February 2023

Q-Rex HRM Analysis Plug-in User Manual

For use with the Q-Rex Software v1.0 for determination of genotypes using High-Resolution Melting analysis

Sample to Insight

QIAGEN

Contents

Introduction

1 Introduction

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

The Q-Rex HRM Analysis Plug-in is intended to be used with the Rotor-Gene Q HRM instruments. High-Resolution Melt (HRM) analysis is based on the melting of DNA in the presence of a double-strand DNA-binding fluorescent dye. The fluorescence signal increases as the dye (e.g., EvaGreen) binds to a double-stranded DNA. By increasing the temperature in very small steps and detecting the fluorescence signal, it is possible to determine the melting temperature (*T*m) of the amplified product. In particular, as a change in nucleotide sequence of the PCR product can influence the *T*m, it is now made possible to distinguish different variants e.g., CpG methylation, insertions, deletions or single nucleotide polymorphisms (SNP) using the HRM analysis. Combined with the outstanding thermal and optical performance of the Rotor-Gene Q instrument, it is therefore capable of discriminating even difficult class 4 SNPs (A/T exchange) which result in a *T*m difference of 0.4°C only.

The Q-Rex HRM Analysis Plug-in visualizes the HRM results and enables autocalling of genotypes based on control samples and a defined limit of confidence.

1.1 About this user manual

This user manual provides information about the functions and features of the Q-Rex HRM 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 HRM 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 HRM 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 Manual for complete information about the proper care, maintenance and use of the Rotor-Gene Q instrument.

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

- Introduction
- Working with HRM experiments
- Set up 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, Q-Rex Software, the Q-Rex HRM 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 HRM Analysis Plug-in User Manual, which provides information about the Q-Rex HRM Analysis Plug-in, version 2.0.0.30.

1.2.4 Intended use

The Q-Rex HRM 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 instrument 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 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 HRM experiments

2 Working with HRM 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 HRM Analysis Plug-in to determine genotypes based on High-Resolution Melt results:

Add an analysis

View plots

Define analysis parameters

Define genotypes

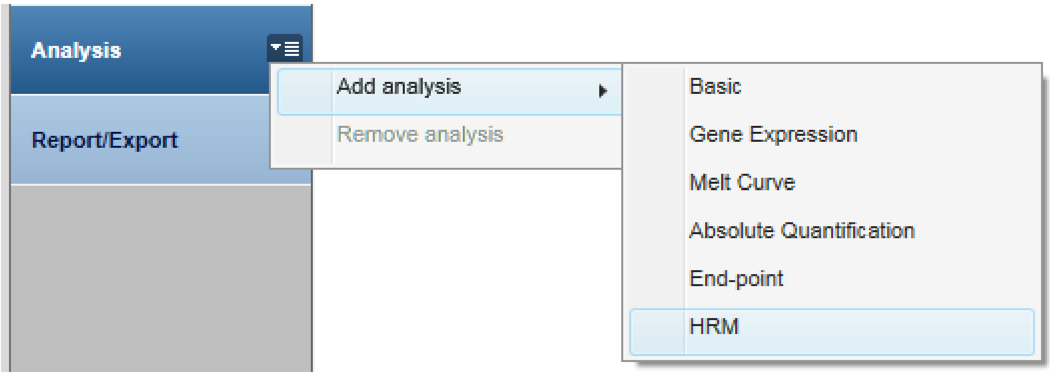
View results

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 HRM 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 HRM Analysis Plug-in to view HRM data.

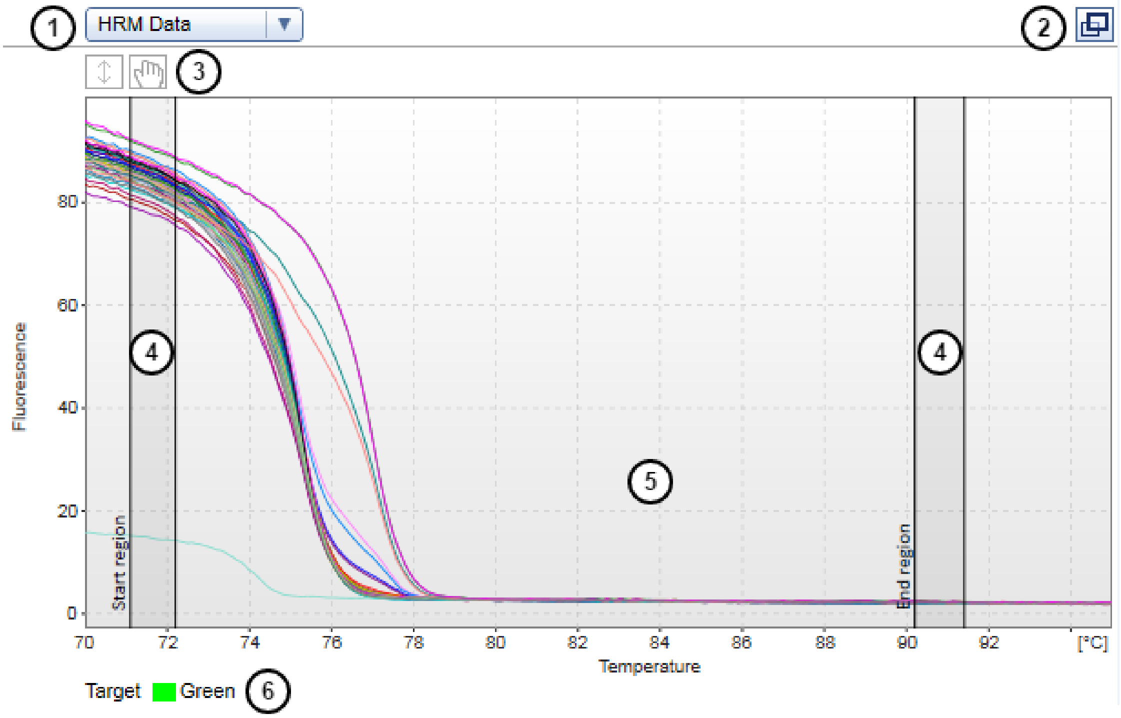
There are three different types of plots available:

HRM data plot – This shows the fluorescence value plotted against temperature.

Norm. HRM data plot – Using the starting and ending regions for normalization, minor differences between the tubes are compensated, leading to comparability.

Difference plot – In order to better visualize small differences between individual HRM curves, each value for each sample is normalized against a single reference (typically an intended control tube e.g., wild-type control).

2.3.2.1 View HRM data plot

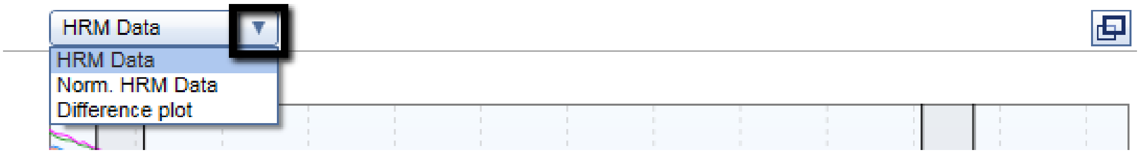


An **HRM data plot** has the following elements:

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

To view **HRM data** of an experiment:

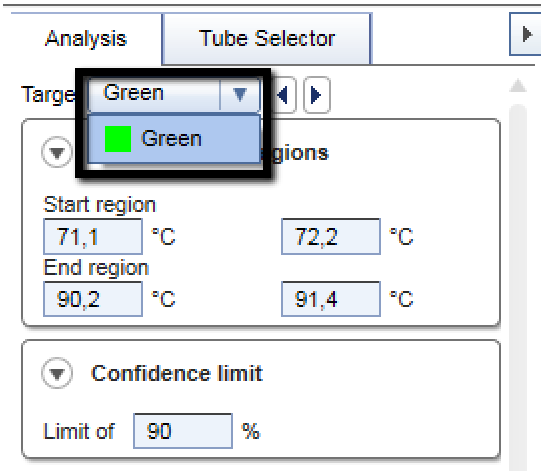
1. Select the plot type you want displayed in the active plot. Click the drop-down menu of the **Plot Selector** to display the available plot types and select one.



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

2. Select a target to display from the drop-down menu in the **Analysis** tab of the **Drawer**.

You can browse through a list of targets using the **Forward** and **Back** buttons.



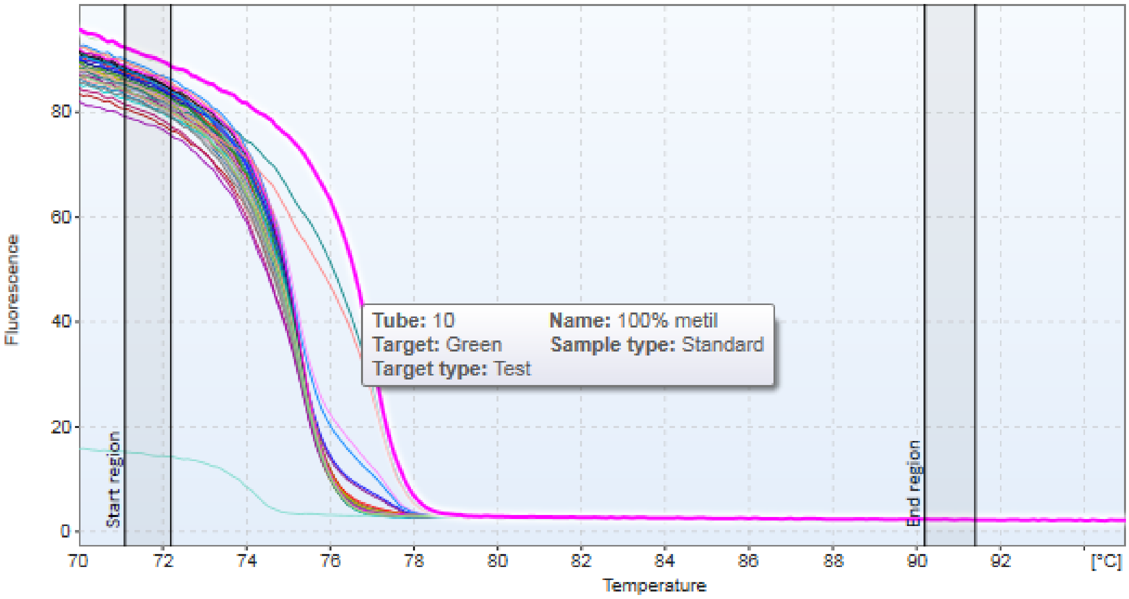
Targe Green

Start region
71,1 °C 72,2 °C
End region
90,2 °C 91,4 °C

Limit of 90 %

3. To view details about a particular curve in the active **HRM data plot**, roll the mouse pointer over the curve.

The curve is highlighted and details appear in a tooltip.



Tube: 10 **Name:** 100% metil
Target: Green **Sample type:** Standard
Target type: Test

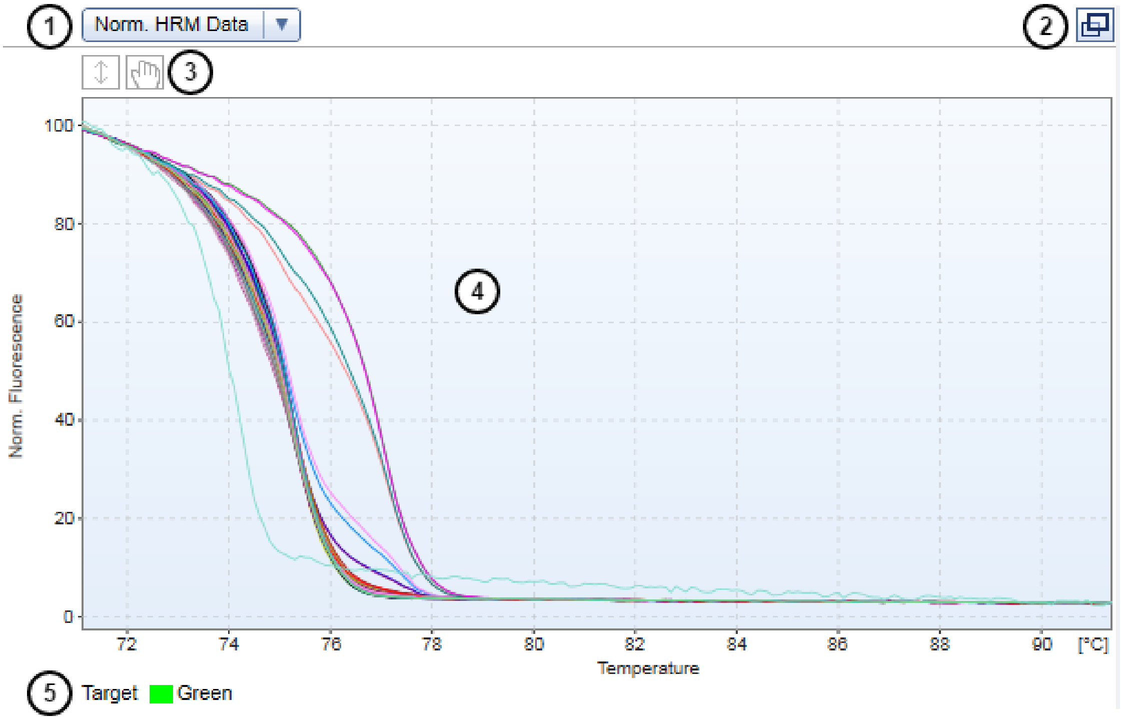
4. The toolbar at the top of the plot includes icons that allow you to alter the way in which the data in the HRM plot are displayed.

① ②

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.

5. The normalization regions that you can see in the **HRM data plot** are used for HRM normalization and can be resized and moved inside the plot area. When regions are changed in the plot, the values in the analysis parameters tab get updated accordingly.

2.3.2.2 View normalized HRM data plot



A **normalized HRM data plot** has the following elements:

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

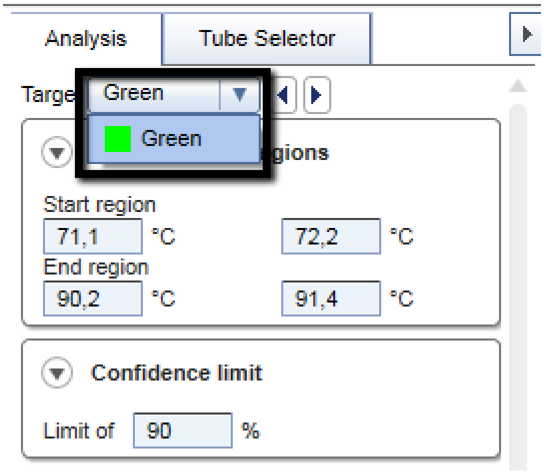
To view **normalized HRM data** of an experiment:

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 **Analysis** tab of the **Drawer**.

You can browse through a list of targets using the **Forward** and **Back** buttons.



Targe Green

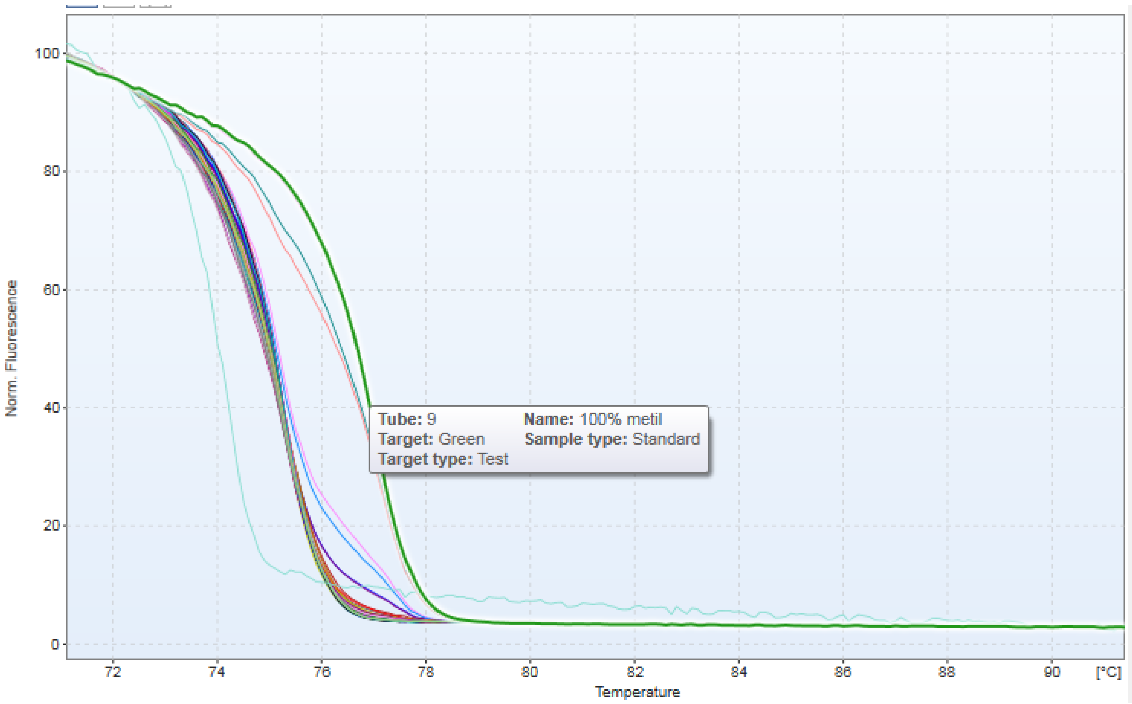
🟩 Green

Start region
71,1 °C 72,2 °C
End region
90,2 °C 91,4 °C

Limit of 90 %

3. To view details about a particular curve in the active **HRM data plot**, roll the mouse pointer over the curve.

The curve is highlighted and details appear in a tooltip.



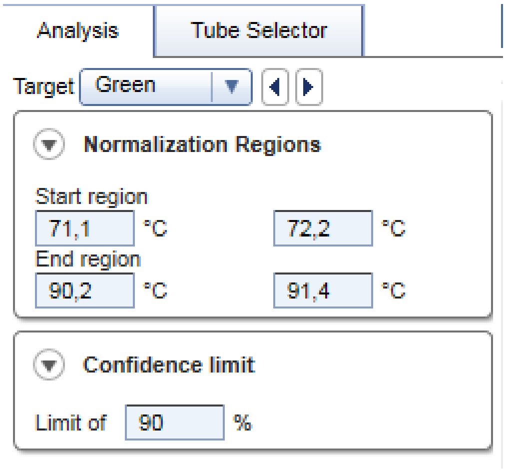
4. The toolbar at the top of the plot includes icons that allow you to alter the way in which the data in the HRM plot are displayed.

↕ 🖐
① ②

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.



Start region
71,1 °C 72,2 °C
End region
90,2 °C 91,4 °C

Limit of 90 %

The active target for which the analysis parameters are defined is displayed at the top of the tab. The target can be selected in the drop-down menu in the **Analysis** tab and it is also possible to navigate through all the valid targets using the **Back** and **Next** buttons.

Normalization Regions

The normalization region is used to generate a common baseline for all tubes and to normalize minimal differences between the samples.

The **Normalization Regions** expander allows you to manually define the normalization region for the HRM analysis by entering temperature values for the start and end regions.

Confidence Limit

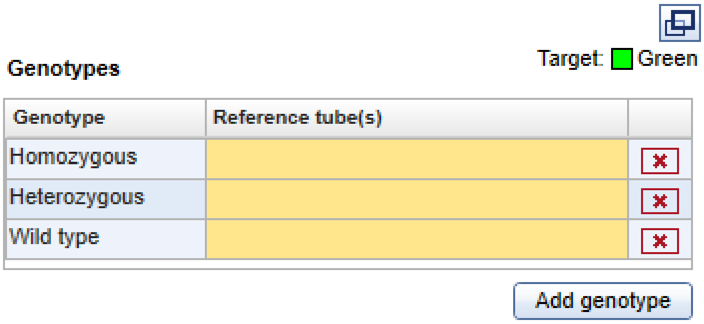
In the **Confidence Limit** expander, you can define the confidence limit, which is the limit of confidence that a sample is showing the same genotype as the control. This analysis is based on a mathematical confidence calculation for each data point of HRM analysis and it is performed against every defined genotype.

Note: Reasonable default values are defined for most analysis parameters. 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 HRM analysis, genotypes can be defined. Upon creation of an HRM analysis, three default genotypes (Homozygous, Heterozygous, and Wild type) are created automatically. The names of the default genotypes can be changed, new genotypes can be added and existing ones can be removed. For each genotype, one or more reference tubes need to be selected.



Genotype	Reference tube(s)	
Homozygous		☒
Heterozygous		☒
Wild type		☒

Adding new genotypes

To add a new genotype, click on the **Add genotype** button below the table. A maximum of ten genotypes are allowed for each HRM target in the analysis.

Editing genotype names

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

Selecting reference tubes for genotypes

In order to perform the HRM analysis, you are required to select one or more reference tubes for each genotype defined. You can select reference tubes in the combo-box from the list of available tubes by clicking on a corresponding checkbox. You can select multiple tubes as references but the same tubes cannot be selected for different genotypes.

Removing genotypes

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

2.3.5 View results

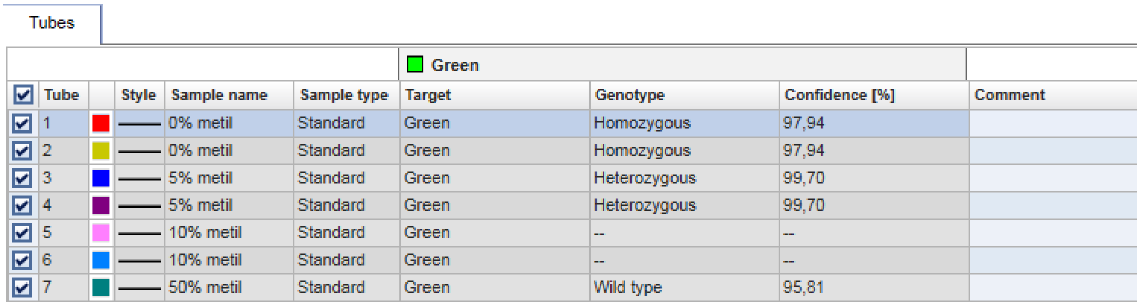
Once the required analysis parameters are defined, the **Result** table in the lower left part of the **Analysis** screen displays the results. Often, the results can be viewed as graphical representations in the difference plot.

- **Result** table**:** This view shows results for each tube in the experiment, with all acquisitions listed next to each other in a single row (see Result table).

- **Difference plot:** Displays the genotyping results in a graphical format (see View difference plot).

2.3.5.1 Result table

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



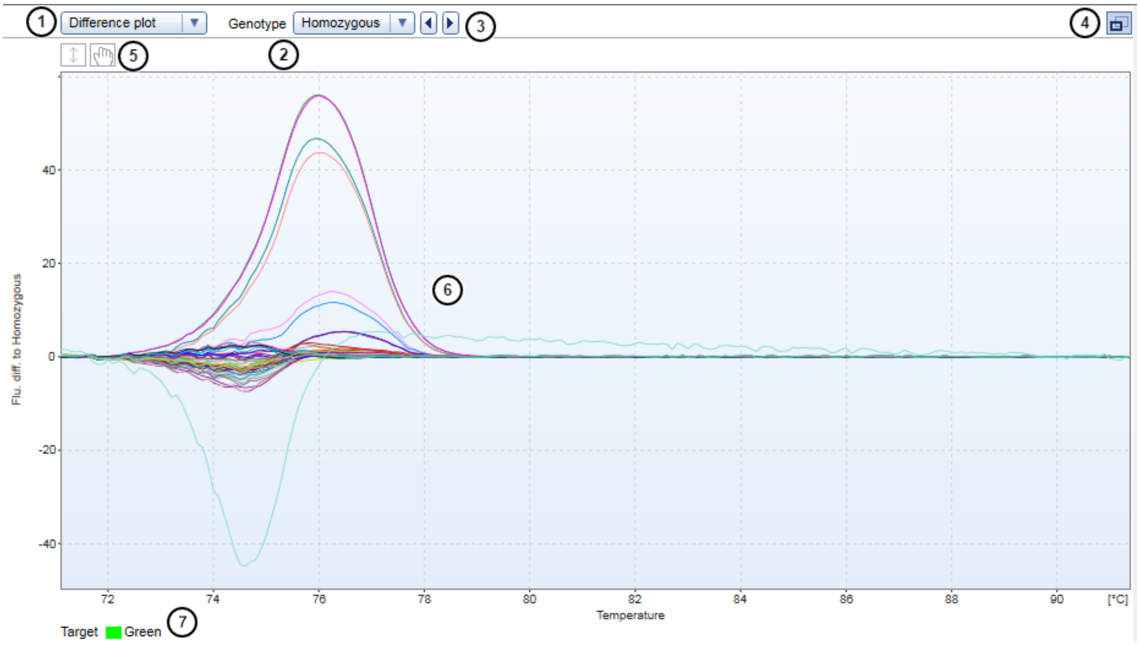
						🟩 Green			
☑	Tube		Style	Sample name	Sample type	Target	Genotype	Confidence [%]	Comment
☑	1	🟥	——	0% metil	Standard	Green	Homozygous	97,94	
☑	2	🟨	——	0% metil	Standard	Green	Homozygous	97,94	
☑	3	🟦	——	5% metil	Standard	Green	Heterozygous	99,70	
☑	4	🟪	——	5% metil	Standard	Green	Heterozygous	99,70	
☑	5	🟪	——	10% metil	Standard	Green	--	--	
☑	6	🟦	——	10% metil	Standard	Green	--	--	
☑	7	🟩	——	50% metil	Standard	Green	Wild type	95,81	

Data are organized into the following columns:

Column label	**Description**
–	The first column contains a checkbox to select or deselect a tube for analysis. The selection is synchronized with the data in the **Tube Selector** and the **HRM plots**.
Tube	Indicates the tube position in the rotor.
–	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, NC).
Target	Lists the target assigned to the tube for the specific acquisition.
Genotype	Lists the genotype(s) that have been identified for that tube based on the defined confidence limit.
Confidence [%]	The confidence with which the genotype has been identified.
Comment	Allows you to enter comments relevant to each sample.

Note: If more than one genotype is detected above the defined confidence limit, the genotype will be shown as a "variation." An empty value "–" will indicate that no genotype was detected above the defined confidence limit.

2.3.5.2 View difference plot



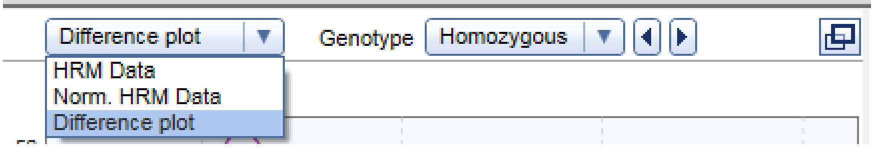
A **difference plot** has the following elements:

- A **Plot Selector** (**1**)
- A **Genotype Selector** (**2**)
- Back and next buttons to step through the genotypes (**3**)
- A button to maximize and minimize the plot window (**4**)
- A toolbar to modify the display of the plot (**5**)
- The plot area (**6**). This area has a darker background color when the plot is active.
- A legend (**7**)

To view the **difference plot** for a specific genotype:

1. Select the plot type you want displayed in the active plot. Click the drop-down menu of the **Plot Selector**

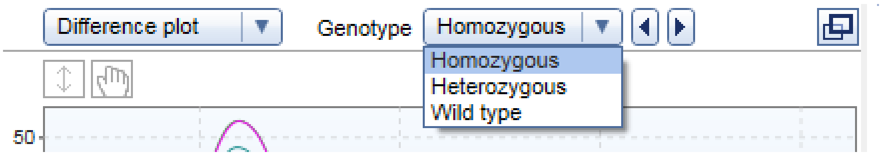
to display the available plot types and select one.



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

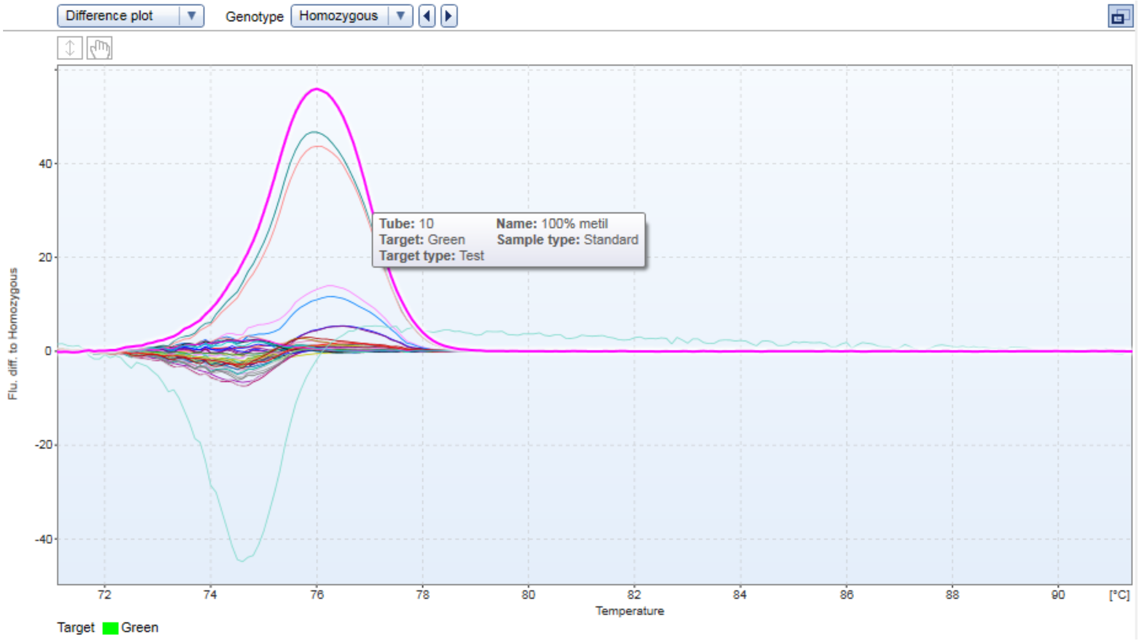
2. Select a genotype from the drop-down menu for which you want to have the **difference plot** displayed.

You can browse through a list of genotypes using the **Forward** and **Back** buttons.



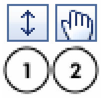
3. To view details about a particular curve in the active **difference plot**, roll the mouse pointer over the curve.

The curve is highlighted and details appear in a tooltip.



Tube: 10
Name: 100% metil
Target: Green
Sample type: Standard
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 HRM 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.

2.4 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 HRM 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 a 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 HRM Analysis Plug-in Software License Agreement

TERMS AND CONDITIONS of an LEGAL AGREEMENT (the "**Agreement**") by and between QIAGEN GmbH, QIAGEN Strasse 1, D-40724 Hilden, Germany, ("**QIAGEN**") and you (either an individual or a legal entity), the licensee of the software (hereinafter referred to as "**SOFTWARE**")

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5. LIMITED WARRANTY

QIAGEN warrants that the SOFTWARE will perform substantially in accordance with the accompanying printed materials for a period of ninety (90) days from the date of receipt. Any implied warranties on the SOFTWARE are limited to ninety (90) days. Some states/jurisdictions do not allow limitations on duration of an implied warranty, so the above limitation may not apply to you.

6. CUSTOMER REMEDIES

QIAGEN's entire liability and your exclusive remedy shall be, at QIAGEN's option, either (a) return of the price paid or (b) repair or replacement of the SOFTWARE that does not meet QIAGEN's Limited Warranty and that is returned to QIAGEN with a copy of your receipt. This Limited Warranty is void if failure of SOFTWARE has resulted from accident, abuse or misapplication. Any replacement of SOFTWARE will be warranted for the remainder of the original warranty period or thirty (30) days, whichever is longer.

7. LIMITED LIABILITY

In no event shall QIAGEN or its suppliers be liable for any damages whatsoever (including, without limitation, damages for loss of business profits, business interruption, loss of business information, or other pecuniary loss, unforeseeable damage, lack of commercial success, indirect damage or consequential damage - in particular financial damage – or for damage resulting from third party claims) arising out of the use or inability to use the SOFTWARE, even if QIAGEN has been advised of the possibility of such damages.

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.

8. NO SUPPORT

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.

Any provision of support by QIAGEN for the SOFTWARE (including network installation support), if any, shall solely be governed by an according separate support agreement.

9. TERMINATION

If you fail to comply with the terms and conditions of this Agreement, QIAGEN may terminate this Agreement and your right and license to use the SOFTWARE. You may terminate this Agreement at any time by notifying QIAGEN. Upon the termination of this Agreement, you must delete the SOFTWARE from your computer(s) and archives.

YOU AGREE THAT UPON TERMINATION OF THIS AGREEMENT FOR ANY REASON, QIAGEN MAY TAKE ACTIONS SO THAT THE SOFTWARE NO LONGER OPERATES.

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This Agreement shall be construed and interpreted in accordance with the laws of Germany, without giving effect to conflict of laws provisions. The application of the provisions of the UN Sales Convention is excluded. Notwithstanding any other provision under this Agreement, the parties to this Agreement submit to the exclusive jurisdiction of the Dusseldorf courts.

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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

QIAGEN shall be released from all obligations under its warranty in the event repairs or modifications are made by persons other than its own personnel, except in cases where the Company has given its written consent to perform such repairs or modifications. All materials replaced under this warranty will be warranted only for the duration of the original warranty period, and in no case beyond the original expiration date of original warranty unless authorized in writing by an officer of the Company. Read-out devices, interfacing devices and associated software will be warranted only for the period offered by the original manufacturer of these products. Representations and warranties made by any person, including representatives of QIAGEN, which are inconsistent or in conflict with the conditions in this warranty shall not be binding upon the Company unless produced in writing and approved by an officer of QIAGEN.

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

Trademarks

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