt curve data and performs the following steps:

- **Negatives Detection** — all wells with sample content type designated NTC or Negative Control in the Plate Editor are automatically considered negatives. Any well with a low starting RFU is also considered a negative. All wells designated as negative are automatically excluded from cluster analysis.
- **RFU Normalization** — all non-negative wells are normalized along the RFU axis (y-axis) such that the average data value at the start of the pre-melt range is one, and the average data value at the end of the post-melt range is zero.
- **Clustering** — Precision Melt Analysis software automatically determines a cluster assignment for each non-negative well.
- **Difference Curves Generation** — for easy visual identification of clusters, the software generates a Difference Curve chart of the data. The difference curve displays the difference in fluorescence between a well and the fluorescence of a reference curve. The reference curve is derived from the average fluorescence of all the curves within a selected reference cluster.

Melt Analysis Profiles

Use the Analysis Options Manager to choose settings for analyzing a melt file in the Data Analysis window. You can save customized analysis settings and apply them to different melt files.



Analysis Options Manager

The Analysis Options Manager provides the following options, which you can customize for different analysis profiles:

- **Auto detect the melt region** — enables the software to automatically define the pre-melt and post-melt temperature ranges.

  To manually define the pre-melt and post-melt ranges, clear the checkbox and provide the appropriate temperature values.

- **Normalized view** — opens the melt file with the melt curve data displayed in normalized view in Precision Melt tab.

- **Temperature shifted view** — opens the melt file with a temperature shift applied to each normalized fluorescence curve along the temperature axis (x-axis).
- **Temperature shift bar height** — specifies the temperature shift bar height by entering a number between 0 (baseline) and 1 (top of melt region). For most applications, the default temperature shift bar height of 0.20 produces acceptable results, with the melt curves clustered into tight groups.
- **Cluster Detection Settings** — sets the cluster detection settings to determine the stringency used to cluster the melt curves. See Melt Curve Shape Sensitivity and Tm Difference Threshold that follow for more information.

Melt Curve Shape Sensitivity

Clustering shape sensitivity determines the stringency used to classify melt curves into different clusters. To refine clustering results, increase or decrease the sensitivity of clustering based on the shape of the melt curve. Enter a numerical value or move the slider bar to the left or to the right. Entering a lower percentage value or sliding the bar to the left reduces stringency and results in fewer heterozygote clusters. Entering a higher percentage value or sliding the bar to the right increases stringency and results in more heterozygote clusters.

If necessary, manually decrease sensitivity to eliminate high numbers of false positives. A high sensitivity value generally produces more groups than a low value. For most applications, the default 50% clustering shape sensitivity value produces acceptable results.

T_m Difference Threshold

T_m difference threshold determines the lowest amount of T_m difference between samples that the software will call as different clusters. Choose the number of degrees Celsius by entering a numerical value from 0.05 to 1.00. Lower values produce more homozygote clusters.

If necessary, manually increase the T_m difference threshold level to eliminate high numbers of false positives. For most applications, the default T_m difference threshold of 0.15 degrees produces acceptable results.

Managing Analysis Profiles

This section explains how to create, rename, and delete analysis profiles.

Creating Analysis Profiles

The procedure for creating an analysis profile differs slightly depending on where in the software you start.

To create a melt analysis profile from the Home window

1. In the Home window, click Analysis Options Manager on the toolbar.
2. Clear or select the appropriate checkboxes and enter specific values where available.
3. Click Create New.
4. In the Enter Name dialog box, type a name for the new profile and click OK to close the dialog box.
5. Click OK to close the Analysis Options Manager dialog box.

To create a melt analysis profile from the Data Analysis window

1. In the Data Analysis window, click Analysis Options on the toolbar to open the Analysis Options dialog box.
2. Clear or select the appropriate checkboxes and enter specific values where available.
3. Click Save to Analysis Options Manager to save the settings for future use.
4. In the Save Analysis Options dialog box, type a name for the new profile and click OK to close the dialog box.
5. Click OK to close the Analysis Options dialog box.

Renaming Analysis Profiles

Use the Analysis Options Manager to rename profiles.

To rename an analysis settings profile

1. In the Home window, open the Analysis Options Manager.
2. Select the analysis profile from the Name dropdown menu.
3. Click Rename.
4. In the Rename dialog box, type the new name for the profile and click OK to close the dialog box.
5. Click OK to close the Analysis Options Manager.

Deleting Analysis Profiles

Use the Analysis Options Manager to delete profiles.

Important: Verify that you want to delete the profile. You are not prompted to confirm the deletion.

To delete an analysis settings profile

1. In the Home window, open the Analysis Options Manager.
2. Select the analysis profile from the Name dropdown menu.
3. Click Delete.
4. Click OK to close the Analysis Options Manager.

Loading an Analysis Profile

Use the Analysis Options dialog box to load an analysis profile into a melt data analysis.

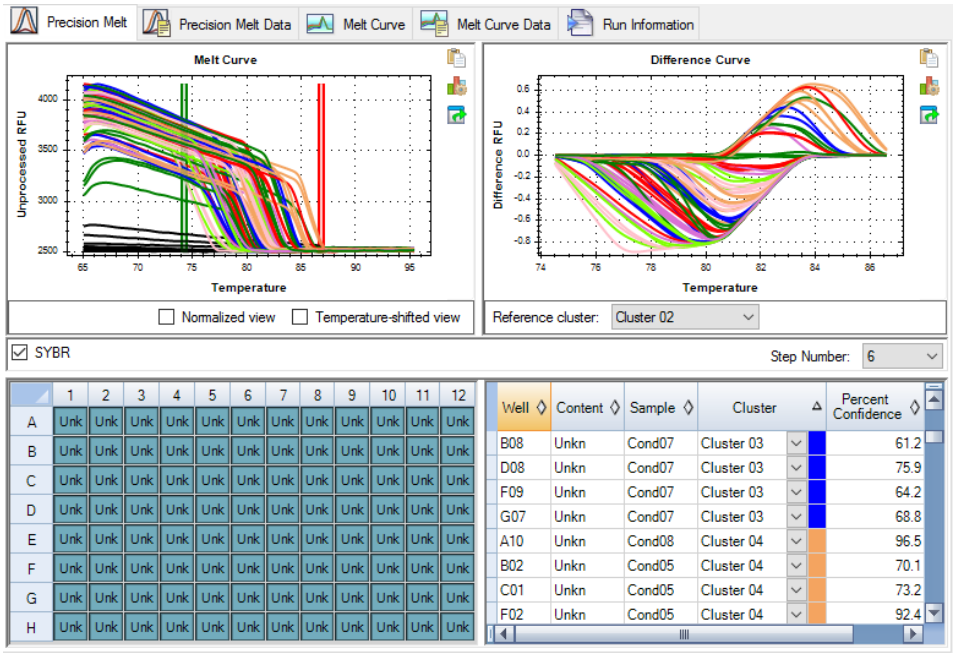
To load an analysis profile

1. In the Data Analysis window, click Analysis Options on the toolbar to open the Analysis Options dialog box.
2. Click Load from Analysis Options Manager.
3. In the Load Analysis Options dialog box, select an analysis profile from the dropdown list.
4. Click OK to apply the analysis settings to the melt file and close the Load Analysis Options dialog box.

Precision Melt Tab

Use the data in the Precision Melt tab to set data analysis conditions, including normalization and, if required, temperature shift. The Precision Melt tab comprises four views:

- Melt Curve chart — displays the relative fluorescense units (RFUs) for each well plotted against temperature. Each trace represents data from a single fluorophore in one well.
- Difference Curve chart — displays the difference RFU plotted on the y-axis against temperature on the x-axis.
- Well selector chart — displays the data you want to show.
- Spreadsheet — displays the data for the selected wells in spreadsheet format.



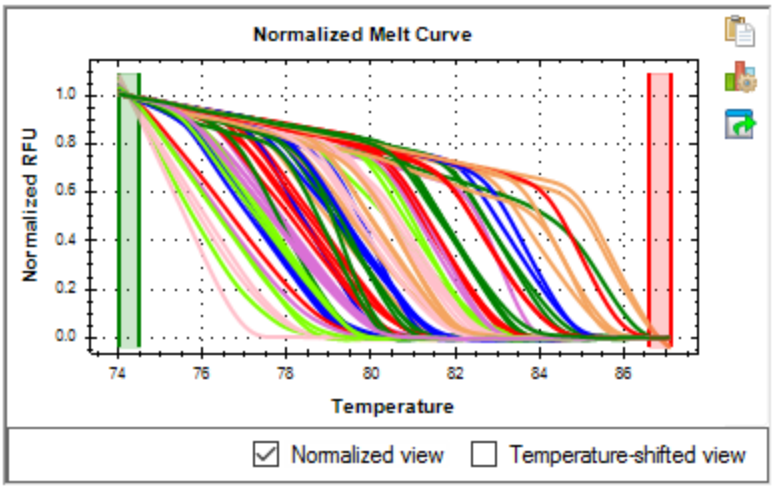
Note: If the protocol includes more than one data collection step , select the step with the data you want to view in the Step Number dropdown list below the Difference Curve chart.

Melt Curve Chart

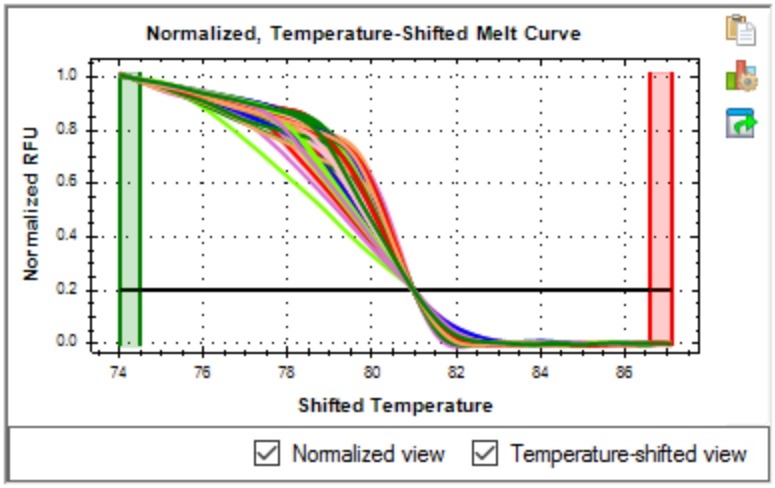
The Melt Curve chart shows RFUs plotted against temperatures for each well. The Melt Curve chart contains two options for displaying the data.

Tip: To magnify any area of the Melt Curve chart, click and drag the mouse across an area of the chart. To return the chart to a full view, right-click and select Set Scale to Default from the menu.

- Normalized view — select Normalized view below the chart to view normalized melting curves. Changing the view of the data in the Melt Curve chart does not change the data clustering.



- Temperature shifted view — select Temperature-shifted view to view temperature shifted melt curves and difference curves. The height of the black temperature shift bar can be changed by dragging the black horizontal line in the RFU chart up or down. The temperature shift bar height corresponds to the y-axis value at which all the melt curves intersect. It appears only when Temperature shifted view is selected. The default value for the temperature shift bar height is specified in the Analysis Options Manager dialog box.



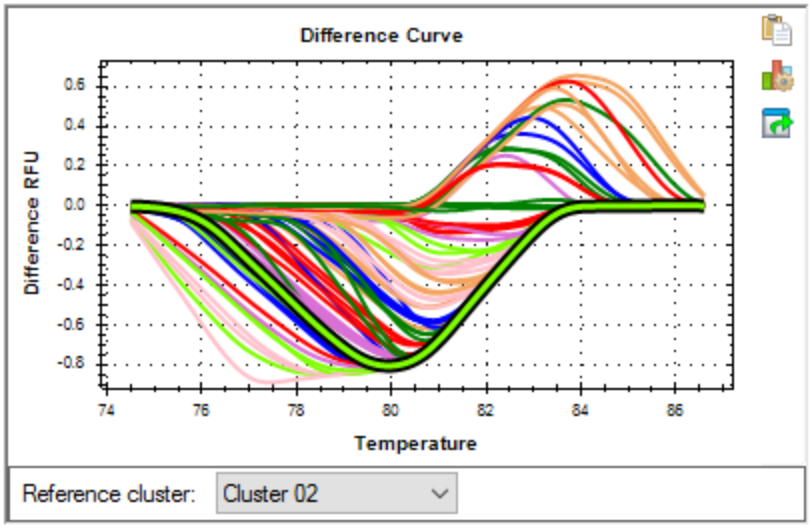
Two pairs of adjustable vertical sliders correspond to the pre-melt region (green) and the post-melt region (red). The colored area between the sliders indicates the melt range.

Precision Melt Analysis software automatically sets the regions before and after the melting transition region. Adjust the pre-melt and post-melt regions by moving the sliders to the left or right by holding down the mouse cursor over a line and dragging the line.

Note: Changing the pre-melt and post-melt temperature regions changes the data clustering.

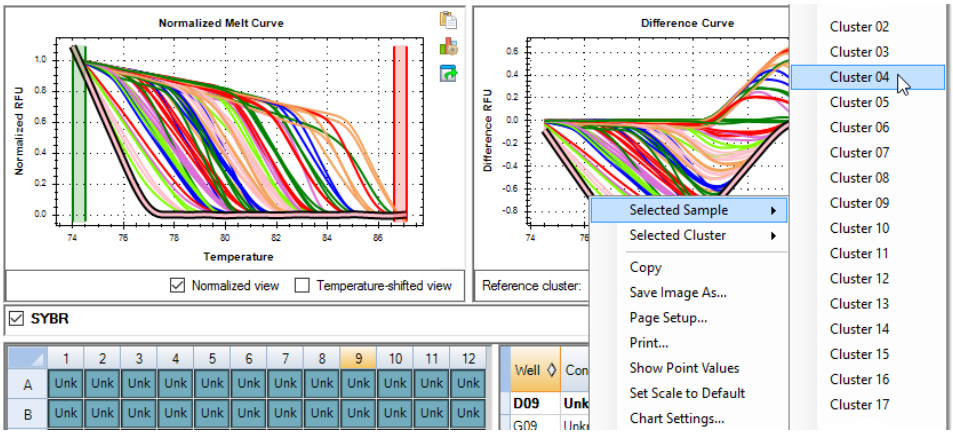
Difference Curve Chart

For easy visual identification of clusters, Precision Melt Analysis software generates a difference curve for each well. The Difference Curve chart displays the difference in fluorescence between a well and the fluorescence of a reference curve. The reference curve is derived from the average fluorescence of all the curves within a selected reference cluster. Select the reference cluster from the Reference cluster dropdown list below the Difference Curve chart.



Manually Assigning Clusters

Right-click on the fluorescence trace of a well in the Melt Curve or Difference Curve chart to override the cluster assignment or change the cluster name.

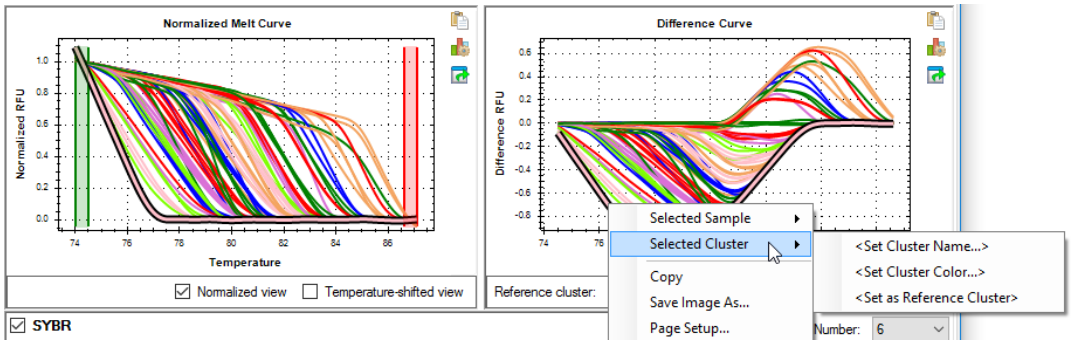


In the Selected Sample menu, do any of the following:

- Select from the list of cluster names to assign the sample well(s) to the cluster.
- Select Create New Cluster to assign the selected well to a new cluster. Enter the name of the new cluster in the Name window and click OK.
- If you previously changed the automatic call of a sample, select Undo Manual Cluster to change the cluster assignment back to the software assigned cluster call.
- Select Exclude from Clusters to exclude the selected sample(s) from cluster analysis. Excluded samples display Excluded in the Cluster column in the data spreadsheet. Excluded samples do not appear in the Normalized Melt Curve chart or the Difference Curve chart. They appear in black in the nonnormalized Melt Curve chart. The raw Melt Curve chart appears when Normalized View is cleared.
- To include an excluded sample, right-click the sample in the Melt Curve chart and select Include in Clusters.

Changing Cluster Parameters

Right-click on the fluorescence trace of a well in the Melt Curve or Difference Curve chart to change cluster parameters.



Changes made to the cluster are applied to all samples in the cluster. Select from the options listed in the Selected Cluster menu:

- Select Set Cluster Name to assign a name to the cluster. Enter the name of the cluster in the Rename window text box and click OK.
- Select Set Cluster Color to change the color of the fluorescence traces for the wells assigned to the selected cluster. Choose the new color from the color options in the Color window and click OK.

- Select Set as the Reference Cluster to use the selected cluster as the Difference Curve reference cluster.

Precision Melt Tab Spreadsheet

Table 14 lists the data shown in the spreadsheet at the bottom right of the Precision Melt tab.

Table 14. Precision Melt tab spreadsheet contents

Information	Description
Well	Well position in the plate
Content	A combination of the Sample Type and Replicate # loaded in the well in the Plate Editor
Sample	Sample name loaded in the well in the Plate Editor
Cluster	Name of the cluster assignment for the well
Confidence	Indication of the relative probability the sample has of being in a cluster

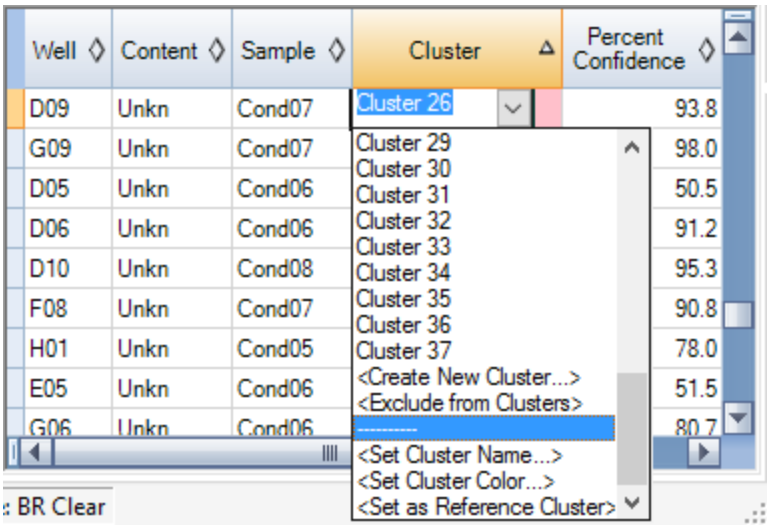
Tip: To make changes to the Content and Sample, click View/Edit Plate to open the Plate Editor.

Confidence Level

Precision Melt Analysis software determines a probability distribution for each cluster based on the standard deviation of the melt curves within the same cluster. Each sample is mapped onto each cluster's probability distribution, based on that sample's similarity to the mean melt curve across each sample in the cluster. The confidence value is an indication of the relative probability the sample has of being in a cluster.

Manually Changing Clusters in the Precision Melt Spreadsheet

In the Precision Melt tab spreadsheet, select options from the dropdown menu in a cell in the Cluster column to override the cluster assignment or change cluster properties.



Note: Changes made to the cluster are applied to all samples in the cluster.

To change the cluster assignment

▶ Do any of the following:

- Select from the list of cluster names to assign the sample well(s) to a new cluster.
- Select Create New Cluster to assign the selected well to a new cluster. Enter the name of the new cluster in the Name window and click OK.
- If you previously changed the automatic call of a sample, select Undo Manual Cluster to change the cluster assignment back to the software-called cluster.
- Select Exclude from Clusters to remove the well from the assigned cluster. Wells excluded from clusters display Excluded in the Cluster column in the data spreadsheet
- Select Include in Clusters to include the sample in the cluster analysis.

To change the cluster parameters

▶ Do any of the following:

- Select Set Cluster Name to assign a name to the cluster. Enter the name of the cluster in the Rename window text box and click OK
- Select Set Cluster Color to change the color of the fluorescence traces for the wells assigned to the selected cluster. Choose the new color from the color options in the Color window and click OK

- Select Set as the Reference Cluster to use the selected cluster as the Difference Curve reference cluster.

Tip: These changes can also be done on the chart.

Precision Melt Data Tab

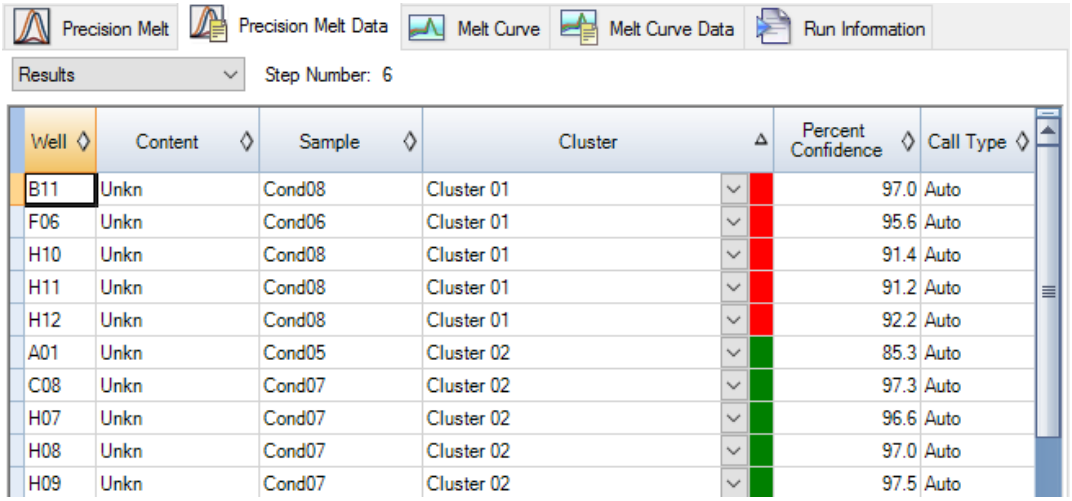
The Precision Melt Data tab displays melt data collected in each well. Precision Melt Analysis software provides six options in which to view the melt curve data:

- Results — displays a spreadsheet view of the data. This is the default view.
- Charts — displays multiple data charts in a single sheet.
- Plate views — displays a view of the data in each well as a plate map.
- Raw RFUs — displays the RFU value in each well for each temperature.
- Normalized RFUs — displays the normalized RFU value in each well for each temperature.
- Difference RFUs — displays the difference RFU value in each well for each temperature.

Tip: Right-click any spreadsheet for options. Sort the data in any spreadsheet by right-clicking and choosing Sort. Click View/Edit Plate to open the Plate Editor and change the contents of any well in the plate.

Results Spreadsheet

The Results spreadsheet displays the data for each well in the plate in spreadsheet format.



Well	Content	Sample	Cluster	Percent Confidence	Call Type
B11	Unkn	Cond08	Cluster 01	97.0	Auto
F06	Unkn	Cond06	Cluster 01	95.6	Auto
H10	Unkn	Cond08	Cluster 01	91.4	Auto
H11	Unkn	Cond08	Cluster 01	91.2	Auto
H12	Unkn	Cond08	Cluster 01	92.2	Auto
A01	Unkn	Cond05	Cluster 02	85.3	Auto
C08	Unkn	Cond07	Cluster 02	97.3	Auto
H07	Unkn	Cond07	Cluster 02	96.6	Auto
H08	Unkn	Cond07	Cluster 02	97.0	Auto
H09	Unkn	Cond07	Cluster 02	97.5	Auto

Table 15 defines the data that appear in the Results spreadsheet.

Table 15. Contents of the Results spreadsheet

Information	Description
Well	Well position in the plate
Content	A combination of the Sample Type and Replicate # loaded in the well in the Plate Editor
Sample	Sample name loaded in the well in the Plate Editor
Cluster	Name of the cluster assignment for the well
Percent Confidence	Confidence percent value as an integrity check for auto-called results
Call Type	Automatic or Manual: ■ Automatic — the software assigned the cluster call for the well ■ Manual — the user assigned the cluster call for the well

Charts View

Charts View displays data for all wells in multiple data charts.

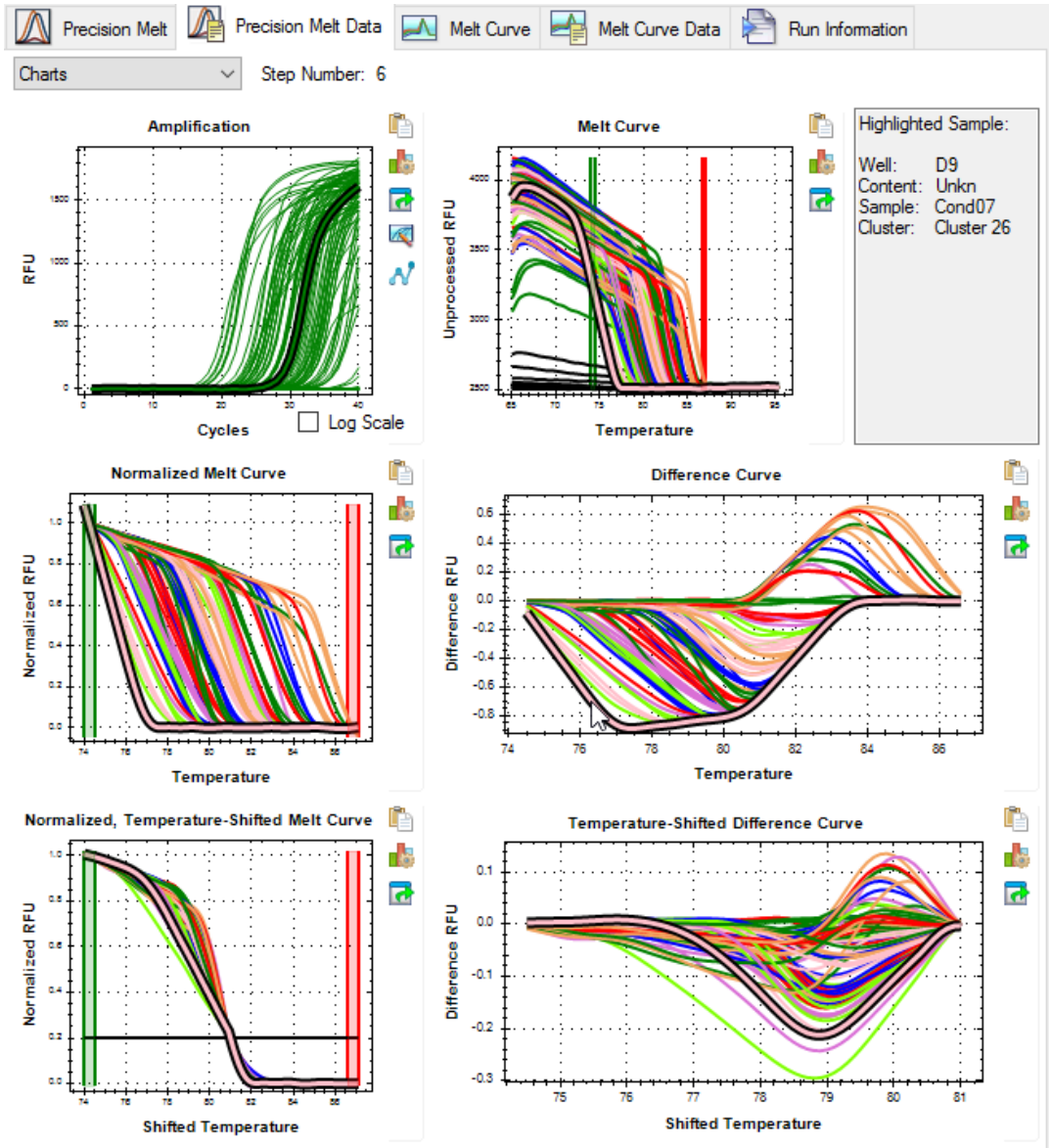


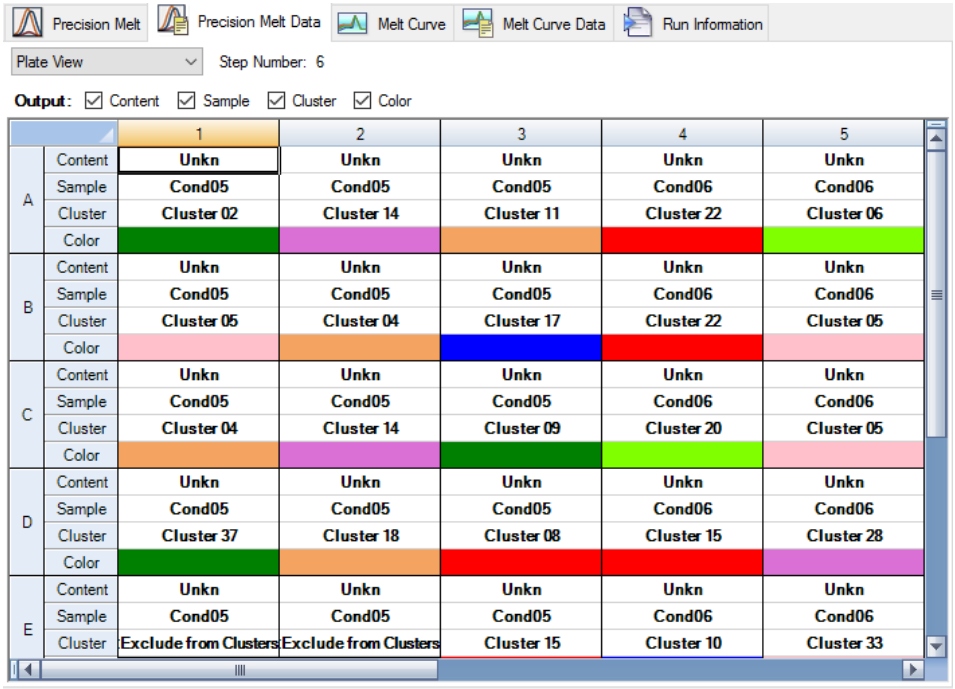
Table 16 on page 58 defines the data that appear in the Highlighted Sample section for a selected fluorescence curve.

Table 16. Information displayed for a selected curve

Information	Description
Well	Well position in the plate
Content	A combination of the Sample Type and Replicate # loaded in the well in the Plate Editor
Sample	Sample name loaded in the well in the Plate Editor
Cluster	Cluster name

Plate View Spreadsheet

The Plate View spreadhsheet displays a plate map of the data.



		1	2	3	4	5
A	Content	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Cond05	Cond05	Cond05	Cond06	Cond06
	Cluster	Cluster 02	Cluster 14	Cluster 11	Cluster 22	Cluster 06
	Color					
B	Content	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Cond05	Cond05	Cond05	Cond06	Cond06
	Cluster	Cluster 05	Cluster 04	Cluster 17	Cluster 22	Cluster 05
	Color					
C	Content	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Cond05	Cond05	Cond05	Cond06	Cond06
	Cluster	Cluster 04	Cluster 14	Cluster 09	Cluster 20	Cluster 05
	Color					
D	Content	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Cond05	Cond05	Cond05	Cond06	Cond06
	Cluster	Cluster 37	Cluster 18	Cluster 08	Cluster 15	Cluster 28
	Color					
E	Content	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Cond05	Cond05	Cond05	Cond06	Cond06
	Cluster	Exclude from Clusters	Exclude from Clusters	Cluster 15	Cluster 10	Cluster 33

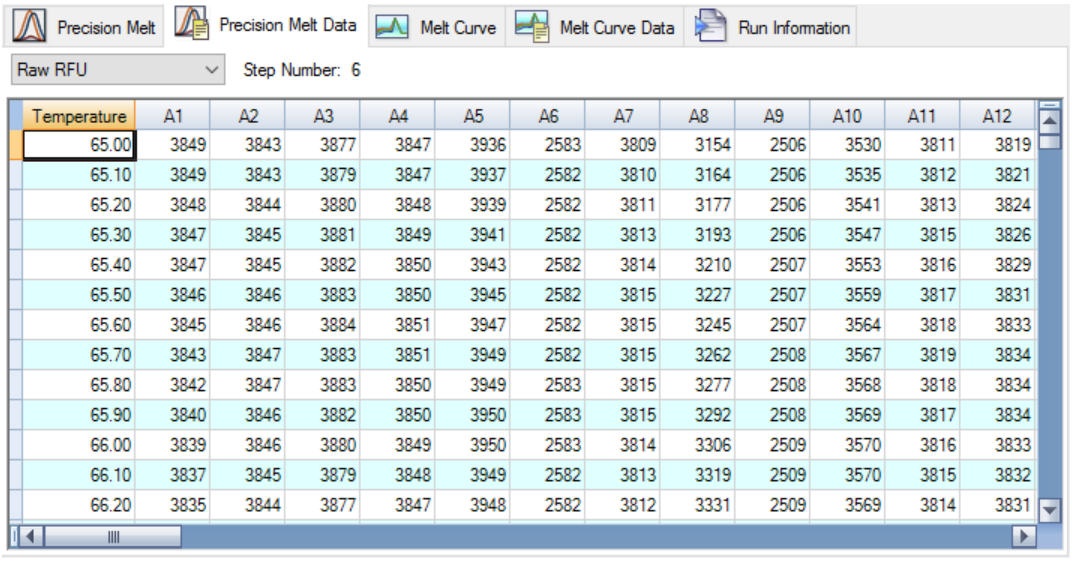
Table 17 defines the data that appear in the Plate View spreadsheet.

Table 17. Plate View spreadsheet contents

Information	Description
Content	A combination of the Sample Type and Replicate # loaded in the well in the Plate Editor
Sample	Sample name loaded in the well in the Plate Editor
Cluster	Cluster name
Color	Cluster color

Raw RFU Spreadsheet

The Raw RFU spreadsheet displays raw fluorescence readings for each well acquired at each temperature step of the melt curve protocol. The well number appears at the top of each column, and temperature appears to the left of each row.



Temperature	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
65.00	3849	3843	3877	3847	3936	2583	3809	3154	2506	3530	3811	3819
65.10	3849	3843	3879	3847	3937	2582	3810	3164	2506	3535	3812	3821
65.20	3848	3844	3880	3848	3939	2582	3811	3177	2506	3541	3813	3824
65.30	3847	3845	3881	3849	3941	2582	3813	3193	2506	3547	3815	3826
65.40	3847	3845	3882	3850	3943	2582	3814	3210	2507	3553	3816	3829
65.50	3846	3846	3883	3850	3945	2582	3815	3227	2507	3559	3817	3831
65.60	3845	3846	3884	3851	3947	2582	3815	3245	2507	3564	3818	3833
65.70	3843	3847	3883	3851	3949	2582	3815	3262	2508	3567	3819	3834
65.80	3842	3847	3883	3850	3949	2583	3815	3277	2508	3568	3818	3834
65.90	3840	3846	3882	3850	3950	2583	3815	3292	2508	3569	3817	3834
66.00	3839	3846	3880	3849	3950	2583	3814	3306	2509	3570	3816	3833
66.10	3837	3845	3879	3848	3949	2582	3813	3319	2509	3570	3815	3832
66.20	3835	3844	3877	3847	3948	2582	3812	3331	2509	3569	3814	3831

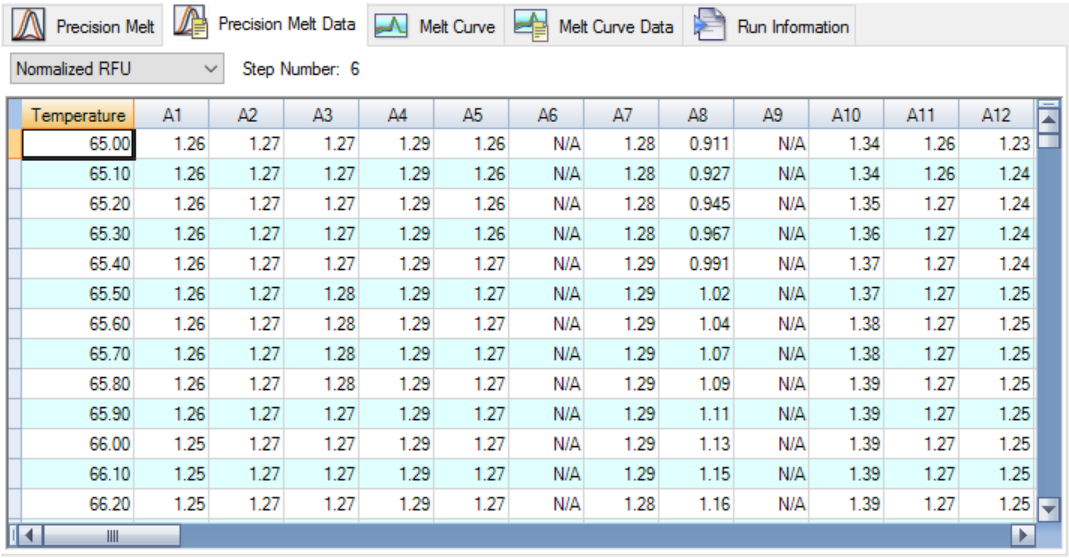
Table 18 on page 60 defines the data that appear in the Raw RFU spreadsheet.

Table 18. Raw RFU spreadsheet contents

Information	Description
Well number (A2, A3, A4, A5, A6)	Well data, listed by position in the plate for all the loaded wells
Temperature	Temperature data listed in ascending order for all the loaded wells

Normalized RFU Spreadsheet

The Normalized RFU spreadsheet displays normalized fluorescence readings for each well. Negative wells do not appear in this spreadsheet because they are excluded from normalized processing.



Temperature	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
65.00	1.26	1.27	1.27	1.29	1.26	N/A	1.28	0.911	N/A	1.34	1.26	1.23
65.10	1.26	1.27	1.27	1.29	1.26	N/A	1.28	0.927	N/A	1.34	1.26	1.24
65.20	1.26	1.27	1.27	1.29	1.26	N/A	1.28	0.945	N/A	1.35	1.27	1.24
65.30	1.26	1.27	1.27	1.29	1.26	N/A	1.28	0.967	N/A	1.36	1.27	1.24
65.40	1.26	1.27	1.27	1.29	1.27	N/A	1.29	0.991	N/A	1.37	1.27	1.24
65.50	1.26	1.27	1.28	1.29	1.27	N/A	1.29	1.02	N/A	1.37	1.27	1.25
65.60	1.26	1.27	1.28	1.29	1.27	N/A	1.29	1.04	N/A	1.38	1.27	1.25
65.70	1.26	1.27	1.28	1.29	1.27	N/A	1.29	1.07	N/A	1.38	1.27	1.25
65.80	1.26	1.27	1.28	1.29	1.27	N/A	1.29	1.09	N/A	1.39	1.27	1.25
65.90	1.26	1.27	1.27	1.29	1.27	N/A	1.29	1.11	N/A	1.39	1.27	1.25
66.00	1.25	1.27	1.27	1.29	1.27	N/A	1.29	1.13	N/A	1.39	1.27	1.25
66.10	1.25	1.27	1.27	1.29	1.27	N/A	1.29	1.15	N/A	1.39	1.27	1.25
66.20	1.25	1.27	1.27	1.29	1.27	N/A	1.28	1.16	N/A	1.39	1.27	1.25

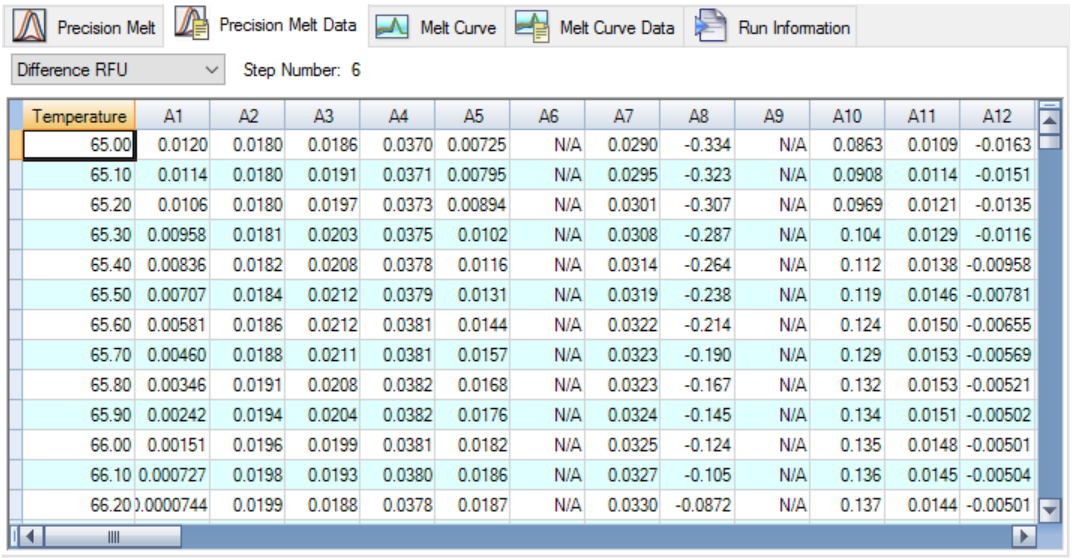
Table 19 defines the data that appear in the Normalized RFU spreadsheet.

Table 19. Normalized RFU spreadsheet contents

Information	Description
Well number (A2, A3, A4, A5, A6)	Well data, listed by position in the plate for all the loaded wells
Temperature	Temperature data listed in ascending order for all the loaded wells

Difference RFU Spreadsheet

The Difference RFU spreadsheet displays the difference fluorescence readings for each well. Negative wells do not appear in this spreadsheet because they are excluded from difference processing.



Temperature	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
65.00	0.0120	0.0180	0.0186	0.0370	0.00725	N/A	0.0290	-0.334	N/A	0.0863	0.0109	-0.0163
65.10	0.0114	0.0180	0.0191	0.0371	0.00795	N/A	0.0295	-0.323	N/A	0.0908	0.0114	-0.0151
65.20	0.0106	0.0180	0.0197	0.0373	0.00894	N/A	0.0301	-0.307	N/A	0.0969	0.0121	-0.0135
65.30	0.00958	0.0181	0.0203	0.0375	0.0102	N/A	0.0308	-0.287	N/A	0.104	0.0129	-0.0116
65.40	0.00836	0.0182	0.0208	0.0378	0.0116	N/A	0.0314	-0.264	N/A	0.112	0.0138	-0.00958
65.50	0.00707	0.0184	0.0212	0.0379	0.0131	N/A	0.0319	-0.238	N/A	0.119	0.0146	-0.00781
65.60	0.00581	0.0186	0.0212	0.0381	0.0144	N/A	0.0322	-0.214	N/A	0.124	0.0150	-0.00655
65.70	0.00460	0.0188	0.0211	0.0381	0.0157	N/A	0.0323	-0.190	N/A	0.129	0.0153	-0.00569
65.80	0.00346	0.0191	0.0208	0.0382	0.0168	N/A	0.0323	-0.167	N/A	0.132	0.0153	-0.00521
65.90	0.00242	0.0194	0.0204	0.0382	0.0176	N/A	0.0324	-0.145	N/A	0.134	0.0151	-0.00502
66.00	0.00151	0.0196	0.0199	0.0381	0.0182	N/A	0.0325	-0.124	N/A	0.135	0.0148	-0.00501
66.10	0.000727	0.0198	0.0193	0.0380	0.0186	N/A	0.0327	-0.105	N/A	0.136	0.0145	-0.00504
66.20	0.0000744	0.0199	0.0188	0.0378	0.0187	N/A	0.0330	-0.0872	N/A	0.137	0.0144	-0.00501

Table 20 defines the data that appear in the Difference RFU spreadsheet

Table 20. Difference RFU spreadsheet contents

Information	Description
Well number (A2, A3, A4, A5, A6)	Well data, listed by position in the plate for all the loaded wells
Temperature	Temperature data listed in ascending order for all the loaded wells

Melt Curve Tab

Precision Melt Analysis software plots the RFU data collected during a melt curve as a function of temperature. To analyze melt peak data, the software assigns a beginning and ending temperature to each peak by moving the threshold bar. The floor of the peak area is specified by the position of the melt threshold bar. A valid peak must have a minimum height relative to the distance between the threshold bar and the height of the highest peak.

The Melt Curve tab displays the T_m of amplified PCR products in four views:

- Melt Curve — displays the real-time data for each fluorophore as RFUs per temperature for each well.
- Melt Peak — displays the negative regression of the RFU data per temperature for each well.
- Well selector — displays wells to show or hide the data.
- Peak spreadsheet — displays the data collected in the selected well.

  Note: This spreadsheet displays up to two peaks for each trace. To see more peaks, click the Melt Curve Data tab.

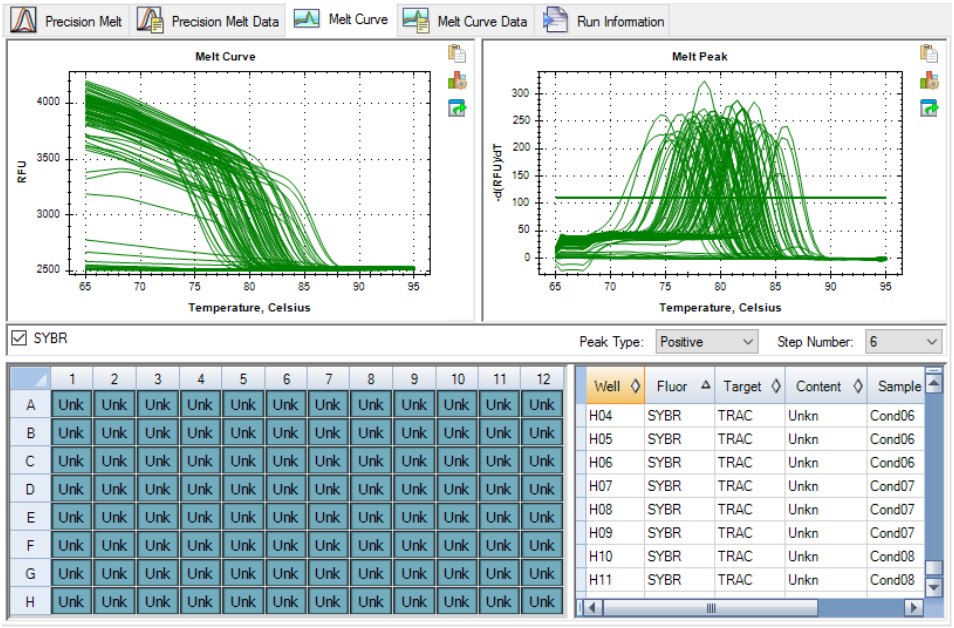


Table 21 on page 63 defines the data that appear in the Melt Curve spreadsheet.

Table 21. Melt Curve spreadsheet contents

Information	Description
Well	Well position in the plate
Fluor	Fluorophore detected
Content	A combination of Sample type and Replicate number
Sample	Name of sample loaded in the Plate Editor
Melt Temp	The temperature of the melt peak for each well **Note:** Only the two highest peaks appear in this spreadsheet.

Adjusting Melt Curve Data

To adjust the Melt Curve data

▶ Do any of the following:

- Click and drag the threshold bars in the Melt Peak chart to include or exclude peaks in data analysis.
- Open the Trace Styles window to change the color of the traces in the Melt Curve and Melt Peak charts.
- Select a number in the Step Number selector to view the Melt Curve data at another step in the protocol. The list shows more than one step if the protocol includes plate reads in more than one melt curve step.
- Select wells in the well selector to focus on subsets of the data.
- Select a well group to view and analyze a subset of the wells in the plate. Select each well group by name in the Well Group dropdown menu in the toolbar.

Melt Curve Data Tab

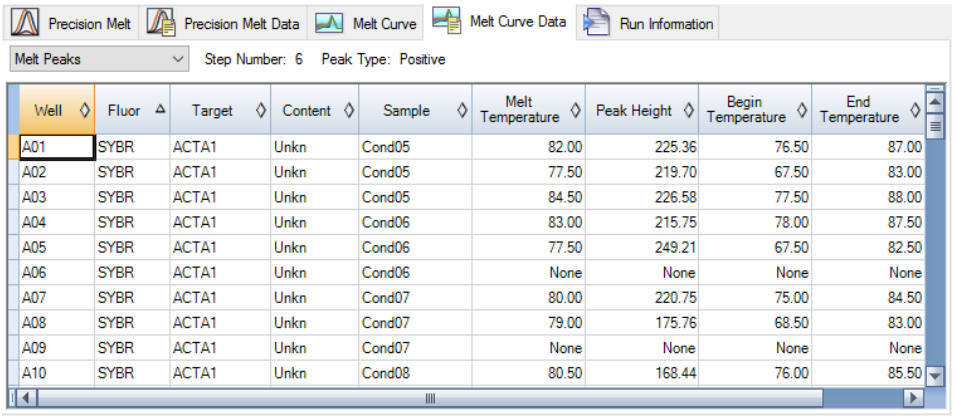
The Melt Curve Data tab displays the data from the Melt Curve tab in multiple spreadsheets that include all the melt peaks for each trace. Precision Melt Analysis software offers four spreadsheet options in which to view the melt curve data:

- Melt Peaks — displays all the data, including all the melt peaks, for each trace. This is the default view.
- Plate — displays a view of the data and contents of each well in the plate.
- RFU — displays the RFU quantities at each temperature for each well.
- -d(RFU)/dT — displays the negative rate of change in RFU as the temperature (T) changes. This is a first regression plot for each well in the plate.

Select each spreadsheet from the dropdown list that appears below the Melt Curve Data tab.

Melt Peaks Spreadsheet

The Melt Peaks spreadsheet displays all melt curve data.



Well	Fluor	Target	Content	Sample	Melt Temperature	Peak Height	Begin Temperature	End Temperature
A01	SYBR	ACTA1	Unkn	Cond05	82.00	225.36	76.50	87.00
A02	SYBR	ACTA1	Unkn	Cond05	77.50	219.70	67.50	83.00
A03	SYBR	ACTA1	Unkn	Cond05	84.50	226.58	77.50	88.00
A04	SYBR	ACTA1	Unkn	Cond06	83.00	215.75	78.00	87.50
A05	SYBR	ACTA1	Unkn	Cond06	77.50	249.21	67.50	82.50
A06	SYBR	ACTA1	Unkn	Cond06	None	None	None	None
A07	SYBR	ACTA1	Unkn	Cond07	80.00	220.75	75.00	84.50
A08	SYBR	ACTA1	Unkn	Cond07	79.00	175.76	68.50	83.00
A09	SYBR	ACTA1	Unkn	Cond07	None	None	None	None
A10	SYBR	ACTA1	Unkn	Cond08	80.50	168.44	76.00	85.50

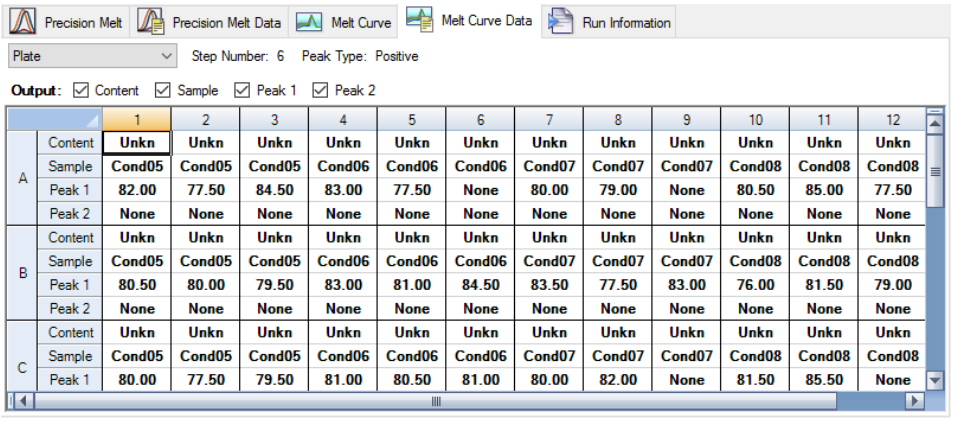
Table 22 on page 66 defines the data that appear in the Melt Peaks spreadsheet.

Table 22. Melt Peaks spreadsheet content

Information	Description
Well	Well position in the plate
Fluor	Fluorophore detected
Content	Sample Type listed in the Plate Editor window
Target	Amplification target (gene)
Sample	Sample Name listed in the Plate Editor window
Melt Temperature	The melting temperature of each product, listed as one peak (highest) per row in the spreadsheet
Peak Height	Height of the peak
Begin Temperature	Temperature at the beginning of the peak
End Temperature	Temperature at the end of the peak

Plate Spreadsheet

The Plate spreadsheet displays melt curve data in a plate format.



		1	2	3	4	5	6	7	8	9	10	11	12
A	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Cond05	Cond05	Cond05	Cond06	Cond06	Cond06	Cond07	Cond07	Cond07	Cond08	Cond08	Cond08
	Peak 1	82.00	77.50	84.50	83.00	77.50	None	80.00	79.00	None	80.50	85.00	77.50
	Peak 2	None	None	None	None	None	None	None	None	None	None	None	None
B	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Cond05	Cond05	Cond05	Cond06	Cond06	Cond06	Cond07	Cond07	Cond07	Cond08	Cond08	Cond08
	Peak 1	80.50	80.00	79.50	83.00	81.00	84.50	83.50	77.50	83.00	76.00	81.50	79.00
	Peak 2	None	None	None	None	None	None	None	None	None	None	None	None
C	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn
	Sample	Cond05	Cond05	Cond05	Cond06	Cond06	Cond06	Cond07	Cond07	Cond07	Cond08	Cond08	Cond08
	Peak 1	80.00	77.50	79.50	81.00	80.50	81.00	80.00	82.00	None	81.50	85.50	None

Note: To adjust the peak that the software calls, adjust the threshold line in the Melt Peak chart on the Melt Curve tab.

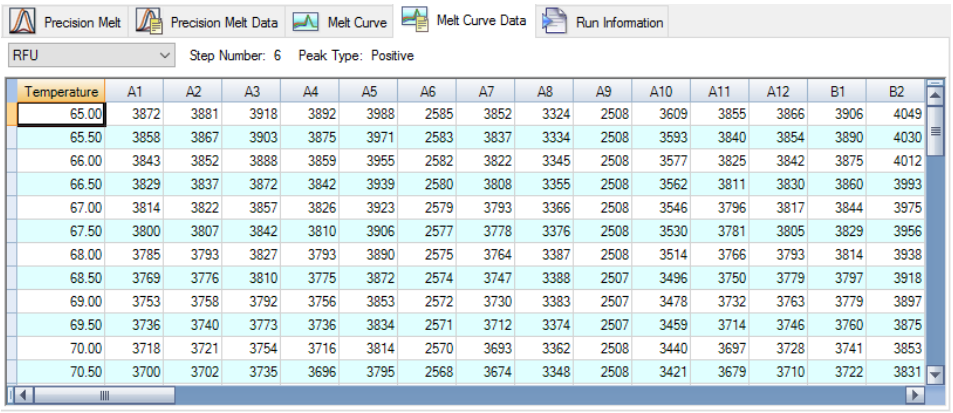
Table 23 on page 67 defines the data that appear in the Plate spreadsheet.

Table 23. Plate spreadsheet content

Information	Description
Content	A combination of Sample Type (required) and Replicate # (optional)
Sample	Sample description
Peak 1	First melt peak (highest)
Peak 2	Second (lower) melt peak

RFU Spreadsheet

The RFU spreadsheet displays the fluorescence for each well at each cycle acquired during the melt curve.



Temperature	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	B1	B2
65.00	3872	3881	3918	3892	3988	2585	3852	3324	2508	3609	3855	3866	3906	4049
65.50	3858	3867	3903	3875	3971	2583	3837	3334	2508	3593	3840	3854	3890	4030
66.00	3843	3852	3888	3859	3955	2582	3822	3345	2508	3577	3825	3842	3875	4012
66.50	3829	3837	3872	3842	3939	2580	3808	3355	2508	3562	3811	3830	3860	3993
67.00	3814	3822	3857	3826	3923	2579	3793	3366	2508	3546	3796	3817	3844	3975
67.50	3800	3807	3842	3810	3906	2577	3778	3376	2508	3530	3781	3805	3829	3956
68.00	3785	3793	3827	3793	3890	2575	3764	3387	2508	3514	3766	3793	3814	3938
68.50	3769	3776	3810	3775	3872	2574	3747	3388	2507	3496	3750	3779	3797	3918
69.00	3753	3758	3792	3756	3853	2572	3730	3383	2507	3478	3732	3763	3779	3897
69.50	3736	3740	3773	3736	3834	2571	3712	3374	2507	3459	3714	3746	3760	3875
70.00	3718	3721	3754	3716	3814	2570	3693	3362	2508	3440	3697	3728	3741	3853
70.50	3700	3702	3735	3696	3795	2568	3674	3348	2508	3421	3679	3710	3722	3831

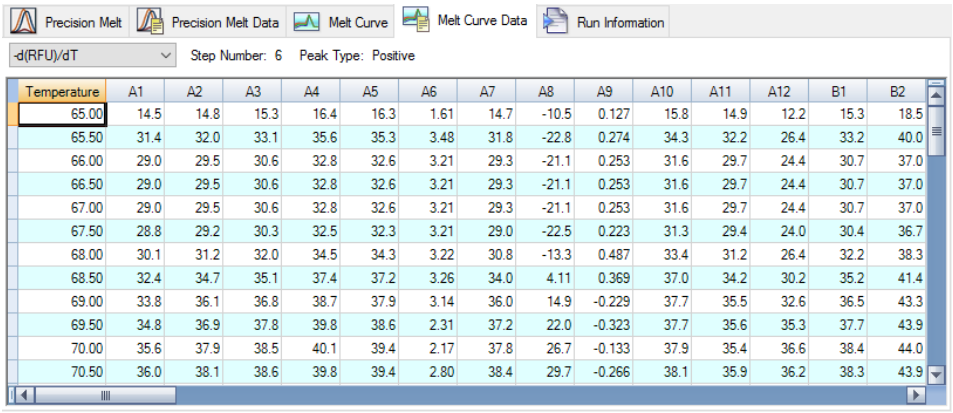
Table 24 defines the data displayed in the RFU spreadsheet.

Table 24. RFU spreadsheet content

Information	Description
Well number (A1, A2, A3, A4, A5)	Well position in the plate for the loaded wells
Temperature	Melting temperature of the amplified target, plotted as one well per row and multiple wells for multiple products in the same well

-d(RFU)/dT Spreadsheet

The -d(RFU)/dT spreadsheet displays the negative rate of change in RFU as the temperature (T) changes.



Temperature	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	B1	B2
65.00	14.5	14.8	15.3	16.4	16.3	1.61	14.7	-10.5	0.127	15.8	14.9	12.2	15.3	18.5
65.50	31.4	32.0	33.1	35.6	35.3	3.48	31.8	-22.8	0.274	34.3	32.2	26.4	33.2	40.0
66.00	29.0	29.5	30.6	32.8	32.6	3.21	29.3	-21.1	0.253	31.6	29.7	24.4	30.7	37.0
66.50	29.0	29.5	30.6	32.8	32.6	3.21	29.3	-21.1	0.253	31.6	29.7	24.4	30.7	37.0
67.00	29.0	29.5	30.6	32.8	32.6	3.21	29.3	-21.1	0.253	31.6	29.7	24.4	30.7	37.0
67.50	28.8	29.2	30.3	32.5	32.3	3.21	29.0	-22.5	0.223	31.3	29.4	24.0	30.4	36.7
68.00	30.1	31.2	32.0	34.5	34.3	3.22	30.8	-13.3	0.487	33.4	31.2	26.4	32.2	38.3
68.50	32.4	34.7	35.1	37.4	37.2	3.26	34.0	4.11	0.369	37.0	34.2	30.2	35.2	41.4
69.00	33.8	36.1	36.8	38.7	37.9	3.14	36.0	14.9	-0.229	37.7	35.5	32.6	36.5	43.3
69.50	34.8	36.9	37.8	39.8	38.6	2.31	37.2	22.0	-0.323	37.7	35.6	35.3	37.7	43.9
70.00	35.6	37.9	38.5	40.1	39.4	2.17	37.8	26.7	-0.133	37.9	35.4	36.6	38.4	44.0
70.50	36.0	38.1	38.6	39.8	39.4	2.80	38.4	29.7	-0.266	38.1	35.9	36.2	38.3	43.9

Table 25 defines the data that appear in the -d(RFU)/dT spreadsheet.

Table 25. -d(RFU)/dT spreadsheet content

Information	Description
Well number (A1, A2, A3, A4, A5)	Well position in the plate for the loaded wells
Temperature -d(RFU)/dT	Negative rate of change in RFU as temperature (T) changes

Chapter 7 Melt Study Analysis

Precision Melt Analysis™ software can compare melt curve data from different melt experiments run on the same instrument. Create a melt study by adding data from one or more melt files to the Melt Study dialog box. The software groups the data into a single melt study file.

Tip: The maximum number of samples that can be analyzed in a melt study is limited by the size of the computer's RAM and virtual memory.

This chapter explains how to create a melt study and how the software analyzes the data.

Melt Study Dialog Box

The Melt Study dialog box includes two tabs:

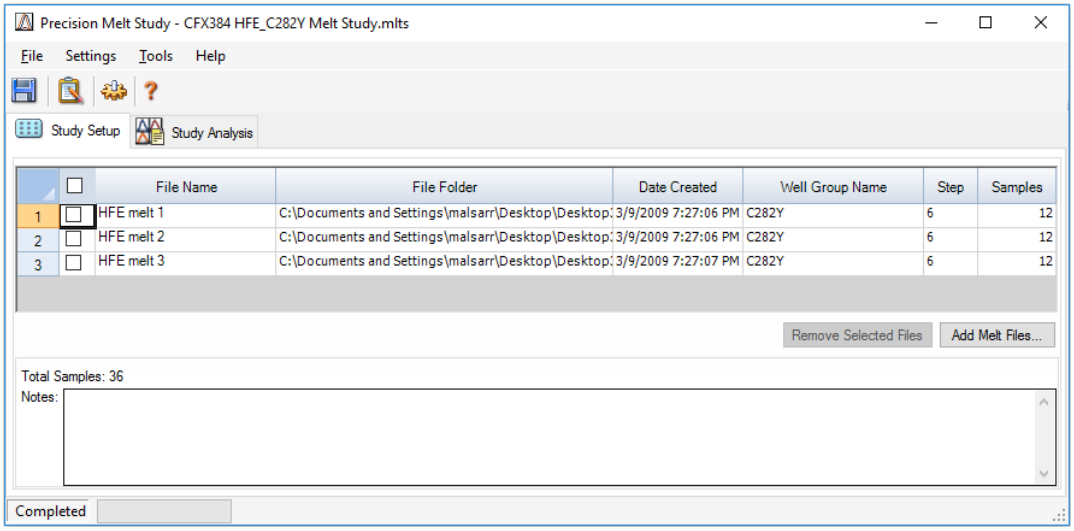
- Study Setup tab — manages the runs in the melt study.

  Important: Adding or removing melt files in a melt study does not change the original data in that file.

- Study Analysis tab — displays the data for the combined runs.

Study Setup Tab

The Study Setup tab displays a list of all the experiments in the melt study.



In the Study Setup tab you can do the following:

- Add experiments — click Add Melt Files to select a file from a browser window

  Tip: To quickly add experiments to a melt study, drag the melt files to the Melt Study dialog box.

  Note: When adding a melt file with multiple well groups, select the well group(s) to add to the study in the Setup tab.

- Remove experiments — select one or more files in the list and click Remove Selected Files
- Add notes — type in the Notes box to add comments about the files and analysis in the melt study

Table 26 on page 73 defines the data that appear in the Study Setup tab.

Table 26. Study Setup tab in the Melt Study dialog box

Column Title	Description
File Name	Name of the melt file
File Folder	Directory that stores the melt file for each experiment in the Melt Study window
Date Created	Date the run data were collected
Well Group Name	Name of the well group selected when the file was added to the Melt Study window
Step	Protocol step that includes the plate read to collect data
Samples	Number of samples

Study Analysis Tab

The Study Analysis tab displays the data from all experiments that are added to the nelt study. The Study Analysis tab includes the same features as the Precision Melt Analysis tab. For example, highlighting a sample in one of the melt study charts highlights the corresponding cell in the spreadsheet below the chart.

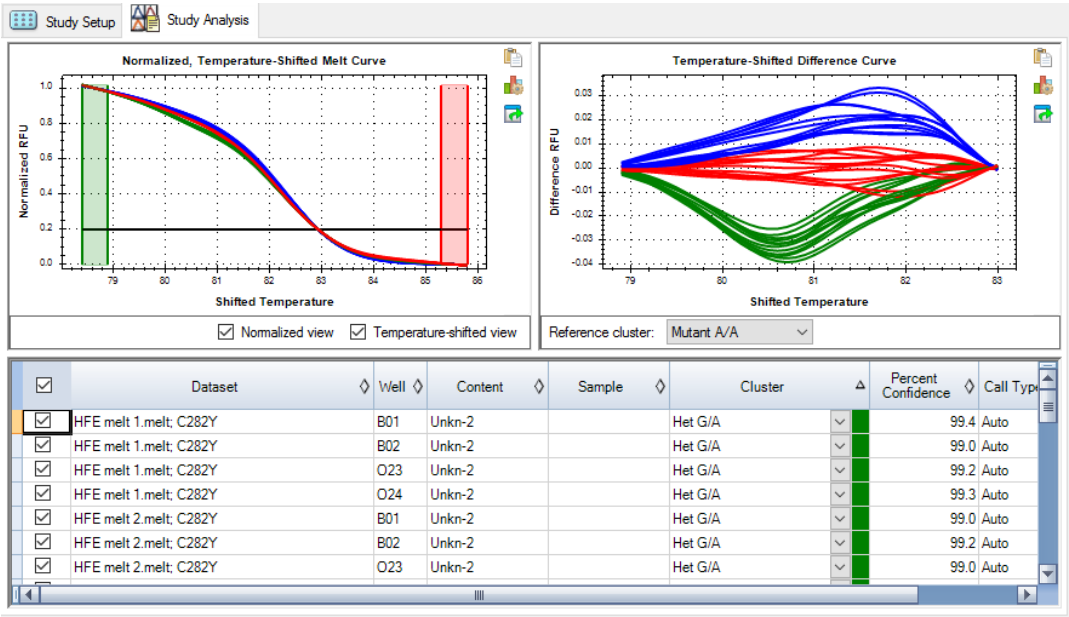


Table 27 defines the data that appear in the Study Analysis tab.

Table 27. Study Analysis tab in the Melt Study dialog box

Information	Description
Dataset	Melt data from one fluorophore in one melt file
Well	Well position in the plate
Content	A combination of Sample Type (required) and Replicate Number (optional) loaded in the Plate Editor
Sample	Sample name loaded in the Plate Editor wells
Cluster	Cluster name

Table 27. Study Analysis tab in the Melt Study dialog box, continued

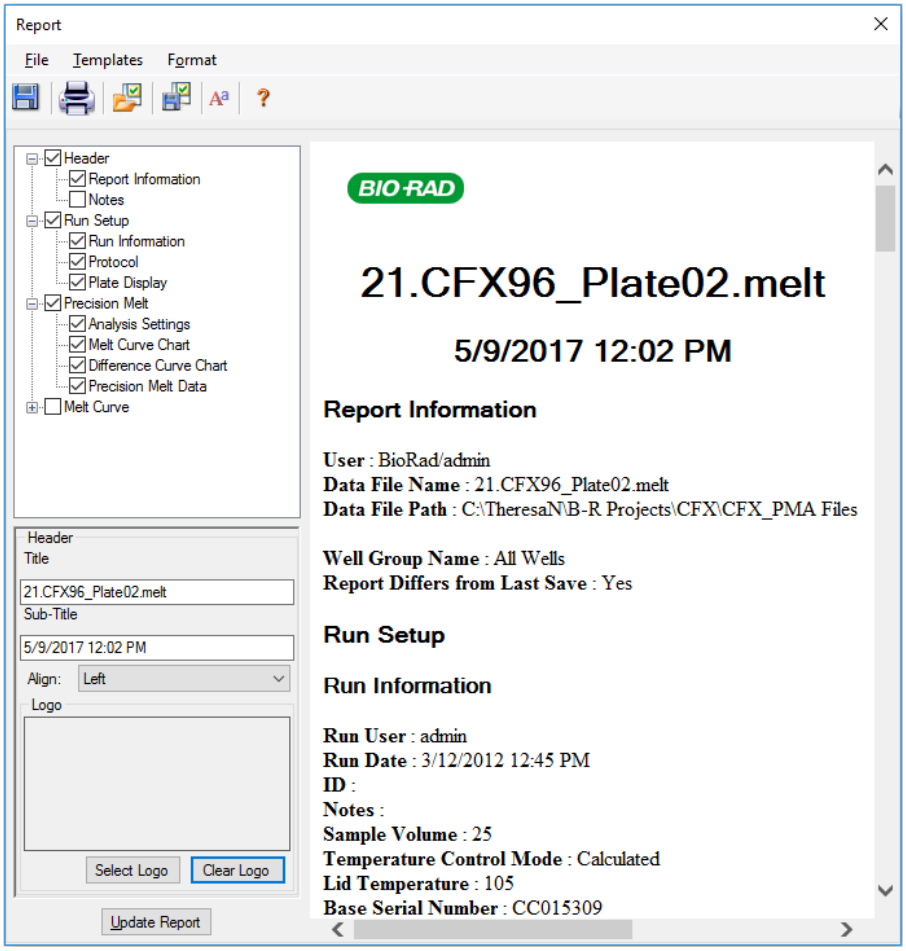
Information	Description
Percent Confidence	Indication of the relative probability the sample has of being in a cluster
Call Type	Auto or manual call of the cluster

Chapter 8 Melt Analysis Reports

The Report dialog box displays information about the current data file in the Data Analysis or Melt Study window. To open a report, select Tools > Reports or click Reports on the toolbar.

The Report dialog comprises the following sections:

- Menu and toolbar — provides options to format, save, and print the report or template.
- Options list (top left side of the dialog box) — provides options to display in the report.
- Options pane (bottom left side of the dialog box) — displays text boxes in which you can enter information about a selected option.
- Preview pane (right side of the dialog box) — displays a preview of the current report.



Creating a Melt Analysis Report

You can save the report layout as a template, which you can use again for similar reports.

To create a melt analysis report

1. Make final adjustments to the well contents, selected wells, charts, and spreadsheets in the Data Analysis window before creating the report.
2. Select Tools > Reports in the Data Analysis menu bar to open the Report dialog box.
3. Choose the options you want to include in the report. The report opens with default options selected. Select or clear the checkboxes to change whole categories or individual options within a category.

   Table 28 on page 80 lists the available options to display.

Note: The data that appear in the report depend on the current selections within the tabs of the Data Analysis or Melt Study window.

4. Change the order of categories and items in a report. Drag the options to the relative position. Items can be reordered only within the categories to which they belong.
5. (Optional) In the Report Options pane, enter information relevant to the selected option:
   - Choose a subset of information to display in the report.
   - Choose specific settings for the selected option.
   - Change the text to display for the selected option.
6. Click Update Report to update the Report Preview with any changes.
7. Print or save the report. Click the Print Report button in the toolbar to print the current report. Select File > Save to save the report in PDF (Adobe Acrobat Reader file) format and select a location in which to save the file. Select File > Save As to save the report with a new name or in a new location.
8. (Optional) Create a report template with the information you want. To save the current report settings in a template, select Template > Save or Save As. Then load the report template the next time you want to make a new report.

Report Categories

Table 28 lists the options available for a melt analysis report, depending on the type of data in the Data Analysis or Melt Study window.

Table 28. Report categories

Category	Option	Description
Header		Title, subtitle, and logo for the report
	Report Information	Experiment date, user name, data file name, data file path, and selected well group
	Notes	Notes about the data report
Experiment Setup		
	Run Information	Includes the experiment date, user, data file name, data file path, and the selected well group
	Protocol	Text view of the protocol steps and options
	Plate Display	Plate view of the information in each well of the plate
Melt Curve		
	Analysis Settings	Includes the melt step number and threshold bar setting
	Melt Curve Chart	Copy of the melt curve chart
	Melt Peak Chart	Copy of the melt peak chart
	Data	Spreadsheet listing the data in each well

Appendix A Guidelines for Successful HRM Analysis

The success of HRM analysis highly depends on the quality of the individual PCR product and the specific sequence under investigation. All experimental parameters must be controlled and highly reproducible from sample to sample to ensure successful HRM analysis.

Recommended Guidelines for Successful HRM Analysis

1. Analyze small DNA amplicons

Analyzing amplicons smaller than 150 bp is preferable, especially when sites with a known polymorphism are investigated. It is possible to detect sequence variations with longer amplicons, however, a single base variation influences the melting behavior of a 100 bp amplicon more than a 600 bp amplicon.

2. Analyze a single pure product

Avoid sequences that are likely to form non-specific products or primer dimers. Always run a BLAST search (www.ncbi.nlm.nih.gov/BLAST) to check the specificity of the primers sequences to the target species and gene. In addition, bad resolution or poor grouping may occur when secondary structures in single-stranded or partially denatured DNA are present. The amplicon sequences should be entered into MFOLD (mfold.rna.albany.edu/?q=mfold/dna-folding-form) to assure that they do not form any secondary structures during PCR.

3. Use sufficient pre-amplification template

Analyzing real-time PCR amplification data can be extremely useful when troubleshooting HRM analyses. Samples should have a quantification threshold (C_q) less than 30 cycles. Products that amplify late due to too little starting template amount or template degradation produce variable HRM results.

4. Normalize template concentration

The amount of template added to the reaction should be consistent. Normalize the starting concentrations so that all amplification plots are within three C_qs of each other, a 10-fold range.

5. Check for aberrant amplification plots

Examine amplification data carefully for abnormal amplification curve shapes. A curve with a jagged log-linear phase or one that reaches a low signal plateau compared to other reactions can indicate poor amplification or a fluorescence signal too low for analysis. Unsuccessful amplification can be caused by reaction inhibitors, too little dye, or incorrect reaction setup. HRM analysis from such samples can cause low resolution or poor grouping.

6. Keep post-amplification sample concentrations similar

Minimizing reaction-to reaction-variability is critical, and using the same sample preparation procedure will minimize this variability. Since the concentration of a DNA fragment affects its melting temperature, ensure every reaction has amplified to the plateau phase. Poor reactions might not reach plateau with the same amplified quantity due to inconsistent assay setup.

7. Ensure sample-to-sample uniformity

Samples must be of equal volume and with the same concentration of dye. DNA melting behavior is affected by salts in the reaction mix, so the concentration of buffer, Mg^{2+} and other salts should be as uniform as possible in all samples.

8. Allow sufficient data collection for pre-and post-melt regions

For easier data interpretation and results with tighter replicates, ensure enough baseline data points were collected. This can be easily accomplished by capturing HRM data points over at least a 10°C (or greater) window, centered around the observed T_m of the amplified product.

Appendix B Tips and Tricks

This appendix lists tips for using Precision Melt Analysis software.

- Open any melt or melt study file by dragging it from a folder to an open software window.
- Right-click a chart, spreadsheet, or well selector to print or export the data.
- Click and drag the edges of a dialog box to change its .
- Add melt files to a melt study by dragging from a folder to an open Melt Study window.
- Open multiple melt and melt study files at the same time.
- In the Plate Editor, double-click a well to open the Well Info dialog box and view detailed information about the well.
- Right-click any graph or chart to change viewing and data analysis options.
- Select a well group to view and analyze a subset of the wells in the plate. Select each well group by name in the Well Group pull-down menu in the toolbar.

Appendix C References

Review the following references to learn more about HRM analysis.

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