

High Resolution Melt Analysis Module

USER GUIDE

for use with QuantStudio™ Design and Analysis Software 2

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Revision	Date	Description
D00	7 March 2024	<p>The document was updated for QuantStudio™ Design and Analysis Software v2.8 and the High Resolution Melt Analysis Module v1.5.</p> <ul style="list-style-type: none">• Permissions were added for the following tasks, when the security settings are enabled:<ul style="list-style-type: none">– Selecting the location when a file is saved (“Select a system template or existing plate file to set up a new plate file” on page 7).– Selecting a passive reference (“Select a passive reference” on page 10).• Instructions were added to export the results (“Export the results” on page 19).
C.0	7 July 2023	<p>The document was updated for QuantStudio™ Design and Analysis Software v2.7 and the High Resolution Melt Analysis Module v1.4.</p> <ul style="list-style-type: none">• The list of compatible data files was removed. See the documentation for the main software for this information.• The instructions to send the plate file to the instrument run queue were removed. See the documentation for the main software for this information.• The instructions to set up the high resolution melt analysis settings were updated to include a manual call.• Instructions were added to select a manual call for a well.• Instructions were added to show or hide the grid for a plot.
B.0	15 April 2020	Update ramp mode and passive reference.
A.0	20 December 2019	New document.

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

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About the High Resolution Melt Analysis Module

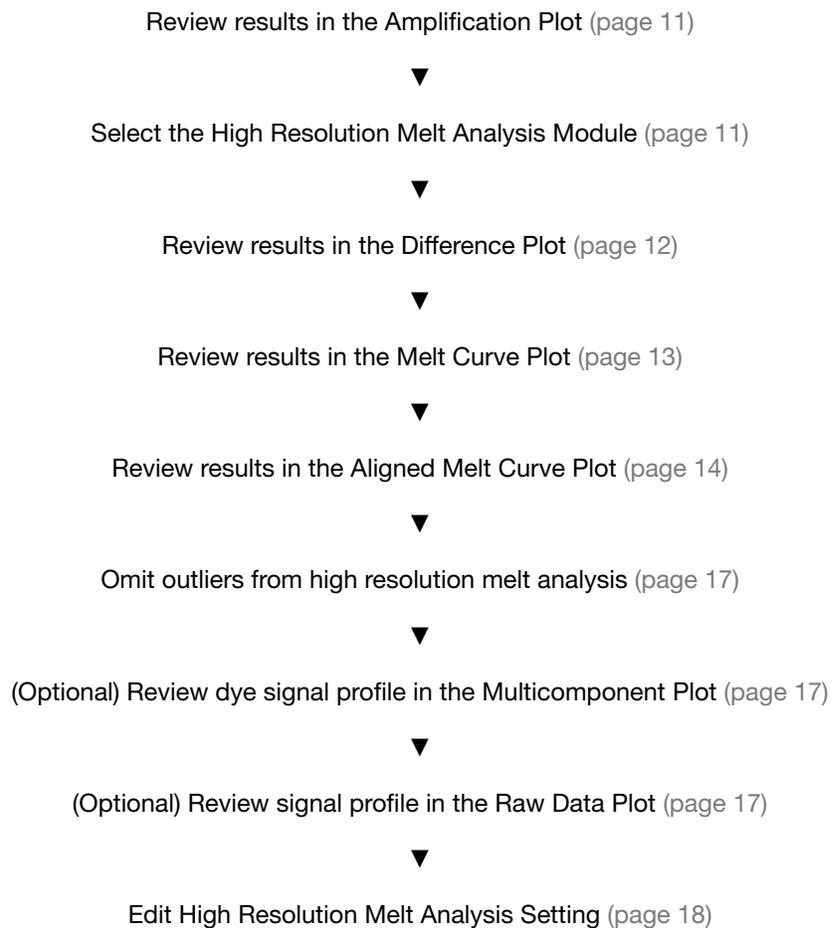
The High Resolution Melt Analysis Module for QuantStudio™ Design and Analysis Software 2 is used to identify genetic variation in nucleic acid sequences.

For more information about high resolution melt analysis, see Chapter 5, “About high resolution melt analysis”.

2

Workflow: High resolution melt analysis

IMPORTANT! If you omit wells or configure the analysis settings, click **Analyze** to reanalyze the data.





Set up a plate file for high resolution melt analysis

For detailed instructions about setting up a plate file, see [? Help ▶ Help Contents](#).

Select a system template or existing plate file to set up a new plate file

A plate file contains the information that is necessary to perform an instrument run, including instrument setup, run method, plate setup, and analysis setting.

A system template is a non-editable plate file that is included with the software.

A new plate file must be created from a system template or a previously created plate file.

For detailed information about system templates and plate files, see [? Help ▶ Help Contents](#).

1. In the home screen, click  **Set Up Plate**.
The **Plate Gallery** opens to the **System Templates** tab.

2. **IMPORTANT!** Select a system template or a plate file that corresponds to your instrument, block, and run mode. These properties are not editable after the plate file has been created.

In the left pane, select the appropriate options to filter the system template and plate file lists.

- **Instrument**
- **Block**
- **Run Mode**
- **Analysis**

Note: Thermal protocol, plate setup, and post-run analysis options are independent of analysis module selection. Analysis module selection can be changed at any point during plate file set or post-run analysis (see “Select the High Resolution Melt Analysis Module” on page 11).

3. Navigate to, then select a system template or plate file.

Tab	Description
System Templates	Contains system templates, non-editable plate files that are included with the software. Select a system template to automatically generate a new plate file that can be edited, then saved.
My Plate Files	Contains plate files that were previously saved to My Plate Files . plate files that are included with the software. Select an existing plate file to edit, then save, or to save as a new plate file.
Recents	Contains plate files that were recently opened. Recently opened plate files from System Templates and My Plate Files do not populate this tab. Select an existing plate file to edit, then save, or to save as a new plate file.

Note: To view all options for opening the plate file, hover over the plate file, then click ... **(Actions)**.

Selecting the location where the file is saved is a controlled function. If the **Browse** button in the **Save As** dialog box is inactive, the security has been enabled in the software. The user role does not have the permission to select the location where the file is saved. The user role must have the permission of **Edit Save As Destination**.

The plate file opens in the **Run Method** tab.

Confirm or edit the run method for high resolution melt analysis

For most analysis, the default run method is appropriate. The following options are compatible for high resolution melt analysis.

- PCR
- 1-step RT-PCR
- 2-step RT-PCR
- In a plate file, in the **Run Method** tab, adjust the run method elements as needed.

Note: Confirm that the ramp mode is set to 0.025°C/second in continuous mode.

For detailed instructions about editing the run method, see [?](#) **Help** ▶ **Help Contents**.

- Click ... **(Actions)** ▶ **Filter Settings** to confirm or edit filter settings.

Confirm or edit the plate setup for high resolution melt analysis

For detailed instructions about plate setup, or to download example plate setup files, see [?](#) **Help** ▶ **Help Contents**.

Add samples and assign to wells

For detailed instructions about plate setup, see [?](#) **Help** ▶ **Help Contents**.

1. In the **Plate Setup** tab, add samples and assign to wells using the following options.
 - Import a plate setup file
 - Manually add samples to the **Samples** table
 - Manually add samples to wells in the plate layout
2. Confirm or edit sample information in the **Samples** table.

Column	Description
Name	Sample name
Color	Sample color
Type ^[1]	High resolution melt analysis uses the following sample types. <ul style="list-style-type: none">• Unknown• Positive Control• Negative Control

^[1] For more information, see “Sample types for high resolution melt analysis” on page 21.

3. Confirm or edit sample well assignments in the plate layout.

Add targets and assign to wells

For detailed instructions about plate setup, see [?](#) **Help** ▶ **Help Contents**.

1. In the **Plate Setup** tab, add targets and assign to wells using the following options.
 - Import an Assay Information File (AIF)
 - Import a plate setup file
 - Manually add targets to the **Targets** table
 - Manually add targets to wells in the plate layout

2. Confirm or edit target information in the **Target** table.

Column	Description
Name	Target name
Color	Target color
Reporter	Reporter dye
Task ^[1]	The software automatically assigns a task to the target in a well based on the sample type in that well. The following tasks are used for high resolution melt analysis. <ul style="list-style-type: none"> • Unknown • Positive Control • Negative Control

^[1] For more information, see “Sample types for high resolution melt analysis” on page 21.

3. Confirm or edit target well assignments in the plate layout.

Edit reagent information

1. In the **Plate Setup** tab, in the **Targets/SNP Assays** table pane, click **Reagents**.
2. In the **Reagents** table, click **+ (Add)**.

Note:

- Click **⋮ (Actions) ▶ Export Reagents** to export reagents.
 - Click **⋮ (Actions) ▶ Import Reagents** to import reagents.
 - Click **⋮ (Actions) ▶ Scan Reagents** to scan reagents.
-

3. Enter the following information for each reagent.

<ul style="list-style-type: none"> • Name • Type • Barcode 	<ul style="list-style-type: none"> • Part Number • Lot Number • Expiration Date
---	--

Note: If the master mix that you enter is not compatible with the current run method, you have the option to apply the recommended run method for your master mix, instrument, block, and run mode.

4. (Optional) Click **✕ (Remove)** in the row of a reagent to delete it from the table.

Select a passive reference

Editing the passive reference is a controlled function. If security has been enabled in the software, the user role must have the permission of **Edit Passive Reference**.

1. In the upper-left corner of the **Plate Setup** tab, select **NONE** as the passive reference from the dropdown list.
2. (Optional) Save the plate file or data file.



Perform high resolution melt analysis

Show or hide the grid

The grid of a plot can be shown or hidden.

1. Click  (**Settings**).
2. In the **General** pane, select or deselect the **Grid** checkbox.
3. Click outside of the pane to apply the settings and close the pane.

Review results in the Amplification Plot

For detailed instructions about reviewing results in the **Amplification Plot**, see [? Help ▶ Help Contents](#).

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, in the plot pane, select **Amplification Plot** from the dropdown list.
2. Review the amplification status for each well.
3. Review or edit threshold settings.
4. Review or edit baseline settings.

Select the High Resolution Melt Analysis Module

1. In an open data file, click **Actions ▶ Analysis Modules**.
2. In the **Analysis Modules** window, select **High Resolution Melt**, then click **Ok**.
The High Resolution Melt Analysis Module opens.

Click **Analyze**, then review the results in the **High Resolution Melt** tab.

Review results in the Difference Plot

For more information about the difference plot, see “Difference Plot overview” on page 21.

1. In the **High Resolution Melt** tab, in the upper-left corner of the plot pane, select **Difference Plot** from the dropdown list.
2. In the upper-middle of the plot pane, select a target from the dropdown list.
3. In the upper-right corner of the plot pane, click **⚙ (Settings)**, then select an option from the **Color By** dropdown list: **Call**, **Sample**, or **Well**.

The plot is displayed. The temperature slider displays the temperature at the position of the line in the plot.

4. Set manual calls.
 - a. Select a row in the **Well Table**, then click **⋮ (Actions) ▶ Set manual call** in the upper-right corner of the table.
Control-click or shift-click to select multiple rows.
 - b. In the **Manual Call** dialog box, select a value from the dropdown list, then click **OK**.
The options in the dropdown list include the manual calls that were added in the analysis settings (see “Edit High Resolution Melt Analysis Setting” on page 18).

5. (*HRM mutation detection data and HRM methylation data only*) Review the **Difference Plot** for outliers.
 - a. In the **Well Table**, select a control or any well as the reference (click **⋮ (Actions) ▶ Set as reference**).

Note: **⋮ (Actions) ▶ Set As Reference** is also available in the **Difference Plot**.

- b. Review:
 - Variant clusters—How many distinct clusters are displayed?
 - Outliers—How tight are the curves within each variant cluster?

Note: Try selecting different reference samples to find the optimal display of the clusters.

6. (*HRM methylation data only*) Confirm the software calls.
 - a. In the **Well Table**, click the **Well** column header to sort the results according to the well position.

- b. For the methylation standard controls, review:
- **Variation Call** column—Do all of the methylation standard controls have the correct call?
 - **Silhouette Score** column—Are there any outliers within the replicate group? Do the values for the replicate group differ from the confidence values for the other replicate groups in the plate?

Note: If any of the controls are outliers, omit them from the HRM analysis, then reanalyze. See “Omit outliers from high resolution melt analysis” on page 17.

- c. Select the rows in the **Well Table** to view the corresponding fluorescence data in the **Difference Plot**.

7. (HRM genotyping data only) Confirm the genotype calls.

- a. In the **Well Table**, click the **Well** column header to sort the results according to the well position.
- b. For the positive controls, review:
- **Variation Call** column—Do all of the positive control replicates have the correct call?
 - **Silhouette Score** column—Are there any outliers within the replicate group? Do the values for the replicate group differ greatly from the confidence values for other replicate groups in the plate?

Note: If any of the controls are outliers, omit them from the HRM analysis, then reanalyze. See “Omit outliers from high resolution melt analysis” on page 17.

- c. For each replicate group, review:
- **Variation Call** column—Do all replicates have the same call?
 - **Silhouette Score** column—Are there any outliers within the replicate group? Do the values for the replicate group differ greatly from the confidence values for other replicate groups in the plate?
- d. Select the rows in the **Well Table** to view the corresponding fluorescence data in the **Difference Plot**.
- e. In the **Well Table**, click the **Variation Call** column header to sort the results according to the variation call. For each variation call, review the samples that are assigned that call.

Review results in the Melt Curve Plot

For more information about the melt curve plot, see “Melt Curve Plot overview” on page 22.

1. In the **High Resolution Melt** tab, in the upper-left corner of the plot pane, select **Melt Curve Plot** from the dropdown list.
2. In the upper-middle of the plot pane, select a target from the dropdown list.

3. In the upper-right corner of the plot pane, click  (**Settings**), then select an option from the:
 - a. **Color By** dropdown list: **Sample**, **Target**, **Call** or **Well**.
 - b. **Plot Type** dropdown list: **Derivative** or **Normalized**.

The plot is displayed.

4. Review and adjust the Pre and Post Melt regions.
 - a. In the **Derivative Melt Curve Plot**, set the Pre Melt region:
 1. Drag the blue Pre Melt End temperature line adjacent to the start of the melt transition region.
 2. Drag the blue Pre Melt Start temperature line approximately 0.2°C to 0.5°C from the Pre Melt End temperature line.

Note:

- Alternatively, to manually enter the Pre Melt End temperature and the Pre Melt Start temperature, click the temperature label.
 - The Pre Melt region should be within a flat area where there are no large spikes or slopes present in the fluorescence levels.
-

- b. Set the Post Melt region:

1. Drag the red Post Melt Start temperature line adjacent to the end of the melt transition region.
 2. Drag the red Post Melt End temperature line approximately 0.2°C to 0.5°C from the Post Melt Start temperature line.
-

Note:

- Alternatively, to manually enter the Post Melt Start temperature and the Post Melt End temperature, click the temperature label.
 - The Post Melt region should be within a flat area where there are no large spikes or slopes present in the fluorescent levels.
-

- c. Click **Analyze**.

The software reanalyzes the data using the new Pre and Post Melt regions.

Review results in the Aligned Melt Curve Plot

For more information about the aligned melt curve plot, see “Aligned Melt Curve Plot overview” on page 24.

1. In the **High Resolution Melt** tab, in the upper-left corner of the plot pane, select **Aligned Melt Curve Plot** from the dropdown list.
2. In the upper-middle of the plot pane, select a target from the dropdown list.

3. In the upper-right corner of the plot pane, click  (**Settings**), then select an option from the **Color By** dropdown list: **Call**, **Sample**, or **Well**.
The plot is displayed. The temperature slider displays the temperature at the position of the line in the plot.
4. *(HRM genotyping data only)* If using positive controls, confirm the calls for the positive controls:
 - a. From the **Well Table**, select the wells containing a positive control to highlight the corresponding melt curve in the **Aligned Melt Curve Plot**.
 - b. Confirm that the color of the line corresponds to the correct genotype.
 - c. Repeat substep 4a and substep 4b for the wells containing the other positive controls.
5. *(HRM mutation detection data only)* If using Wild type controls, confirm the calls for the positive controls:
 - a. From the **Well Table**, select the wells containing a positive control to highlight the corresponding melt curve in the **Aligned Melt Curve Plot**.
 - b. Confirm that the wild type controls cluster well and review the population for outliers.
 - c. Repeat substep 5a and substep 5b for the wells containing the other positive controls.
6. Screen the negative controls to ensure that samples failed to amplify:
 - a. From the **Well Table**, select the wells containing a negative control to highlight the corresponding melt curve in the **Aligned Melt Curve Plot**.
 - b. Confirm that the selected wells in the **Well Table** are negative controls, and not unknown samples.

Samples that grouped with the negative controls may:
 - Contain no DNA
 - Contain PCR inhibitors
 - Be homozygous for a sequence deletion
7. *(HRM genotyping data only)* Confirm the results of the samples that did not group tightly or are grouped with negative controls by retesting them.
8. If you select to run replicate reactions, carefully review your data set for curves that do not align tightly with the other samples in the group (outliers) to ensure the accuracy of the genotype calls. If outliers are present, confirm the results of the associated samples by retesting them.

9. Review the data.

- HRM genotyping data:
 - Variant groups (different colors)—How many different variant groups are displayed? Does this number correspond to the number of variants you were expecting? If you see more than you were expecting, you may have sample contamination or may need to modify the analysis settings.
 - Outliers—Are there any curves within a variant group that do not cluster tightly with the other samples in that group?
- HRM mutation detection data:
 - Possible mutations—Review the curves for samples with melt curves that are different from the wild type melt curves.
 - Unexpected peaks—Confirm that the Derivative Melt Curve shows no unexpected T_m peaks. If the sequence you amplified contains more than one variant or a more complex mutation, you may see more than one T_m peak. Unexpected peaks can indicate contamination, primer dimers, or non-specific amplification.
- HRM methylation data:
 - Methylated DNA standards—Do the melt curves for the methylated DNA standards cluster well? Are there any outliers?
 - Define methylation range for unknowns—Which methylated standard melt curves are above and below the melt curves for the unknowns? For example, if the melt curve for an unknown sample lies between the melt curves for the 5% and 10% methylated standards, the unknown sample contains between 5% and 10% methylated nucleotides.

10. (HRM mutation detection data only) Confirm the software calls.

- a. In the **Well Table**, click the **Well** column header to sort the results according to the well position.
- b. For each replicate group, review:
 - **Variant Call** column—Do all replicates have the same call?
 - **Silhouette Score** column—Are there any outliers within the replicate group? Do the values for the replicate group differ greatly from the confidence values for other replicate groups in the plate?
- c. Select the rows in the **Well Table** to view the corresponding fluorescence data in the **Aligned Melt Curve Plot**.
- d. In the **Well Table**, click the **Variant Call** column header to sort the results according to the variant call. Scan the results for samples that were not assigned the same call as the wild type control.

Omit outliers from high resolution melt analysis

Outlier wells have C_q values that differ significantly from the average for the associated replicate wells. To ensure C_q precision, consider omitting the outliers from analysis.

1. In the **High Resolution Melt** tab, select an option to omit wells from analysis.

Option	Description
Omit wells in the Plate Layout	Select outlier wells, then click ... (Actions) ▶ Omit Wells .
Omit wells in the Well Table	Select Omit in the row of the outlier well.

2. Click **Analyze** to reanalyze the data with any outliers removed.

(Optional) Review dye signal profile in the Multicomponent Plot

For more information about the **Multicomponent Plot**, see [?](#) **Help** ▶ **Help Contents**.

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, in the plot pane, select **Multicomponent Plot** from the dropdown list.
2. Review the signal profiles for the passive reference dye, reporter dye, and negative control wells.
3. Review the plot to ensure that there are no irregularities in the dye signals.

(Optional) Review signal profile in the Raw Data Plot

For detailed instructions about reviewing results in the **Raw Data Plot**, see [?](#) **Help** ▶ **Help Contents**.

If no data are displayed in the **Quality Check** tab, or if reanalysis is required, click **Analyze**.

1. In the **Quality Check** tab, in the plot pane, select **Raw Data Plot** from the dropdown list.
2. Click-drag the **Cycle Number** slider through all of the cycles, then confirm that each filter displays the characteristic signal increase.

Edit High Resolution Melt Analysis Setting

Open the High Resolution Melt Analysis Module.

1. Click **Actions** ▶ **High Resolution Melt Analysis Setting**.
2. In the **Target Call** tab, edit the analysis settings if needed.

Option	Description
Edit default analysis settings	<ul style="list-style-type: none"> • In the table, in the Default Setting row, deselect Auto Variants to edit the Variants setting. • In the table, in the Default Setting row, deselect Auto Pre/Post to edit the Pre Melt Start, Pre Melt End, Post Melt Start, and Post Melt End settings. • To apply the default analysis settings to a target, in the target row, select Default.
Edit analysis settings for an individual target	In the table, in the target row, click in a cell to edit the setting.

3. In the **Control** tab, in the table:
 - Click **+** (**Add**) to add the settings.
 - Click in a cell to edit the following settings if needed.
 - **Genotype**
 - **Color**
 - **Target Name**
 - **Samples**
 - Click **×** (**Remove**) to delete the settings.
4. In the **Manual Call** tab, in the table:
 - Click **+** (**Add**) to add a manual call.
 - Click in a cell to edit the following settings if needed.
 - **Genotype**
 - **Color**
 - Click **×** (**Remove**) to delete the settings.

The new manual call is available to select when setting up a manual call in the well table.

5. Click **Apply**.

The data are reanalyzed using the updated analysis settings.

Export the results

1. In the table pane, click ⋮ **(Actions)** ▶ **Export**.
2. *(Optional)* In the **Export CSV** dialog box, edit the file name in the **File Name** field.
The **File Name** field is populated with a default file name.
3. Click **Browse** to select a location to save the file.
Selecting the location where the file is saved is a controlled function. If the **Browse** button in the **Export CSV** dialog box is inactive, the security has been enabled in the software. The user role does not have the permission to select the location where the file is saved. The user role must have the permission of **Edit Export Destination**.
4. Click **Export**.



About high resolution melt analysis

Overview of high resolution melt analysis

High resolution melt (HRM) analysis is used to identify genetic variation in nucleic acid sequences.

HRM analysis is based on PCR melt (dissociation) curve techniques and is enabled by the recent availability of improved double-stranded DNA (dsDNA)-binding dyes along with next-generation real-time PCR instrumentation and analysis software. HRM analysis can discriminate DNA sequences based on their composition, length, GC content, or strand complementarity.

For HRM analysis, the software can perform the following:

- Mutation scanning experiments—Screen DNA samples for new single-base changes, insertions/deletions, or other unknown mutations. The mutation scanning experiment product can be used for subsequent sequencing reactions.
- Methylation studies—Determine the percentage of methylated DNA in unknown samples.
- Genotyping experiments—Determine the genotype of a DNA sample. For all types of experiments, the software compares the melt curves of unknown samples against the melt curves of positive controls to identify groups of variants.

Sample types for high resolution melt analysis

High resolution melt analysis includes the following sample types.

Sample type (Type column in Samples table)	Sample description	Automatic target task assignment ^[1] (Task column in Targets table)
Unknown	A sample in which the genotype, variant content, or percentage methylation is unknown.	Unknown
Positive Control	<p>A sample that contains a known genotype, target sequence, or standard. The type of samples used as positive controls depends on the type of experiment:</p> <ul style="list-style-type: none"> • Mutation scanning experiments—One or more samples with the wild type sequence are used as the control. For unknowns, the call is either “wild type” (if it matches the control) or “variant X”. • Methylation studies—Methylated DNA standards that contain from 0% to 100% methylated DNA are used as the positive controls. The software identifies the percentage methylation of the variants based on their comparison to the standards. • Genotyping experiments—Three samples are used as controls: one homozygous for Allele 1, one homozygous for Allele 2, and one heterozygous for both alleles (Allele 1 and Allele 2). The software identifies the genotypes of the unknown variants. 	Positive Control
Negative Control	Wells that contain water or buffer instead of sample template. No amplification of the target should occur in negative control wells.	Negative Control

^[1] The software automatically assigns a task to the target in a well based on the sample type in that well.

Difference Plot overview

The **Difference Plot** displays the melt curve data for a single experiment in a graph that contrasts reference-normalized fluorescence versus temperature. To generate the differenced melt curve data, the software subtracts the fluorescence of the well specified in ... **(Actions) ▶ Set As Reference** from each well in the experiment.

The **Difference Plot** allows you to more easily see small differences between curves and identify outliers among replicate populations. The plot can be used to confirm the uniform performance of control populations and technical replicates. For example, wells that contain positive controls should exhibit similar differenced melt curves.

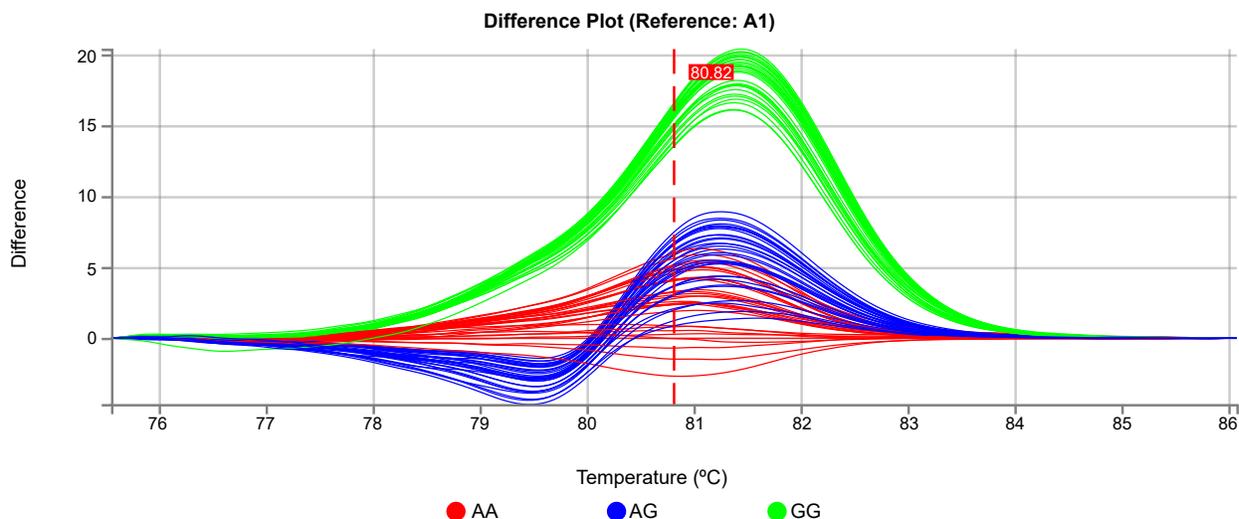


Figure 1 Example Difference Plot

Melt Curve Plot overview

The **Derivative Melt Curve Plot** displays the melt curve data for a single experiment in a graph that contrasts the negative derivative ($-Rn'$) of normalized fluorescence of each well versus temperature. The **Derivative Melt Curve Plot** allows you to visualize the rate of change in normalized fluorescence throughout the course of the temperature ramp. Each peak present within the plot corresponds to a maximum rate of change in fluorescence for a particular well, from which the software estimates the T_m for the related sample.

The **Derivative Melt Curve Plot** contains vertical bars that allow you to review and adjust the Pre and Post Melt regions for optimizing separation and variant calls. For more information on viewing and adjusting the Pre and Post Melt regions, see “Edit High Resolution Melt Analysis Setting” on page 18.

For all experiments, note that:

- Unexpected peaks may indicate possible contamination, primer dimers, or non-specific amplification.
- The data might appear noisy because more data is collected during a high-resolution melt curve than during a standard melt curve.

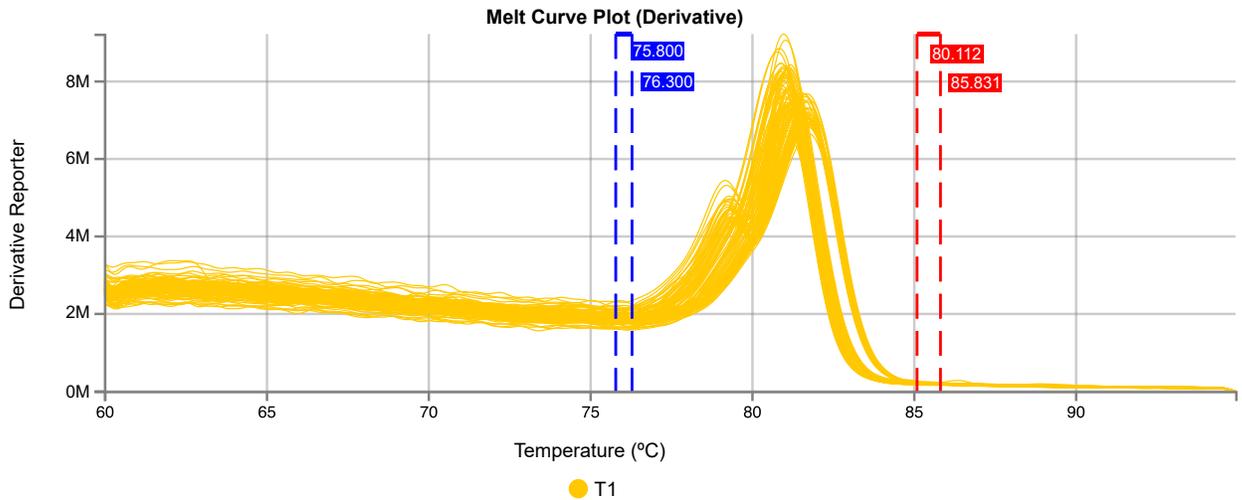


Figure 2 Example Derivative Melt Curve Plot

The **Raw Melt Curve Plot** displays the melt curve data for a single experiment in a graph that contrasts normalized fluorescence (Rn) versus temperature. The **Raw Melt Curve Plot** allows you to visualize the decrease in normalized fluorescence for each well throughout the course of the temperature ramp. The normalized reporter (Rn), displayed on the y-axis, is calculated as the fluorescence signal from the reporter dye normalized to the fluorescence signal of the passive reference.

The **Raw Melt Curve Plot** contains vertical bars that allow you to review and adjust the Pre and Post Melt regions for optimizing separation and variant calls. For more information on viewing and adjusting the Pre and Post Melt regions, see “Edit High Resolution Melt Analysis Setting” on page 18.

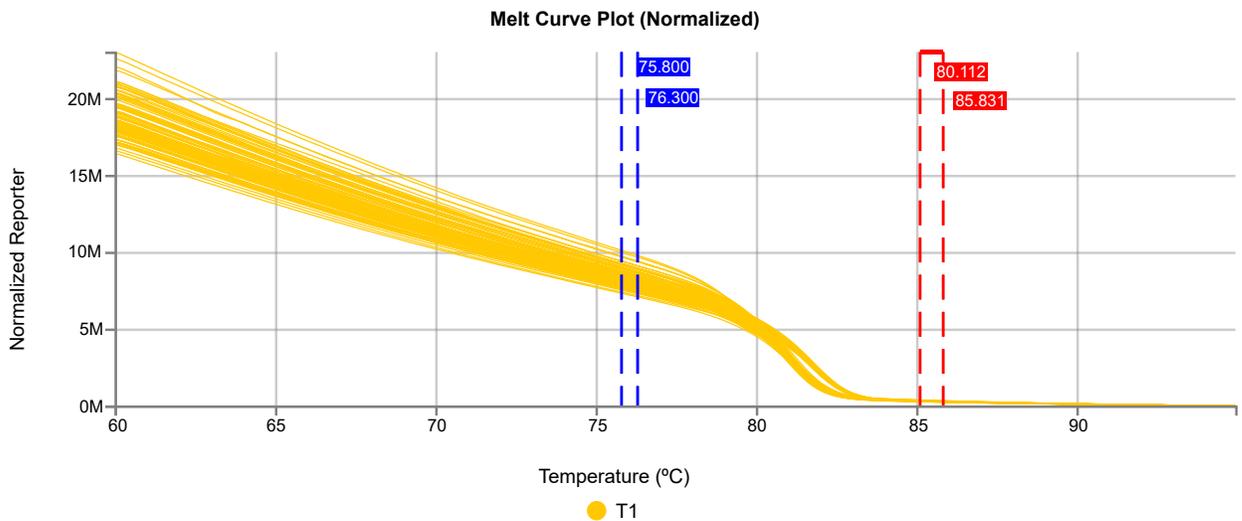


Figure 3 Example Raw Melt Curve Plot

Table 1 Regions to review in the Derivative Melt Curve Plot and the Raw Melt Curve Plot

Regions	Description
Pre Melt region	The pair of lines to the left of the peak indicate the Pre Melt Start and End temperatures when every amplicon is double-stranded. Fluorescence data from the Pre Melt region corresponds to 100% fluorescence in the Aligned Melt Curve Plot .
Active Melt region	The data peak indicates the Active Melt region of the plot. Data from the Active Melt region are used to plot the Aligned Melt Curve Plot .
Post Melt region	The set of lines to the right of the peak indicate the Post Melt Start and End temperatures when every amplicon is single-stranded. Fluorescence data from the Post Melt region correspond to 0% fluorescence in the Aligned Melt Curve Plot .

Aligned Melt Curve Plot overview

The **Aligned Melt Curve Plot** displays the melt curve data for a single experiment in a graph that contrasts percent normalized fluorescence (0-100%) versus temperature. The software uses the data that are captured during the Pre and Post Melt regions to define the limits of the fluorescence range, where signal that is captured during the Post Melt region defines the 0% limit and the signal that is captured during the Pre Melt region defines the 100% limit.

In the **Aligned Melt Curve Plot**, the shape of the melt profile of a PCR product is influenced by its guanine-cytosine content, length, sequence, and heterozygosity (if performing genotyping). Consequently, the characteristic slopes of melt curves can indicate differences in the nucleic acid content of the targeted amplicon.

Application	Note
Genotyping	<ul style="list-style-type: none"> Heterozygous samples have a characteristically different curve shape when compared to the homozygous samples. The shape of the melt curve is an indicator of heteroduplex formation. Homozygous samples are distinguishable from each other based on the difference in T_m values.
Mutation detection	The curves of samples that vary from the wild type samples can contain mutations.
Methylation study	The positions of the melt curves for the unknowns relative to those for the methylated standards can be used to estimate the percentage methylation of the samples. For example, if the melt curve for an unknown sample lies between the melt curves for the 5% and 10% methylated standards, the unknown sample contains between 5% and 10% methylated nucleotides.

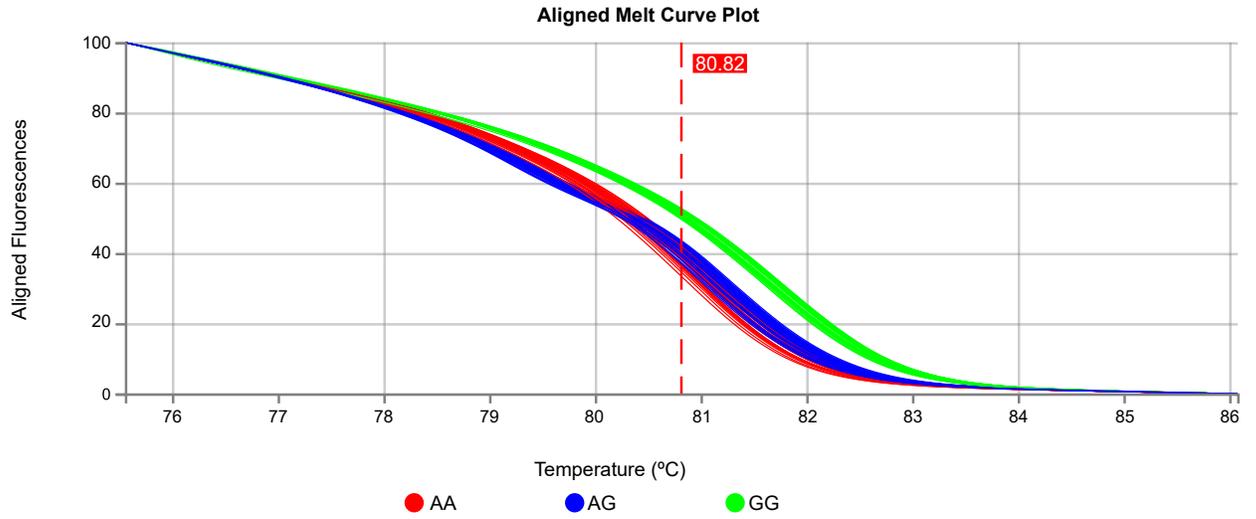


Figure 4 Example Aligned Melt Curve Plot



Documentation and support

Related documentation

Document	Publication number
<i>QuantStudio™ Design and Analysis Software 2 User Guide</i>	MAN0018200
<i>QuantStudio™ Design and Analysis v2 User Guide (Thermo Fisher™ Connect Platform)</i>	MAN0018202

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