February 2023

Q-Rex Melt Curve Analysis Plug-in User Manual

For use with the Q-Rex Software v2.0 for Rotor-Gene® Q and QIAquant® for determination of genotypes using Melt Curve Analysis

Sample to Insight

Contents

Introduction

1 Introduction

The plug-ins for the Q-Rex Software grant additional analysis capabilities. The plug-ins for the Q-Rex Software cannot be used without the main software component. Familiarize yourself with the Q-Rex Software before installing and using plug-ins.

The Q-Rex Melt Curve Analysis Plug-in detects genetic variants such as single nucleotide polymorphisms (SNPs) on the basis of melting temperature (*Tm*) generated by probe-based melting. There are several possibilities of designing an experiment, including use of molecular beacons or TaqMan Minor Groove Binder (MGB). The probes are sequence specific for each variation, which enables a clearer differentiation at the end of the analysis. MGB as well as locked nucleic acids (LNA) increase the melting temperature and stabilize probe-target hybrids, allowing use of shorter probes and enabling a better differentiation. To monitor the thermal dissociation from their targets, the probes are labeled with dyes. Fluorescence resonance energy transfer (FRET) hybridization probes are a pair of fluorescent probes that are differently labeled such that the emission spectrum of one overlaps with the excitation spectrum of the other. As the fluorescent probes come closer to each other, the energy is transferred from one dye molecule to the other. On the other hand, the so-called SimpleProbe experiments require only one hybridization probe labeled with only one dye. This probe emits more fluorescence when bound to the target DNA.

The Q-Rex Melt Curve Analysis Plug-in displays the recorded fluorescence intensity values and plots them with respect to the sample temperature. The melt peak plot shows the negative first derivative of the melting curve to highlight the characteristic melting temperature (*Tm*) of the samples as a peak. The peaks are assigned to bins which are then applied to identify the genotype of each sample.

1.1 About this user manual

This user manual provides information about the functions and features of the Q-Rex Melt Curve Analysis Plug-in. You will find general information about the functions and features of the Q-Rex Software in the Q-Rex Software User Manual.

Installing the Q-Rex Melt Curve Analysis Plug-in affects only the analysis aspects of the Q-Rex Software. This user manual describes changes to settings and functionalities necessary to perform the analyses enabled by the Q-Rex Melt Curve Analysis Plug-in. All other aspects of the Q-Rex Software remain unchanged, and therefore, instructions in the Q-Rex Software User Manual remain valid. Make sure to read the Q-Rex Software User Manual and pay particular attention to the listed limitations and warnings before working with the software.

Please refer to the Rotor-Gene Q User Manual or QIAquant User Manual for complete information about the proper care, maintenance, and use of the Rotor-Gene Q or QIAquant instrument.

This user manual provides information about the Q-Rex Melt Curve Analysis Plug-in in the following sections:

- Introduction
- Working with Melt Curve experiments
- Set up an experiment
- Run an experiment

- Analyze an experiment
- Report and export results
- Troubleshooting
- Glossary
- Appendices

1.2 General information

1.2.1 Technical assistance

At QIAGEN®, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the Rotor-Gene Q, QIAquant, Q-Rex Software, the Q-Rex Melt Curve Analysis Plug-in, or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**.

1.2.2 Policy statement

It is the policy of QIAGEN to improve products as new techniques and components become available. QIAGEN reserves the right to change specifications at any time.

In an effort to produce useful and appropriate documentation, we appreciate your comments on this user manual. Please contact QIAGEN Technical Services.

1.2.3 Version management

This document is the Q-Rex Melt Curve Analysis Plug-in User Manual, which provides information about the Q-Rex Melt Curve Analysis Plug-in, version 2.0.0.29.

1.2.4 Intended use

The Q-Rex Melt Curve Analysis Plug-in is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

The Q-Rex Software and the Rotor-Gene Q and QIAquant instruments are intended for use by professional users, such as technicians and physicians trained in molecular biological techniques and the operation of the Rotor-Gene Q or QIAquant instrument.

1.3 Getting help

Please refer to "Getting help" in the Q-Rex Software User Manual for a description of the available help function.

Working with Melt Curve experiments

2 Working with Melt Curve experiments

2.1 Set up an experiment

For all general information on how to set up a new experiment, refer to "Set up a new experiment" or "Set up an experiment via wizard" in the Q-Rex Software User Manual.

2.2 Run an experiment

To run an experiment, see "Run an experiment" in the Q-Rex Software User Manual.

2.3 Analyze an experiment

The following sections describe using the Q-Rex Melt Curve Analysis Plug-in to determine genotypes based on Melt results:

Add an analysis

View plots

Define analysis parameters

Define genotypes

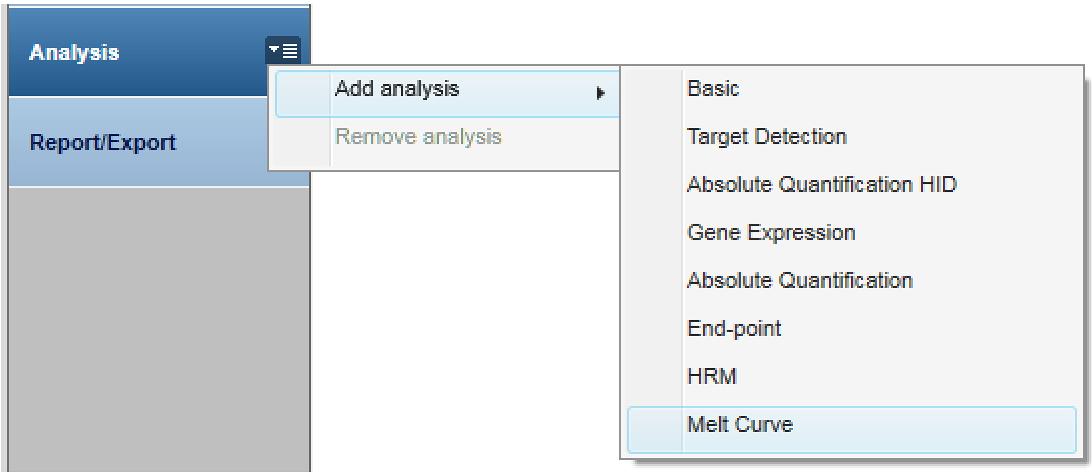
View results

For a description of general use concepts of the Q-Rex Software, see "Analyze an experiment" in the Q-Rex Software User Manual.

2.3.1 Add an analysis

For general information on how to add an analysis in the Q-Rex Software, see "Add an analysis" in the Q-Rex Software User Manual.

If the Q-Rex Melt Curve Analysis Plug-in was installed correctly, a menu item for the plug-in will appear in the list of available analyses:



2.3.2 View plots

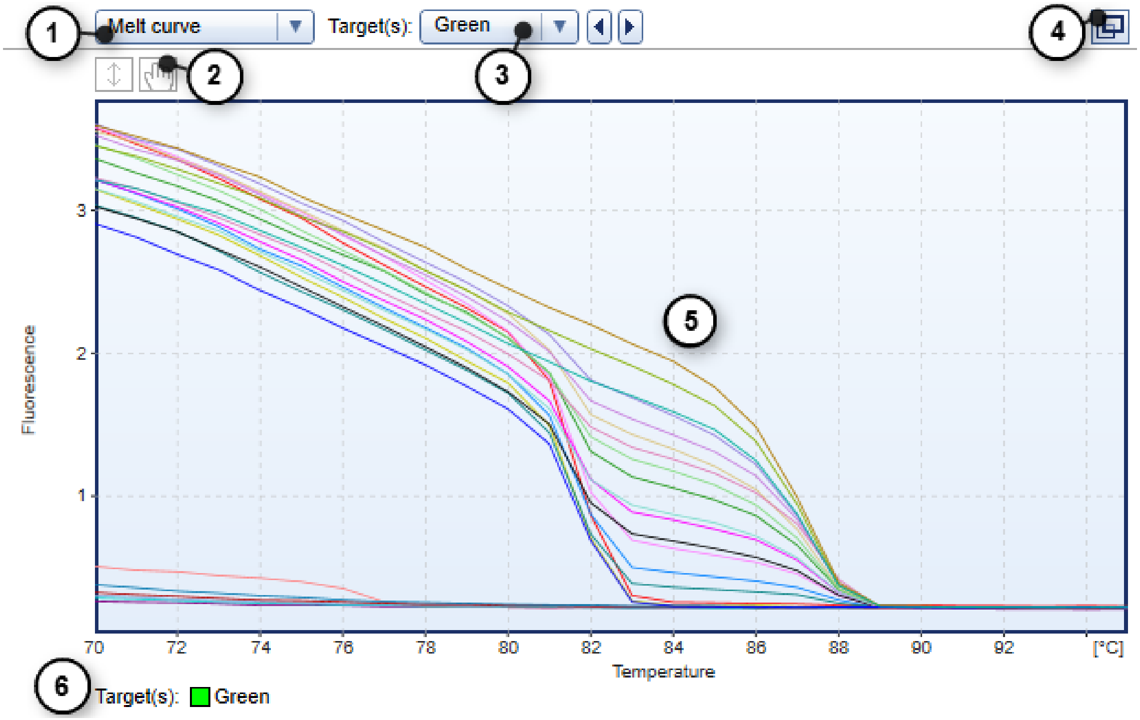
The following sections describe using the Q-Rex Melt Curve Analysis Plug-in to view Melt data.

There are two different types of plots available:

Melt curve — This shows the fluorescence value plotted against the temperature.

Melt peak — This displays the change in fluorescence value plotted against the temperature.

2.3.2.1 View Melt curve plot



A **Melt curve plot** features the following elements:

- A **Plot Selector** (1)
- A toolbar to modify the display of the plot (**2**)
- A **Target Selector** (**3**)
- A button to maximize and minimize the plot window (**4**)
- The plot area (**5**). This area has a darker background color when the plot is active.
- A legend (**6**)

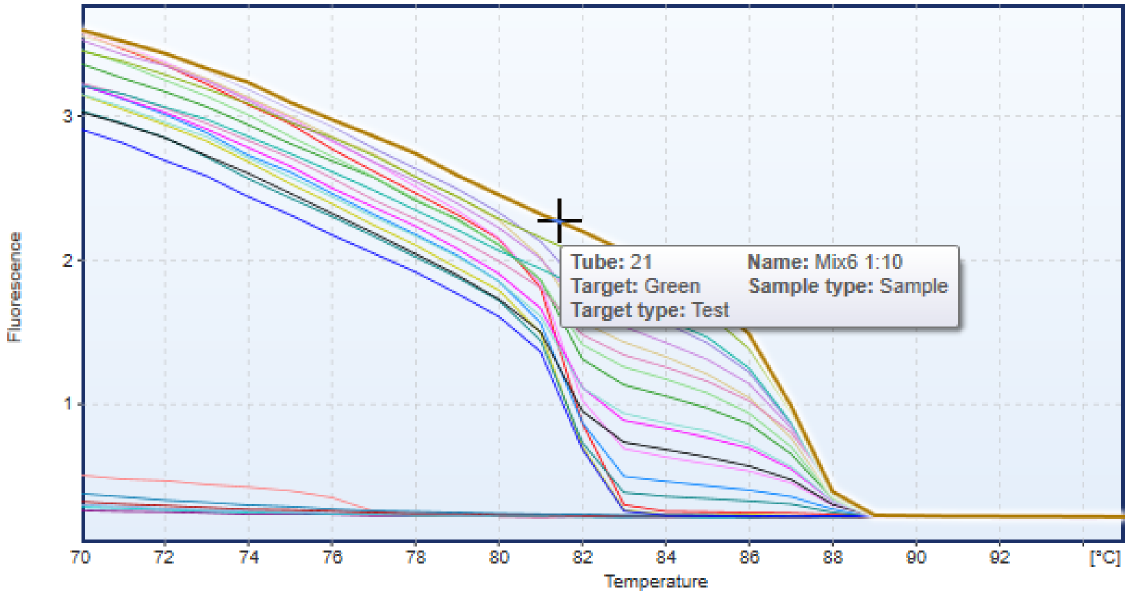
To view **Melt curve** data:

1. Select the plot type you want displayed in the active plot. Click the drop-down menu of the **Plot Selector** to display the available plot types and select one.

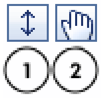
Note: The active plot is highlighted in light blue.

2. Select a target to display from the drop-down menu in the **Target Selector**.
   You can browse through a list of targets using the **Forward** and **Back** buttons.

3. To view details about a particular curve in the active **Melt curve plot**, roll the mouse pointer over the curve.
   The curve is highlighted and details appear in a tooltip.


Tube: 21
Target: Green
Target type: Test
Name: Mix6 1:10
Sample type: Sample

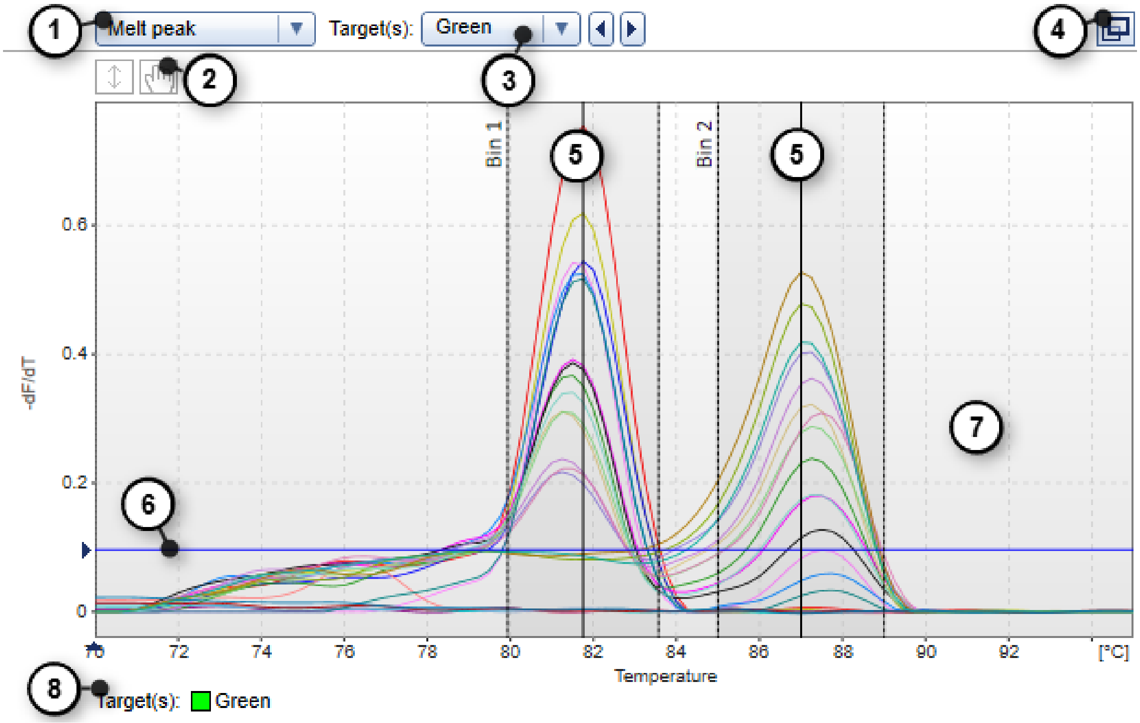
4. The toolbar at the top of the plot includes icons that allow you to alter the way in which the data in the **Melt curve plot** are displayed.



Auto-scale (1)	Automatically scales the plot to use space optimally. Both axes are restricted to available minimum and maximum values.
Shift zoomed region (2)	Clicking this icon allows you to shift the visible area of the plot (to pan) when you are zoomed in on a specific region. Click and drag the plot in the desired

direction.

2.3.2.2 View Melt peak plot



A **Melt peak plot** features following elements:

- A **Plot Selector** (1)
- A toolbar to modify the display of the plot (**2**)
- A **Target Selector** (3)
- A button to maximize and minimize the plot window (**4**)
- A bin representation (**5**). Bins can be moved and scaled right inside the plot.
- A melt peak threshold line (**6**). Threshold line can be moved inside the plot.
- The plot area (**7**). This area has a darker background color when the plot is active.
- A legend (**8**)

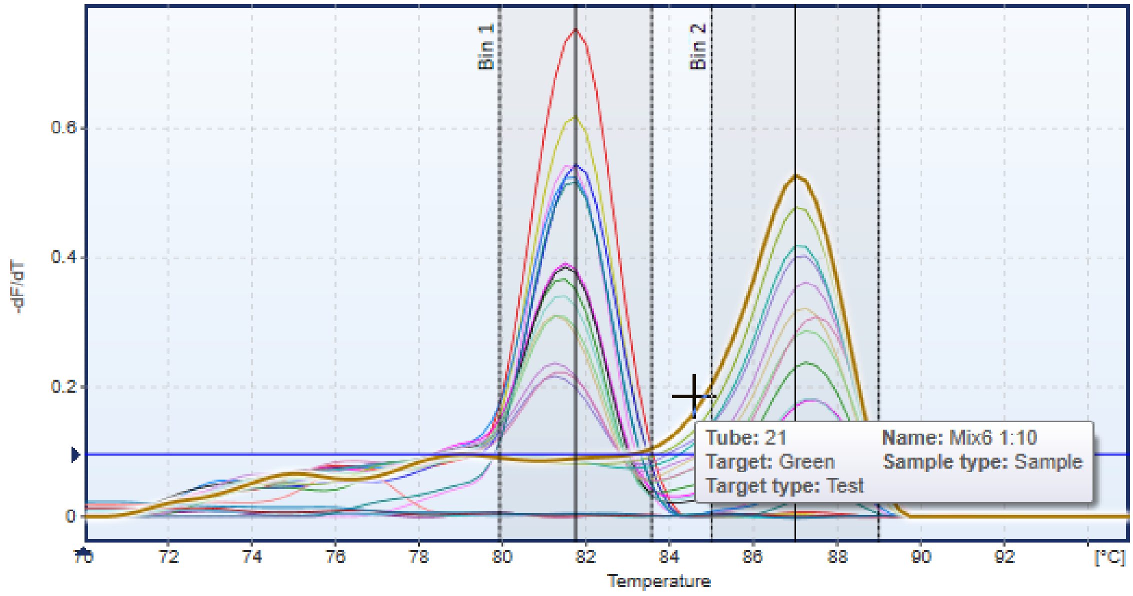
To view **Melt peak** data:

1. Select the plot type you want displayed in the active plot. Click the drop-down menu of the **Plot Selector** to display the available plot types and select one.

Note: The **active plot** is highlighted in light blue.

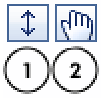
2. Select a target to display from the drop-down menu in the **Target Selector**.
   You can browse through a list of targets using the **Forward** and **Back** buttons.

3. To view details about a particular curve in the active **Melt peak plot**, roll the mouse pointer over the curve.
   The curve is highlighted and details appear in a tooltip.



Tube: 21
Name: Mix6 1:10
Target: Green
Sample type: Sample
Target type: Test

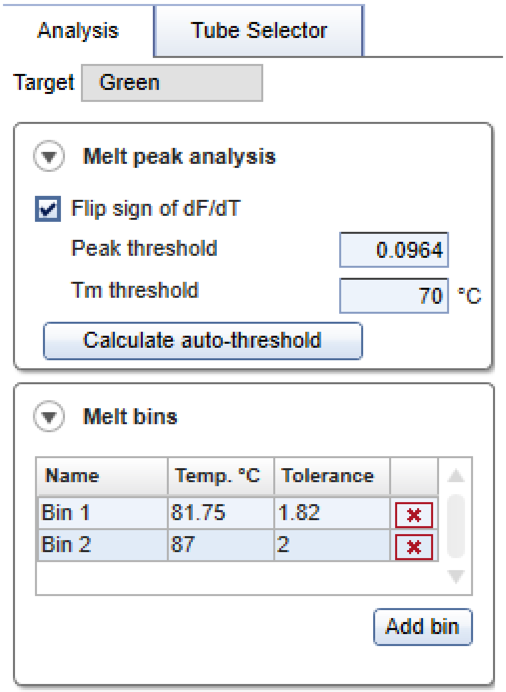
4. The toolbar at the top of the plot includes icons that allow you to alter the way in which the data in the **Melt peak plot** are displayed.



Auto-scale (1)	Automatically scales the plot to use space optimally. Both axes are restricted to available minimum and maximum values.
Shift zoomed region (2)	Clicking this icon allows you to shift the visible area of the plot (to pan) when you are zoomed in on a specific region. Click and drag the plot in the desired direction.

2.3.3 Define analysis parameters

To define analysis parameters for each target, open the **Analysis** tab of the drawer in the **Experiment** environment.



Target: Green

☑ Flip sign of dF/dT

Peak threshold: 0.0964

Tm threshold: 70 °C

Name	Temp. °C	Tolerance	
Bin 1	81.75	1.82	☒
Bin 2	87	2	☒

The active target for which the analysis parameters are defined is displayed at the top of the tab. This is the target selected in the **active plot** window. If multiple targets are selected in the **active plot** window, this field remains empty and analysis parameters cannot be edited.

Melt peak analysis

To define melt peak threshold, either enter the value of the threshold manually or use **Calculate auto-threshold** button. Defined melt threshold can be altered by dragging with the mouse over the plot.

Temperature threshold (shown in software as ***Tm* threshold**) can be used to remove the starting area of the **Melt peak plot** data, where most of the noise appears.

Whenever new **Melt Curve** analysis is added to the experiment (and the run has finished), software will automatically calculate and present a melt peak threshold.

Melt bins

This section outlines how to define melt bins for the selected target. Bins can be added with the **Add bin** button and can be removed using the delete button in the last column of the bins table.

Because bins are required for melt curve analysis, a lack of bins for any target results in an invalid analysis.

There is a limit to the number of bins that can be created and bins cannot overlap within one target. Any overlap within one target will be highlighted in yellow, suggesting some correction.

The names of bins can be changed and should be unique within one target.

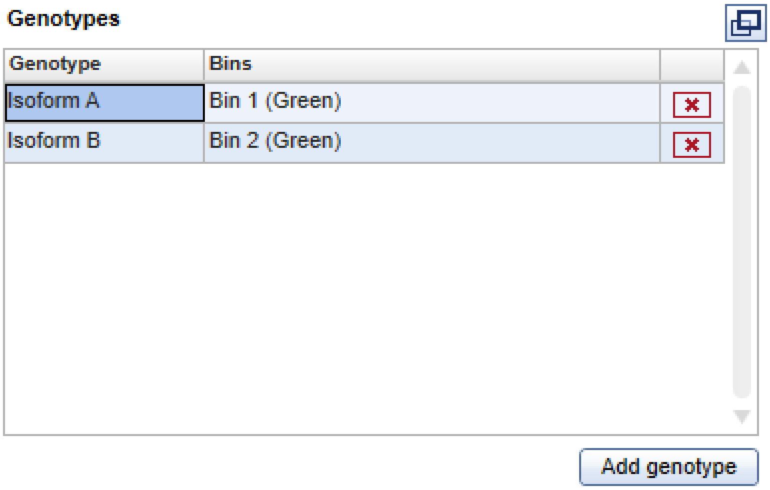
Melt bins can also be altered in the **Melt peak plot** – they can be moved and scaled by dragging with the mouse over the plot area. However, they cannot be added or removed within the plot.

Note: If parameters must be defined, their entry fields are highlighted in yellow. Unless these required parameters are defined, the corresponding input fields appear as invalid and results cannot be displayed. If an invalid input field is hidden, the surrounding parameter group, the **Analysis** tab or even the drawer itself are shown as invalid.

2.3.4 Define genotypes

Genotypes table

In the lower part of the **Melt curve** analysis, genotypes can be defined. Upon creation of a Melt curve analysis, three default genotypes (Homozygous, Heterozygous and Wild type) are created automatically. The names of the genotypes can be changed; new genotypes can be added and existing ones can be removed.



Genotype	Bins	
Isoform A	Bin 1 (Green)	☒
Isoform B	Bin 2 (Green)	☒

Adding new genotypes

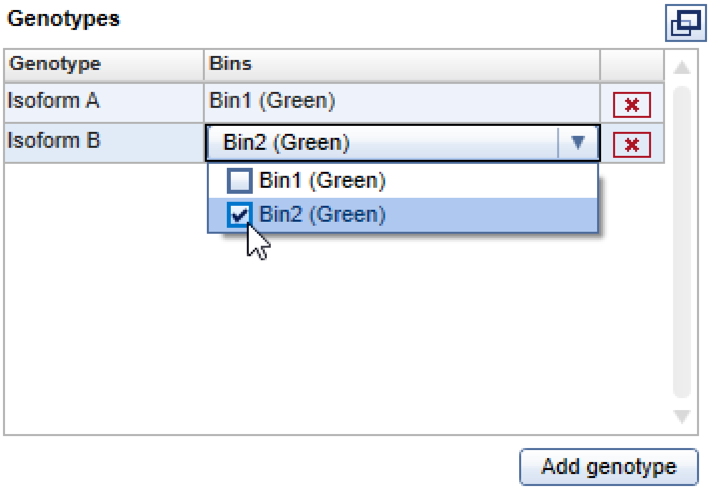
To add a new genotype, click on the **Add genotype** button below the table. There is a maximum number of genotypes that can be specified.

Editing genotype names

You can edit the name of any genotype by clicking on the cell showing the current name under the **Genotype** column.

Bin assignment to genotypes

To perform the Melt curve analysis, you are required to assign one or more bins to each genotype defined, but no other genotype can use the same combination of bins. You can select bins in the combo-box from a list of available bins by clicking the corresponding checkbox.



Genotypes

Genotype	Bins
Isoform A	Bin1 (Green)
Isoform B	Bin2 (Green)

☐ Bin1 (Green)
☑ Bin2 (Green)

Bin assignment to genotypes is predefined according to the incidence of melt peaks within the assigned bin. However, no peak should appear within an unassigned bin.

Removing genotypes

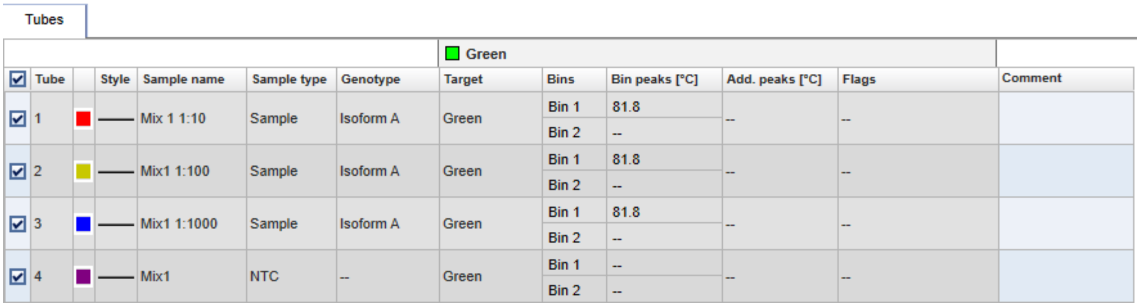
You can delete genotypes from the analysis by clicking on the delete icon next to the genotype.

2.3.5 View results

Once all required analysis parameters are defined, the **Results** table in the lower part of the **Analysis** screen displays the results. This view shows results for each tube or well in the experiment, with all acquisitions listed next to each other in a single row.

2.3.5.1 Result table

The **Tubes** view of the **Results** table shows results for each tube or well laid out in rows.



							🟩 Green					
☑	Tube		Style	Sample name	Sample type	Genotype	Target	Bins	Bin peaks [°C]	Add. peaks [°C]	Flags	Comment
☑	1	🟥	——	Mix 1 1:10	Sample	Isoform A	Green	Bin 1	81.8	--	--	
								Bin 2	--			
☑	2	🟨	——	Mix1 1:100	Sample	Isoform A	Green	Bin 1	81.8	--	--	
								Bin 2	--			
☑	3	🟦	——	Mix1 1:1000	Sample	Isoform A	Green	Bin 1	81.8	--	--	
								Bin 2	--			
☑	4	🟪	——	Mix1	NTC	--	Green	Bin 1	--	--	--	
								Bin 2	--			

Data are organized into the following columns:

Column label	Description
–	The first column contains a check box to select or deselect a tube or well for analysis. The selection is synchronized with the data in the **Tube Selector** and the **Melt plots**.
Tube/Well	Indicates the tube position in the rotor or well position on the plate.
–	The third column displays the color used for the corresponding curve in a fluorescence plot.
Style	Indicates the line style used for the corresponding curve in a fluorescence plot.
Sample name	Lists the sample name.
Sample groups	**Optional**: If you have defined sample groups, this column displays all the groups to which a sample is assigned.
Sample type	Lists the assigned sample type (Sample, Standard, PC, NTC, or NC).
Genotype	Lists the genotype that has been identified for that tube or well, based on the defined bins where peaks should appear.
Target	Lists the target assigned to the tube or well for the specific acquisition.
Bins	One row is shown for each (valid) bin that has been defined for the target.
Bin peaks (°C)	Lists melt peaks that appear inside its corresponding bin.
Add. peaks (°C)	Lists all melt peaks that appeared outside their defined bins for the mentioned target.
Flags	Lists possible flags that mark the current state of the peaks for a specific tube or well (e.g., **Multiple peaks** flag appears when more than one peak appears inside a bin).
Comment	Allows you to enter comments relevant to each sample.

2.4 Report and export results

To create a report or to export results, see "Reports and exports" in the Q-Rex Software User Manual.

2.5 Troubleshooting

The Q-Rex Melt Curve Analysis Plug-in displays error messages and warnings when unexpected events or behavior occur during use.

For information about error messages of the Q-Rex Software and troubleshooting, see "Troubleshooting" in the Q-Rex Software User Manual.

When contacting QIAGEN Technical Service for support, please ensure that you provide the following information to the Service Specialist:

- Steps and events leading to the error message.
- The Message ID. This number uniquely identifies the source of an error or warning and helps QIAGEN Technical Services to resolve the problem faster.

2.6 Glossary

For definitions of general terms used in the Q-Rex Software, refer to the "Glossary" in the Q-Rex Software User Manual.

Appendices

3 Appendices

3.1 Appendix A – Limited License Agreement

QIAGEN's Q-Rex Melt Curve Analysis Plug-in Software License Agreement

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The above restrictions of liability shall not apply in cases of personal injury or any damage resulting from willful acts or gross negligence or for any liability based on the Product Liability Act (Produkthaftungsgesetz), guarantees or other mandatory provisions of law.

The above limitation shall apply accordingly in case of:

- delay,
- compensation due to defect,
- compensation for wasted expenses.

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Nothing in this agreement shall obligate QIAGEN to provide any support for the SOFTWARE. QIAGEN may, but shall be under no obligation to, correct any defects in the SOFTWARE and/or provide updates to licensees of the SOFTWARE. You shall make reasonable efforts to promptly report to QIAGEN any defects you find in the SOFTWARE, as an aid to creating improved revisions of the SOFTWARE.

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This Product contains or makes use of the following third party components: Log4Net, Stateless (Apache License v.2.0), DotNetZip, Plossum (zip/libpng license), Mog (BSD license) NHibernate (GNU Lesser General Public License).

3.2 Appendix B – Liability clause

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3.3 Appendix C – Revision history

Document Revision History	
R3 02/2023	Update of the user manual to include changes related to support of QIAquant.

3.4 Copyright information

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