High Resolution Melt Analysis Module

USER GUIDE

for use with Diomni™ Design and Analysis (RUO) Software 3

Publication Number MAN0030165

Revision A

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0030165 A (English)

Revision	Date	Description
A	7 February 2025	New document for the High Resolution Melt Analysis Module v1.5 (Diomni™ Design and Analysis (RUO) Software 3).

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 Diomni™ Design and Analysis (RUO) Software 3 is used to identify genetic variation in nucleic acid sequences.

The module is not compatible with the on-premise configuration of the software.

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

2 Workflow: High resolution melt analysis

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3 Set up a plate file

For detailed instructions about setting up a plate file, see the primary user guides for the software. See “Related documentation” on page 28.

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 the primary user guides for the software.

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.

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1. Click **Run templates**.
   The **Plate Gallery** is displayed.

2. Click the tab at the top of the **Plate Gallery** that is associated with the type of plate file to be used to set up a new plate file.

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

3. 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 setup or post-run analysis (see "Select the High Resolution Melt Analysis Module" on page 13).

4. Open the plate file.
   - Hover over the plate file, then click ··· **(More Options)**.
     The ··· **(More Options)** menu for the plate file displays all of the options to open the plate file.
   - Click the plate file.

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

Confirm or edit the run method

For most analysis, the default run method is appropriate. The following options are compatible:

- 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.

- Click ··· **(More Options)** ▸ **Filter Settings** to confirm or edit filter settings.

Confirm or edit the plate setup

For detailed instructions about plate setup, see the primary user guides for the software.

Add samples and assign to wells

For detailed instructions about plate setup, see the primary user guides for the software.

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
     To assign a sample from the **Samples** table to the well, select a well in the plate layout, then select the checkbox associated with the sample in the **Samples** table.
   - Manually add samples to the **Enter Sample** fields in the wells of the plate layout
     The sample is added to the **Samples** table.
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. • Unknown • Positive Control • Negative Control

[1] For more information, see "Sample types for high resolution melt analysis" on page 23.

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

Add targets and assign to wells

For detailed instructions about plate setup, see the primary user guides for the software.

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. • Unknown • Positive Control • Negative Control

[1] For more information, see "Sample types for high resolution melt analysis" on page 23.

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

Edit reagent information

1. In the **Plate Setup** tab, in the **Target** table pane, click **Reagents**.
2. In the **Reagents** table, perform one of the following actions.
   - Click + **(Add)**.
   - Click ··· **(More Options)** ▸ **Export Reagents** to export reagents.
   - Click ··· **(More Options)** ▸ **Import Reagents** to import reagents.
   - Click ··· **(More Options)** ▸ **Scan Reagents** to scan reagents.
3. If you are manually adding reagents or editing reagents, enter the following information in the table.
   - Name
   - Type
   - Barcode
   - 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. If you are scanning the reagent barcode, in the **Scan Reagent** dialog box, select or deselect the **Enable automatic parsing** checkbox.
5. If you are scanning the reagent barcode, when the **Scan Reagent** dialog box is displayed, use a barcode scanner to scan the reagent label.

   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.

   The fields in the **Scan Reagent** dialog box are populated.

6. In the **Scan Reagent** dialog box, click **Add**.
7. (*Optional*) Click ✕ **(Remove)** in the row of a reagent to delete it from the table.

Select a 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.

Review and save the plate file

In Thermo Fisher™ Connect Platform, instruments are connected by the InstrumentConnect application.

The QuantStudio™ 6 Pro Real-Time PCR Instrument and the QuantStudio™ 7 Pro Real-Time PCR Instrument can connect to the software.

The template file must be transferred to instruments other than the QuantStudio™ 6 Pro Real-Time PCR Instrument and the QuantStudio™ 7 Pro Real-Time PCR Instrument. See the user documentation for your instrument to transfer a template file to start a run.

1. In the **Run Summary** tab, review the run method selections, then edit if needed.
2. Review the plate setup, then edit if needed.
3. (*Optional*) Click the barcode field, then scan the plate barcode.
4. (*Optional*) Select **Add to My Plates**.
   This option allows you to create new plate files using the current plate file as a template.
5. Select an instrument from the list.
   If the instrument does not appear on the list, click **System** ▸ **Instruments** to add a new instrument.
6. Save the plate file.

Start the run on an instrument. For more information about starting a run, see the documentation for the instrument.

4 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 the primary user guides for the software.

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 23.

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 ··· **(More Options)** ▸ **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 20).
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 ··· **(More Options)** ▸ **Set as reference**).

      Note: ··· **(More Options)** ▸ **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:
   - **Variant 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 19.

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:
      - **Variant 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 19.

   c. 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?

   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 **Variant Call** column header to sort the results according to the variant call. For each variant 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 24.

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 26.

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 confirm that they did not 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 run replicate reactions, carefully review your data set for curves that do not align tightly with the other samples in the group (outliers).
   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 Tm peaks. If the sequence you amplified contains more than one variant or a more complex mutation, you may see more than one Tm 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 support 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 ··· **(More Options)** ▸ **Omit Wells**.
Omit wells in the **Well Table**	Select **Omit** in the row of the outlier well.
Omit wells in the plot	In the plot, click and click and drag around the data to omit. The selected data are displayed in the **Well Table** and the **Plate Layout**. Omit the wells in the **Well Table** or the **Plate Layout**.

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 the primary user guides for the software.

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 reporter dye and negative control wells.
3. Review the plot to confirm 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 the primary user guides for the software.

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	• 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

For more information about setting up the export location, see the primary user guides for the software.

In the Thermo Fisher™ Connect Platform, the results are exported to the downloads folder of the computer. The file name and location cannot be selected.

1. In the table pane, click ··· **(More Options)** ▸ **Export**.
   In the Thermo Fisher™ Connect Platform, the file is downloaded.
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.
4. Click **Export**.

5 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	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: • 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 ··· **(More Options)** ▸ **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.

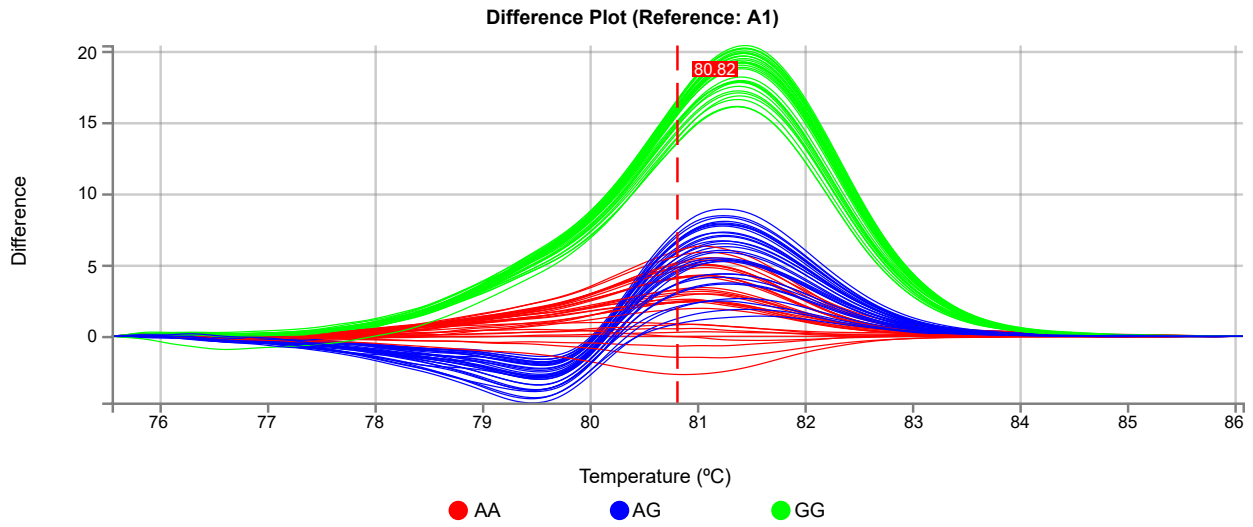

Figure 1 Example Difference Plot

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 Tm 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 20.

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.

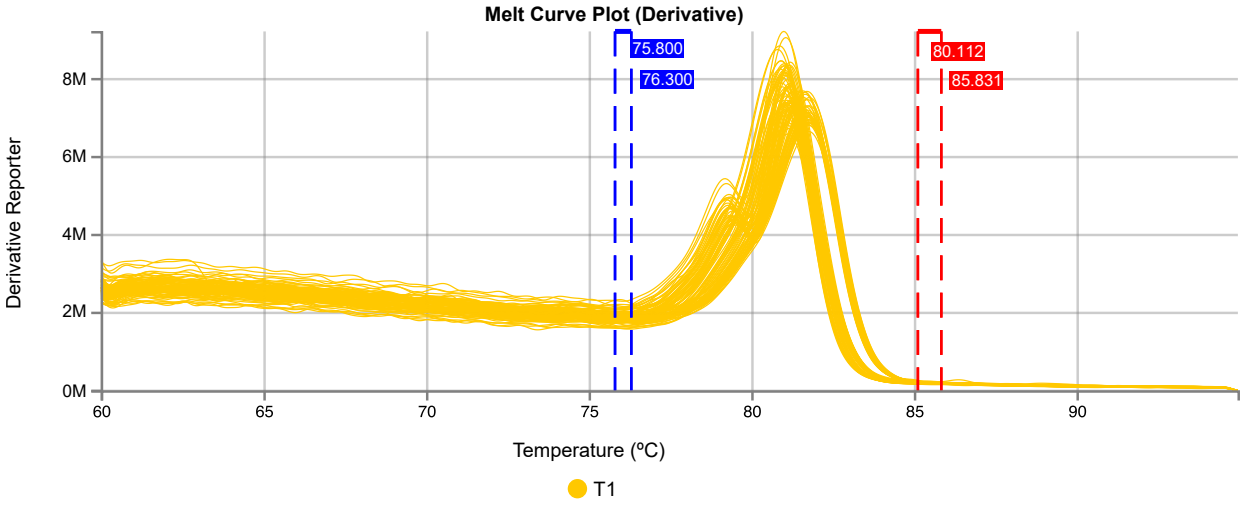

Figure 2 Example Derivative Melt Curve Plot

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 20.

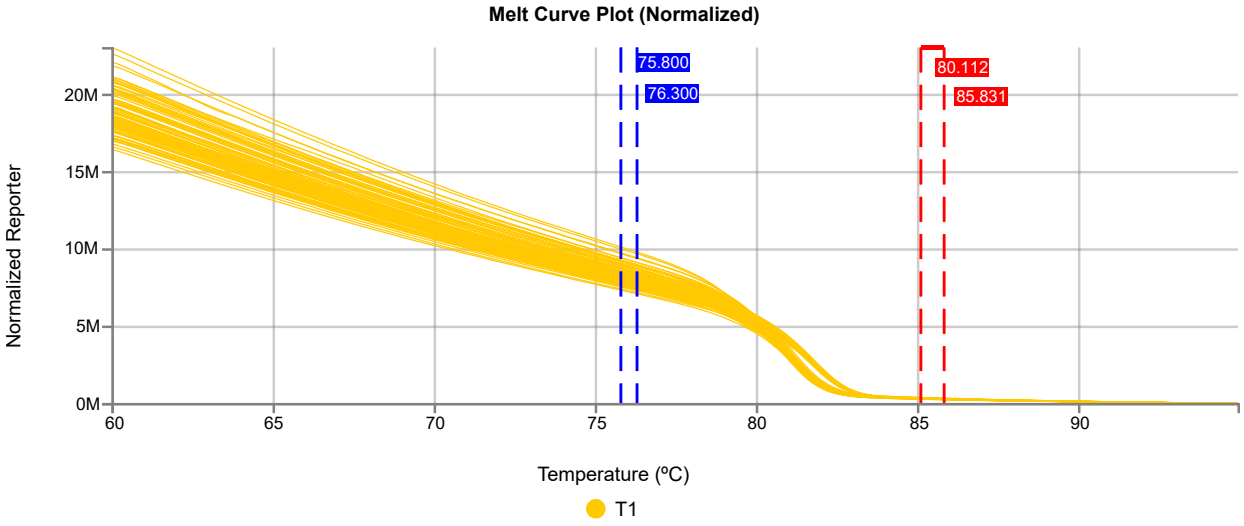

Figure 3 Example Raw Melt Curve Plot

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	• 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 Tm 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.

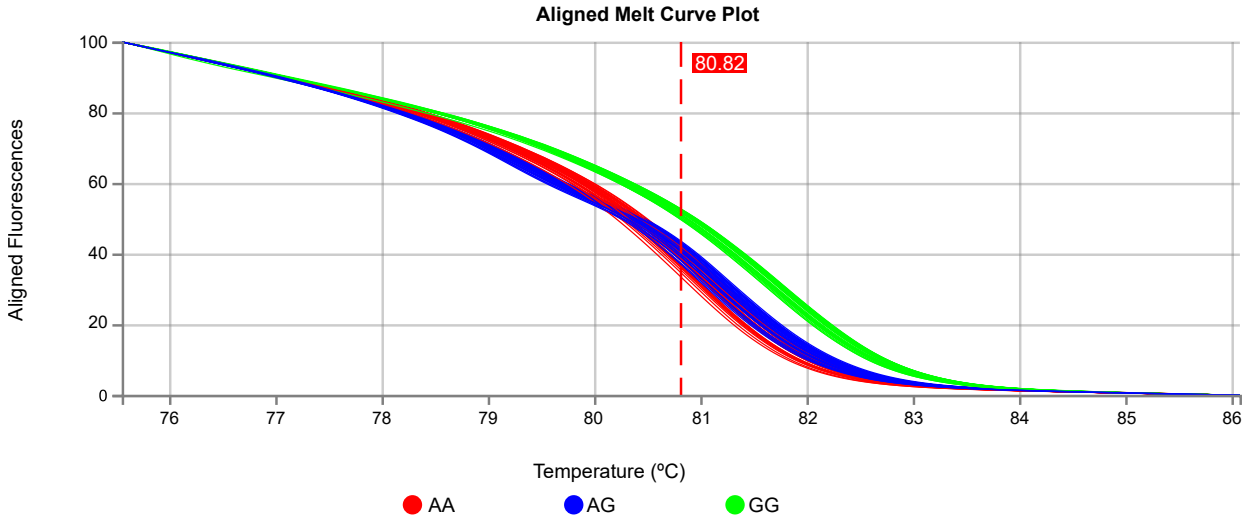

Figure 4 Example Aligned Melt Curve Plot

Documentation and support

Related documentation

Document	Publication number
Diomni™ Design and Analysis (RUO) Software 3 (Desktop) User Guide	MAN0030162
Diomni™ Design and Analysis (RUO) 3 User Guide (Thermo Fisher™ Connect Platform)	MAN0030163

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