

September 2017

Q-Rex End-Point Analysis Plug-in User Manual

For use with the Q-Rex Software v1.0 to classify the genotypes of unknown samples



Contents

1	Introduction.....	1-2
1.1	About this user manual.....	1-2
1.2	General information.....	1-3
1.2.1	Technical assistance	1-3
1.2.2	Policy statement	1-3
1.2.3	Version management	1-3
1.2.4	Intended use	1-3
1.3	Getting help.....	1-3
2	Working with end-point experiments.....	2-2
2.1	Set up an experiment.....	2-2
2.2	Run an experiment.....	2-2
2.3	Analyze an experiment.....	2-2
2.3.1	Add an analysis	2-2
2.3.1.1	Define target pair.....	2-3
2.3.2	View plots	2-4
2.3.2.1	View scatter plot.....	2-5
2.3.3	Define analysis parameters	2-7
2.3.4	Define genotyping parameters	2-8
2.3.5	Compensating crosstalk	2-8
2.3.5.1	Performing crosstalk compensation.....	2-9
2.3.5.2	Defining crosstalk compensation settings.....	2-10
2.3.5.3	Saving compensation factors.....	2-13
2.3.5.4	Loading compensation factors.....	2-15
2.3.6	Copy analysis parameter	2-18
2.3.7	View results	2-20
2.3.7.1	Tubes view.....	2-20
2.4	Report and export results.....	2-22
2.5	Troubleshooting	2-23

2.6	Glossary.....	2-23
3	Appendices.....	3-2
3.1	Appendix A – Limited License Agreement.....	3-2
3.2	Appendix B – Liability clause.....	3-6
3.3	Copyright information.....	3-7

Introduction

1 Introduction

Plug-ins for the Q-Rex Software grant additional analysis capabilities. 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 End-Point Analysis Plug-in is intended for analysis of genetic variations such as single nucleotide polymorphisms (SNPs) and mutations at specific loci in the genome.

An end-point analysis requires at least two differently labeled sequence-specific probes (e.g., hydrolysis probes) that are designed to specifically detect the distinct forms of the allele. Once the qPCR run is complete, the end fluorescence of both the targets are set in relation by plotting each sample on a scatter plot. Based on a k-mean algorithm, the data points lying close to each other are grouped into clusters. Typically, three different clusters are visible in the scatter plot: Homozygous for Allele A, homozygous for Allele B and heterozygous.

The Q-Rex End-Point Analysis Plug-in provides analysis on either raw or normalized end-point fluorescence data.

1.1 About this user manual

This user manual provides information about the functions and features of the Q-Rex End-Point 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 End-Point 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 End-Point 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 End-Point Analysis Plug-in in the following sections:

- [Introduction](#)
- [Working with end-point analysis 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 End-Point 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**](http://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 End-Point Analysis Plug-in User Manual*, which provides information about the Q-Rex End-Point Analysis Plug-in, version 1.0.

1.2.4 Intended use

The Q-Rex End-point 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 end-point experiments

2 Working with end-point 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*.

The Q-Rex End-Point Analysis Plug-in requires acquisitions in two different channels corresponding to the used probes.

Make sure to include the following control samples:

- Positive Controls Allele X/Allele X
- Positive Controls Allele Y/Allele Y
- Positive Controls Allele X/Allele Y
- No Template Controls (NTC)

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 End-Point Analysis Plug-in to determine the genotype of samples based on their end-point fluorescence:

[Add an analysis](#)

[View plots](#)

[Define analysis parameters](#)

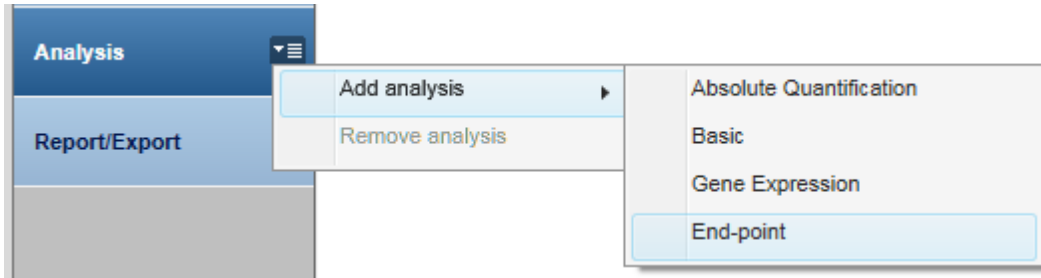
[View results](#)

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*.

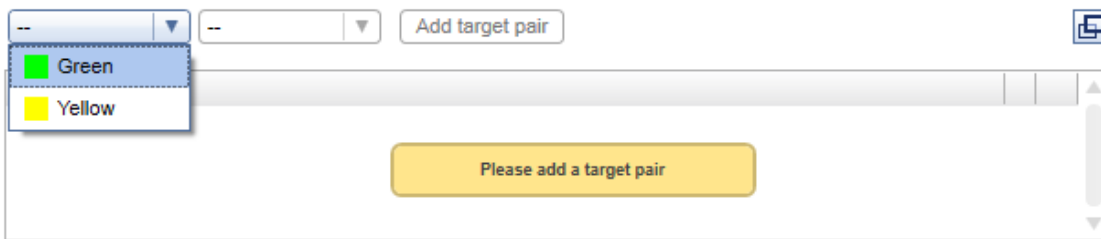
If the Q-Rex End-Point Analysis Plug-in was installed correctly, a menu item for the plug-in will appear in the list of available analyses:



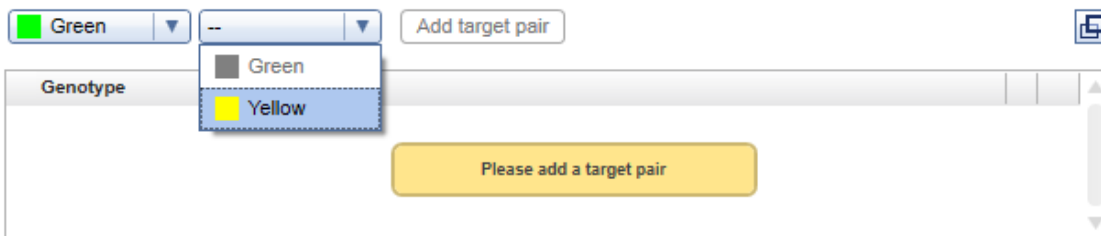
2.3.1.1 Define target pair

To view a **scatter plot**, at least one target pair needs to be defined in the target pair table in the lower right area of the screen in the **Analysis** step:

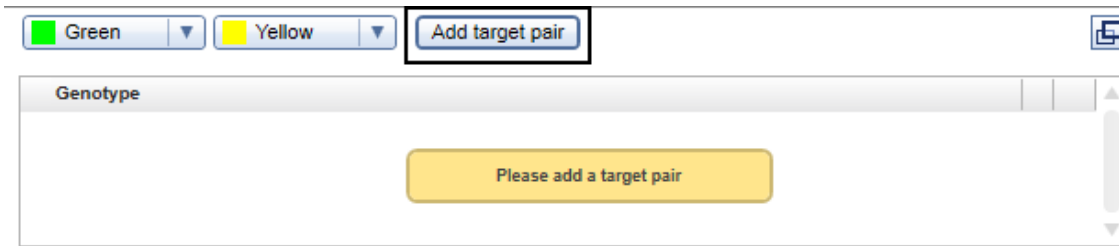
1. Select the first target from a drop-down menu of the first combo box.



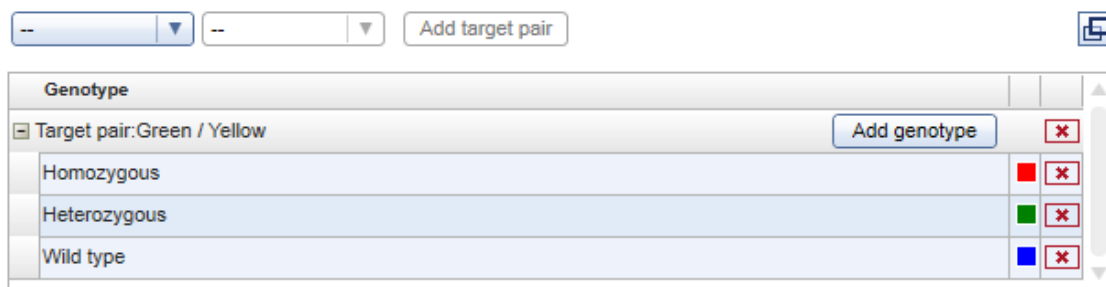
2. Choose the second target from the drop-down menu of the second combo box.



3. Click **Add a target pair** to confirm.



4. Defined target pair is shown along with the default genotypes in the genotype table.



Note: Only targets that are assigned to the same tube are shown in the second combo box for target pair definition.

2.3.2 View plots

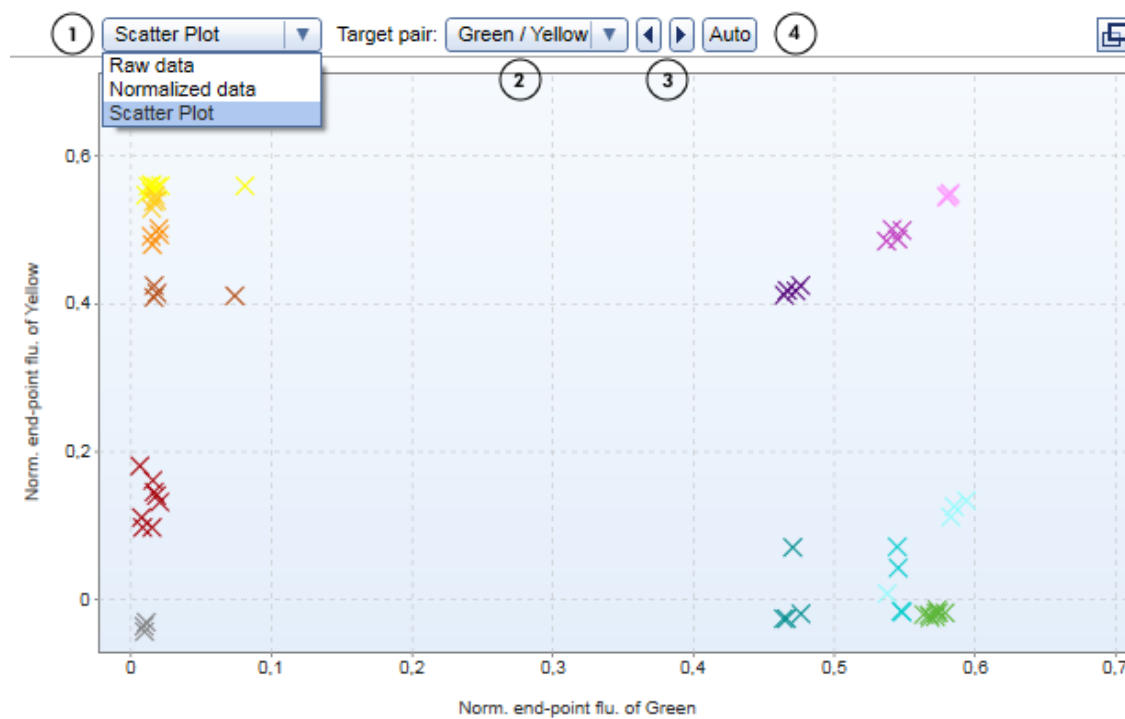
For an overview of fluorescence plots in the Q-Rex Software, see "View fluorescence plots" in the *Q-Rex Software User Manual*.

In addition to the raw and normalized data fluorescence plots, analysis with the Q-Rex End-Point Analysis Plug-in offers a third plot type called the **scatter plot**.

For detailed information regarding the **scatter plot**, refer to the "View scatter plot" section.

2.3.2.1 View scatter plot

The **scatter plot** can be selected in any of the plot windows at the top of the screen in the **Analysis** step.



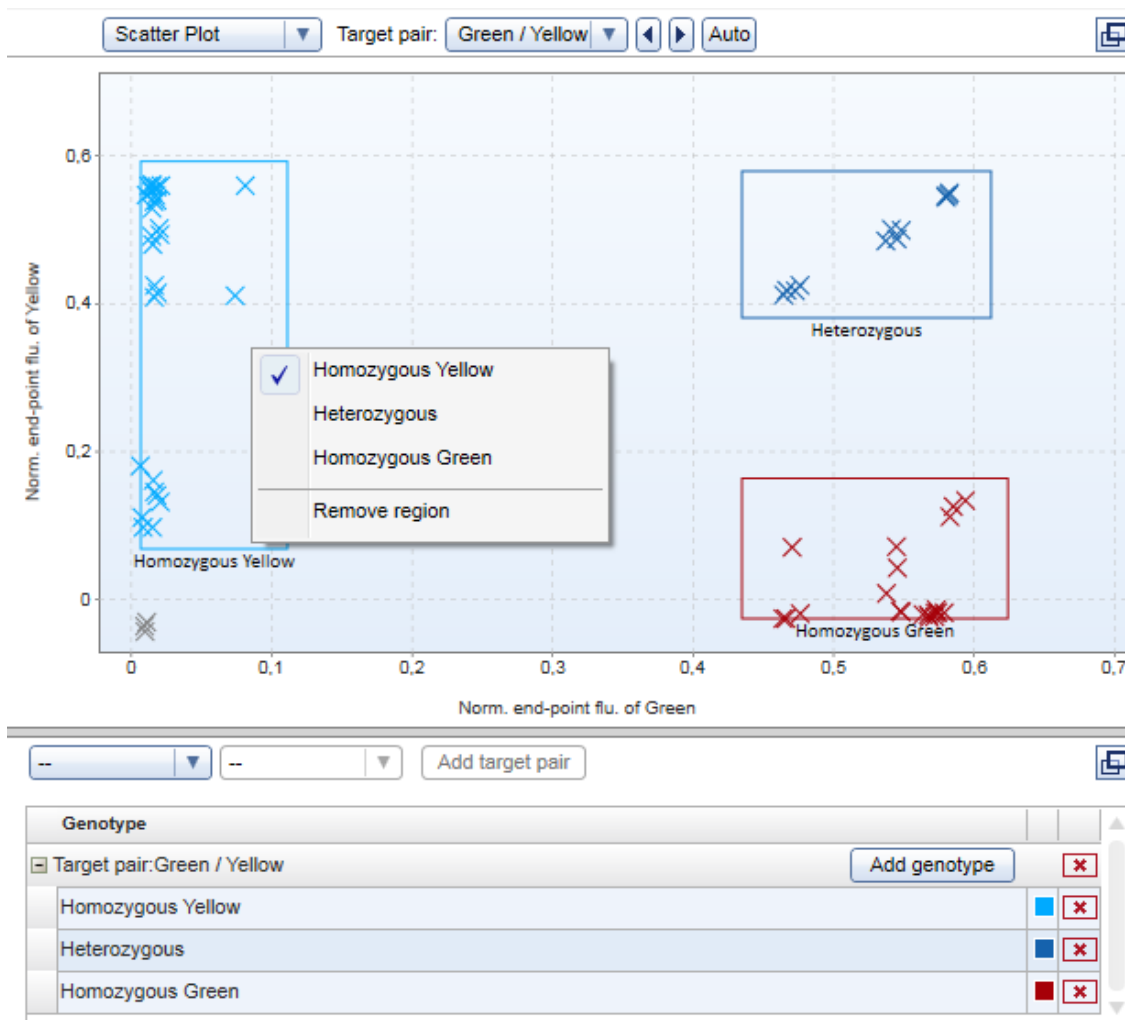
The **scatter plot** includes the following elements:

- A **Plot Selector** (1)
- A **Target Pair Selector** (2)
- Arrows to switch between the available target pairs (3)
- A button to automatically group samples to a genotype for the selected target pair (4)

To view end-point results in the scatter plot, select the **scatter plot** from the **Plot Selector** drop-down menu. Use the **Target Pair Selector** to display the end-point fluorescence of the analyzed target pair. You can browse through a list of target pairs using the **Forward** and **Back** buttons.

Crosses on the plot represent the end-point fluorescence of samples defined for both targets in the selected target pair.

To automatically cluster the samples to genotypes, click the **Auto** button. The samples with the same genotype will be assigned to a region and the corresponding genotype will be displayed. The genotype names are assigned to the target pair table. The names as well as the color of the region can be changed.



However, you can manually change the regions by dragging the rectangle. Select the region you wish to reassign or remove and right-click to open the context menu.

Note: To see a **scatter plot**, a target pair needs to be defined first.

Note: The scale of the axes is automatically set for both axes to the maximal value.

2.3.3 Define analysis parameters

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

The screenshot shows the 'Analysis' tab of the 'Drawer' interface. At the top, there are two tabs: 'Analysis' (selected) and 'Tube Selector'. Below the tabs, the 'Target' is set to 'Green'. There are four main analysis parameter groups, each with a play button icon: 'Filter data', 'Normalization', 'Genotyping', and 'Crosstalk compensation'. The 'Genotyping' group is expanded, showing 'Base analysis on:' with two radio button options: 'Raw fluorescence end-point data' and 'Normalized fluorescent end-point data', with the latter selected. At the bottom, there is a 'Copy settings to ...' dropdown menu and an 'OK' button.

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.

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.

Define the following parameter groups for end-point analysis:

Filter data	See the <i>Q-Rex Software User Manual</i> for details.
Normalization	See the <i>Q-Rex Software User Manual</i> for details.
Genotyping	See "Define genotyping parameters" for details.

Optionally, the following additional analysis features can be used:

Crosstalk compensation	See "Compensating crosstalk."
Copy settings to...	See "Copy analysis parameters."

2.3.4 Define genotyping parameters

The Q-Rex End-Point Analysis Plug-in comes with the option to perform the analysis based on the raw or normalized end-point fluorescence.

By default, the scatter plot is shown for the normalized fluorescence end-point data. However, you can apply the analysis to raw fluorescence end-point data. All regions in the scatter plot will be reset.

If you change the selection of tubes or sample types, the regions are not automatically updated. Click **Auto** again to have the software calculate new regions based on the updated tube selection.

Note: Automatic region detection requires at least four tubes for the active target. The sample type of that tube should not be of the type **Not in use**.

2.3.5 Compensating crosstalk

Using two or more acquisition channels in the same experiment can lead to observed crosstalk. Crosstalk occurs when fluorescent dyes and filters have overlapping wavelength range and the emission from one channel contributes to the acquisition of another channel.

In the Q-Rex End-Point Analysis Plug-in, you can compensate for this signal bleed from one channel into another. **Crosstalk compensation** allows you to remove this signal interference by:

[Performing crosstalk compensation](#)

[Defining crosstalk compensation settings](#)

[Saving compensation factors](#)

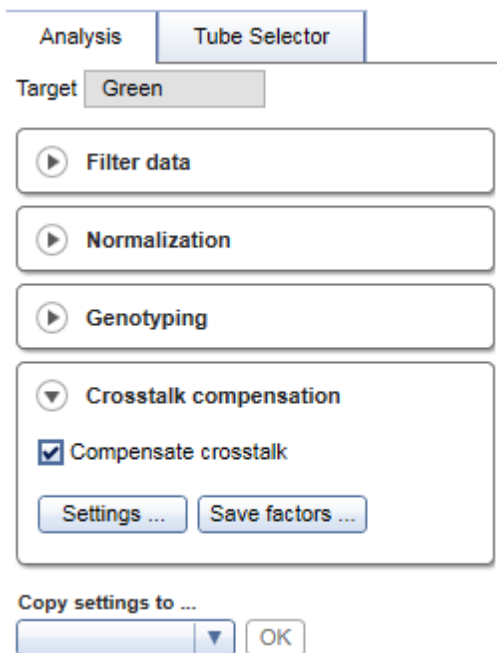
[Loading compensation factors](#)

Note: Crosstalk compensation cannot be a component of a template. Crosstalk compensation is added after execution and review of an experiment, and only if it is required. Crosstalk compensation can only be applied to normalized data.

2.3.5.1 Performing crosstalk compensation

To set up crosstalk compensation, expand the **Crosstalk compensation** section in the **Analysis** tab of the **Drawer**.

Note: Crosstalk compensation cannot be a component of a template. Instead, it is added after execution and review of an experiment and only if it is required.



Click **Settings** to access the [crosstalk compensation settings](#) dialog. Define your crosstalk compensation and click **OK**.

Check the **Compensate crosstalk** checkbox to apply your defined crosstalk compensation settings. The compensation appears on the normalized data of the channels which were specified in the **Settings** dialog.

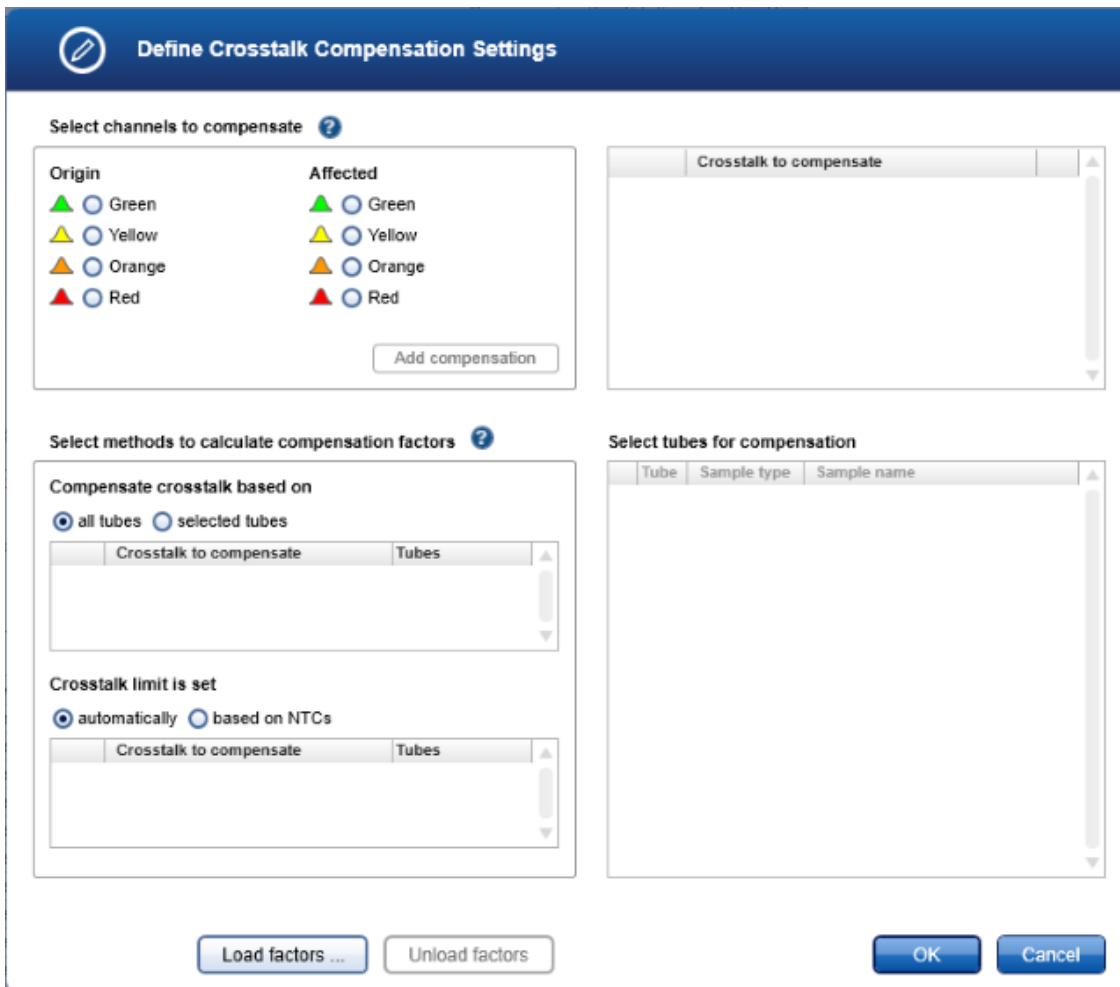
Remove the tick from the checkbox to remove the crosstalk compensation from the normalized data.

Note: Invalid crosstalk compensation settings disable the **Compensate crosstalk** checkbox and the **Settings** button is displayed as invalid.

Click **Save factors** to access the **Save Compensation Factors** dialog. You can save calculated crosstalk compensation factors to the file system and reuse them in a different experiment.

2.3.5.2 Defining crosstalk compensation settings

Set up the compensation settings for your experiment in the **Define Crosstalk Compensation Settings** dialog.



You can [load saved crosstalk compensation factors](#) or define new settings. To define new settings:

1. Select the channels requiring crosstalk compensation. The **Select channels to compensate** section lists all channels in the experiment into two columns labeled **Origin** and **Affected**.

Select channels to compensate ?

Origin	Affected
<input checked="" type="radio"/> Green	<input type="radio"/> Green
<input type="radio"/> Yellow	<input type="radio"/> Yellow
<input type="radio"/> Orange	<input type="radio"/> Orange
<input type="radio"/> Red	<input checked="" type="radio"/> Red

Crosstalk to compensate
<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> Green bleeding into Yellow <input checked="" type="checkbox"/>

Select the channel causing the crosstalk from the **Origin** list and the channel affected by the crosstalk from the **Affected** list.

2. Add your selection to the **Crosstalk to compensate** section to the right by clicking **Add compensation**.

To remove a defined channel compensation, click in the row of the defined crosstalk.

Note: Each combination of **Origin** and **Affected** channels can be added only once to the **Crosstalk to compensate** section. You cannot compensate channels with the same wavelength or channels that undergo data acquisition in different cycling steps.

3. Select the method to calculate compensation factors.

Select methods to calculate compensation factors ?

Compensate crosstalk based on

all tubes selected tubes

Crosstalk to compensate	Tubes
<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> Green bleeding into Yellow	1-22

Crosstalk limit is set

automatically based on NTCs

Crosstalk to compensate	Tubes
<input checked="" type="checkbox"/> <input checked="" type="checkbox"/> Green bleeding into Yellow	--

Select tubes for compensation

Tube	Sample type	Sample name
------	-------------	-------------

The factors for a compensation can be calculated based on **all tubes** or **selected tubes**.

all tubes All tubes with Sample type other than **Not in use** are used for the calculation. This option is preselected by default and is best used in combination with the option to set the crosstalk limit **automatically** for an initial compensation in an experiment.

selected tubes Only selected tubes are used to calculate compensation factors. You must define at least one tube with an expected signal in the **Origin** channel and no expected signal in the **Affected** channel.

This is an advanced option and should be implemented only by experienced users.

To select tubes, click the radio button **selected tubes** and a table of tubes in the experiment appears to the right.

The screenshot shows two panels. The left panel, titled "Select methods to calculate compensation factors", has two sections. The first section, "Compensate crosstalk based on", has radio buttons for "all tubes" (selected) and "selected tubes" (with a warning icon). Below it is a table with columns "Crosstalk to compensate" and "Tubes", containing one row: "Green bleeding into Yellow". The second section, "Crosstalk limit is set", has radio buttons for "automatically" (selected) and "based on NTCs". Below it is a table with columns "Crosstalk to compensate" and "Tubes", containing one row: "Green bleeding into Yellow" with "--" in the "Tubes" column.

The right panel, titled "Select tubes for compensation", is a table with columns "Tube", "Sample type", and "Sample name". It contains 14 rows:

Tube	Sample type	Sample name	
<input type="checkbox"/>	1	Sample	sample 1
<input type="checkbox"/>	2	Sample	sample 1
<input type="checkbox"/>	3	Sample	sample 2
<input type="checkbox"/>	4	Sample	sample 2
<input type="checkbox"/>	5	Sample	sample 3
<input type="checkbox"/>	6	Sample	sample 3
<input type="checkbox"/>	7	Sample	sample 4
<input type="checkbox"/>	8	Sample	sample 4
<input type="checkbox"/>	9	Sample	sample 5
<input type="checkbox"/>	10	Sample	sample 5
<input type="checkbox"/>	11	Sample	sample 6
<input type="checkbox"/>	12	Sample	sample 6
<input type="checkbox"/>	13	NTC	multiplex NTC
<input type="checkbox"/>	14	NTC	multiplex NTC

Check the checkbox of each tube to be used in the calculation. Each tube must have an expected signal in the **Origin** channel and no expected signal in the **Affected** channel.

4. Select the method to set the limit for crosstalk compensation; that is, to what extent should the signals of affected tubes be compensated.

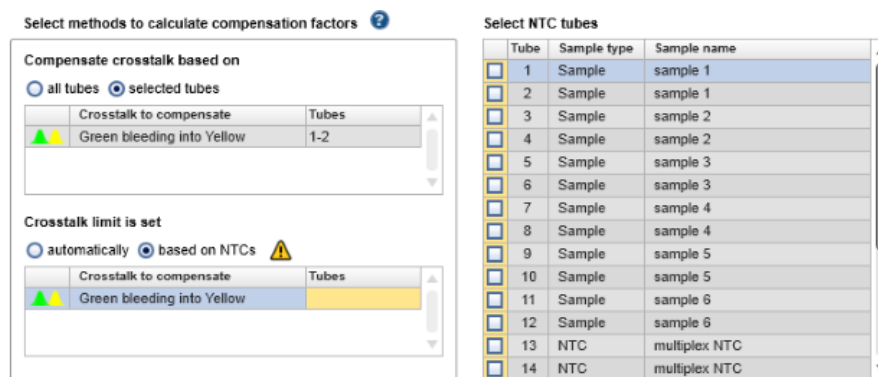
In the **Crosstalk limit is set** section, choose between setting the limit automatically or based on specific no template controls (NTCs).

automatically All tubes with Sample type other than **Not in use** are used to automatically calculate a compensation limit. This option is preselected by default and is best used in combination with the option to compensate crosstalk based on all tubes for an initial compensation in an experiment.

based on NTCs Signals are compensated until the curve reaches that of crosstalk-free NTCs. This option is available if you choose to compensate crosstalk based on selected samples. Fluorescence signals are compensated until the fluorescence of the specified NTC is reached.

This is an advanced option and should be used only by experienced users.

To select tubes for this option, click the radio button **based on NTCs** and a list of tubes in the experiment appears to the right.



In the table, check the checkbox of each tube to be used to set the limit. Each tube must have acquisitions in the **Origin** and **Affected** channels, but no template in either channel (NTC) and therefore no amplification signals.

Note: The options to compensate crosstalk based on **all tubes** and set the crosstalk limit **based on NTCs** cannot be used together.

5. Once you have defined the settings, click **OK** to return to the analysis to apply them.

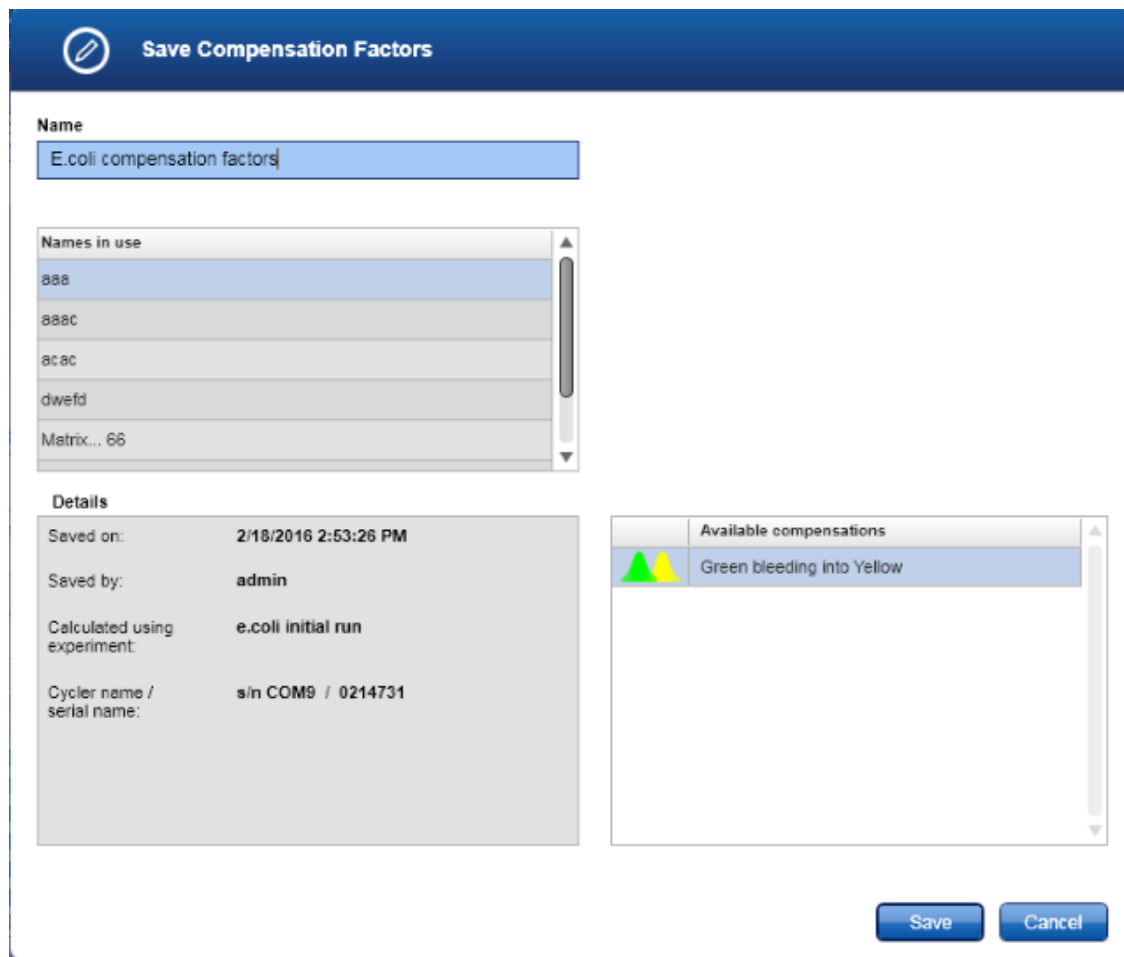
Alternatively, click **Cancel** to discard all entries made and exit the dialog.

2.3.5.3 Saving compensation factors

You can save calculated compensation factors to reuse them in a different experiment. We recommend saving compensation factors only if they will be used for experiments run on the same instrument and with the same experimental setup.

To save your compensation factors:

1. Open the **Save Compensation Factors** dialog by clicking **Save factors** in Crosstalk compensation section of the **Analysis** tab of the **Drawer** (see [Performing crosstalk compensation](#)).



- | | |
|-------------------------|--|
| Names in use | Displays file names of compensation factors already saved and used on your system. |
| Details | Summarizes the experiment from which the compensation factors were calculated. |
| Available compensations | Lists all channels chosen to be compensated. |

2. Click the **Name** field and enter a name for the compensation factors to be saved. If a saved factors file already exists with the same name, a warning message is displayed.
3. Click **Save** to save the compensation factors and exit the dialog.

2.3.5.4 Loading compensation factors

To load compensation factors saved on your system, click **Load factors** in the **Define Crosstalk Compensation Settings** dialog.

Define Crosstalk Compensation Settings

Select channels to compensate ?

Origin

Green Yellow Orange Red

Affected

Green Yellow Orange Red

Add compensation

Select methods to calculate compensation factors ?

Compensate crosstalk based on

all tubes selected tubes

Crosstalk to compensate	Tubes

Crosstalk limit is set

automatically based on NTCs

Crosstalk to compensate	Tubes

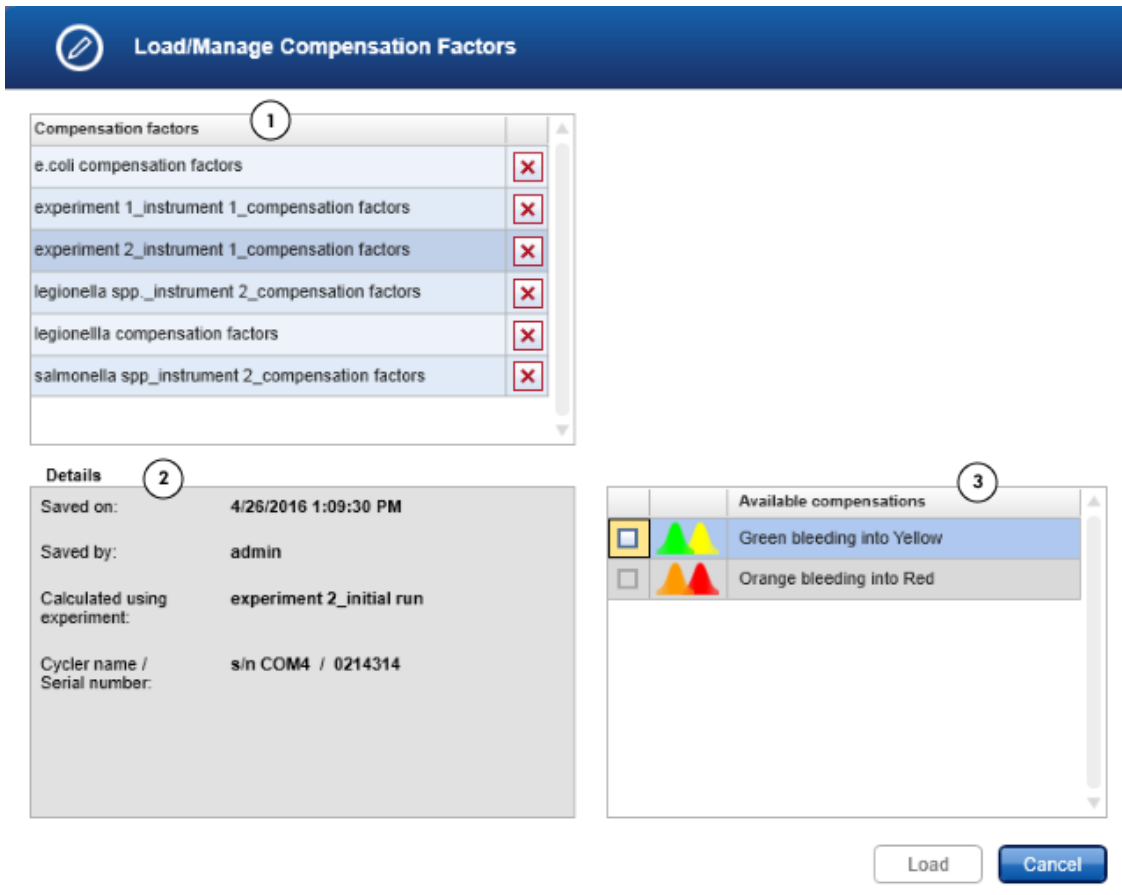
Select tubes for compensation


Tube	Sample type	Sample name

Load factors ... Unload factors OK Cancel

The **Load/Manage Compensation Factors** dialog that appears lists all previously saved compensation factors. In this dialog, you can either load compensation factors or remove unwanted compensation factor files from your system.

Note: We recommend using saved compensation factors only in experiments run on the same instrument and with the same experimental setup.



To remove compensation factor files, find the file in the **Compensation factors** field (1), click  and confirm the deletion of the compensation factors from your system.

To load saved compensation factors:

1. Select the file with the compensation factors you want to load from the **Compensation factors** field (1).
2. Check the information provided in the **Details** field (2) to make sure you have chosen the correct compensation factors.

3. From the **Available compensations** table (3), select at least one compensation in the file to be loaded. Compensations which are not applicable to your experiment are grayed out and cannot be selected.
4. Click **Load** and return to the **Define Crosstalk Compensation Settings** dialog. Details about the loaded compensation factors are displayed in this dialog.
5. Click **OK** to confirm your selection and return to the analysis.

Alternatively, you can load compensation factors from another file by clicking **Load factors** or unload the compensation factors by clicking **Unload factors**. All settings return to default and you can [define new crosstalk compensation settings](#).

Click **Cancel** to return to the analysis step without saving defined compensation settings.

Define Crosstalk Compensation Settings

Select channels to compensate ?

Origin	Affected
<input checked="" type="radio"/> Green	<input checked="" type="radio"/> Green
<input type="radio"/> Yellow	<input type="radio"/> Yellow

Add compensation

Compensation factors loaded ?

Saved on:	4/26/2016 1:09:30 PM
Saved by:	admin
Calculated using experiment:	experiment 2_initial run
Cycler name / Serial number:	s/in COM4 / 0214314

Crosstalk to compensate	

Compensations	
<input checked="" type="radio"/> <input checked="" type="radio"/>	Green bleeding into Yellow

Load factors ... Unload factors OK Cancel

2.3.6 Copy analysis parameter

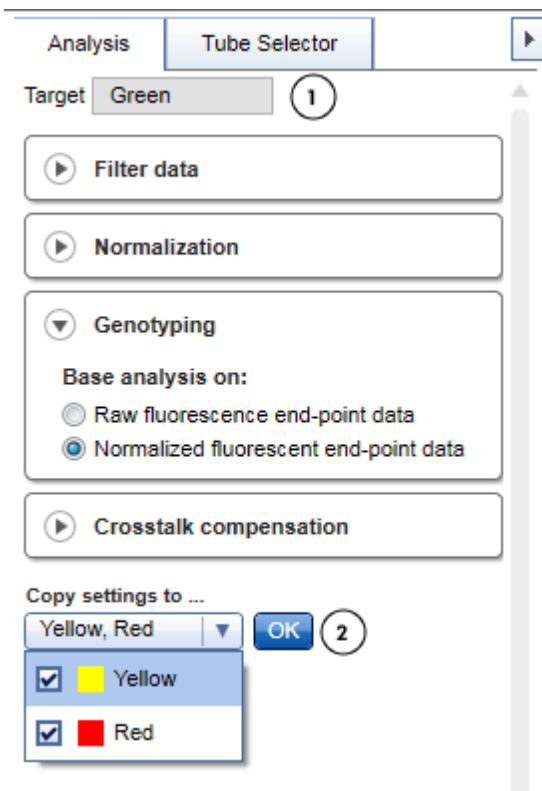
In **End-Point** analysis, parameters from one target can be copied to other targets using the **Copy settings to (2)** function of the **Analysis** tab in the **Drawer**.

To copy analysis parameters to one or more targets:

1. Define analysis parameters for the active target.

The active target for the analysis is visible at the top of the **Drawer (1)** and its editable analysis parameters are listed below. This is also the target selected in the active fluorescence plot.

2. The drop-down menu under **Copy settings to (2)** lists all targets available except for the active target. Use the drop-down menu to indicate the destination targets for the parameters.



3. Click **OK**. Filter data, normalization settings, thresholds and melt settings (if available) from the active target are copied to all selected destination targets.
















Note: If multiple targets are selected in the fluorescence plot, the **Target** field at the top of the **Drawer** remains empty, the analysis parameters cannot be edited and the **Copy settings to** function is disabled.

2.3.7 View results

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

2.3.7.1 Tubes view

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

Tubes				Genotype		Norm. end-point fluorescence	
<input type="checkbox"/>	Tube	Sample name	Sample type	Green / Yellow	Green	Yellow	
<input checked="" type="checkbox"/>	21	 Homozygous Yellow	Sample	Homozygous Yellow	0,08	0,56	
<input checked="" type="checkbox"/>	22	 Homozygous Yellow	Sample	Homozygous Yellow	0,01	0,55	
<input checked="" type="checkbox"/>	23	 Homozygous Yellow	Sample	Homozygous Yellow	0,01	0,56	
<input checked="" type="checkbox"/>	24	 Homozygous Yellow	Sample	Homozygous Yellow	0,01	0,55	
<input checked="" type="checkbox"/>	57	 Heterozygous Green/Yellow	Sample	Heterozygous	0,58	0,54	
<input checked="" type="checkbox"/>	58	 Heterozygous Green/Yellow	Sample	Heterozygous	0,58	0,55	
<input checked="" type="checkbox"/>	59	 Heterozygous Green/Yellow	Sample	Heterozygous	0,58	0,55	
<input checked="" type="checkbox"/>	60	 Heterozygous Green/Yellow	Sample	Heterozygous	0,58	0,55	
<input checked="" type="checkbox"/>	61	 Heterozygous Green/Yellow	Sample	Heterozygous	0,55	0,49	
<input checked="" type="checkbox"/>	62	 Heterozygous Green/Yellow	Sample	Heterozygous	0,55	0,50	
<input checked="" type="checkbox"/>	63	 Heterozygous Green/Yellow	Sample	Heterozygous	0,54	0,50	
<input checked="" type="checkbox"/>	64	 Heterozygous Green/Yellow	Sample	Heterozygous	0,54	0,48	
<input checked="" type="checkbox"/>	65	 Heterozygous Green/Yellow	Sample	Heterozygous	0,46	0,41	
<input checked="" type="checkbox"/>	66	 Heterozygous Green/Yellow	Sample	Heterozygous	0,48	0,42	
<input checked="" type="checkbox"/>	67	 Heterozygous Green/Yellow	Sample	Heterozygous	0,47	0,42	

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 fluorescence plots (see the <i>Q-Rex Software User Manual</i> for details).
Tube	Indicates the tube position in the rotor.
-	The third column displays the color used for the corresponding curve in a fluorescence plot.
Sample name	Lists the sample name.

Sample type Lists the assigned sample type (Sample, Standard, PC, NTC, NC, Not in use).

With this plug-in, two additional columns are displayed:

Column Label	Description
Genotype	Lists the genotypes determined for each tube and each target pair.
Raw/Norm. end-point fluorescence	Displays the end-point fluorescence as raw or normalized values for each target depending on the chosen analysis setting.

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 End-Point Analysis Plug-in displays error messages and warnings when unexpected events or behavior occur during use.

The following list includes the most common error messages or warnings that can occur while using the Q-Rex End-Point Analysis Plug-in and certain troubleshooting suggestions. These are specific to the Q-Rex End-Point Analysis Plug-in. For information about general 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.

Error messages:

Message ID	Error text	Comments and suggestions
20175000	Minimal number of selected tubes required.	Automatic threshold calculation was attempted with an incorrect tubes selection. To use the automatic threshold calculation, at least one tube for the active target with a sample type other than Not in use must be selected.

Warning messages:

Message ID	Warning text	Comments and suggestions
20000003	The same name is already saved for the compensation factors. Would you like to overwrite the existing file?	Use a different name to save the compensation factors. Otherwise, the existing file will be overwritten.
20000002	The selected compensation factors will be removed from your system and will no longer be available.	By confirming this warning, the compensation factors will be removed completely. Do not delete compensation factors that could be used in the future.

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 End-Point 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**")

By installing, having installed and using the SOFTWARE you are agreeing to be bound by the terms of this Agreement. If you do not agree to the terms of this Agreement, promptly return the software package(s) and the accompanying items (including written materials) to the place you obtained them for a full refund of the costs of the SOFTWARE.

1. GRANT OF LICENSE

Scope. Subject to the terms and conditions of this agreement, QIAGEN grants you a worldwide, perpetual, non-exclusive, and nontransferable license to use the SOFTWARE solely for your internal business purposes.

You shall not:

- modify or alter the whole or any part of the SOFTWARE nor merge any part of it with another software nor separate any components of the SOFTWARE from the SOFTWARE nor, save to the extent and in the circumstances permitted by law, create derivative works from, or, reverse engineer, decompile, disassemble or otherwise derive source code from the SOFTWARE or attempt to do any of these things
- copy the SOFTWARE (except as provided above)
- assign rent, transfer, sell, disclose, deal in, make available or grant any rights in the Software Product in any form to any person without the prior written consent of QIAGEN;
- remove alter, obscure, interfere with or add to any proprietary notices, labels, trademarks, names or marks on, annexed to, or contained within the SOFTWARE;
- use the SOFTWARE in any manner that infringes the intellectual property or other rights of QIAGEN or any other party; or
- use the SOFTWARE to provide on-line or other database services to any other person.

Single-Computer Use. This Agreement permits you to use one copy of the SOFTWARE on a single computer.

Trial versions. Trial versions of the SOFTWARE may expire after a period of 30 (thirty) days without prior notice.

Open Software/Third Party Software. This Agreement does not apply to any other software components identified as subject to an open source license in the relevant notice, license and/or copyright files included with the programs (collectively the "Open Software"). Furthermore, this Agreement does not apply to any other software for which QIAGEN is only granted a derived right to use ("Third Party Software"). Open Software and Third Party Software may be supplied in the same electronic file transmission as the

SOFTWARE, but are separate and distinct programs. The SOFTWARE is not subject to the GPL or any other open source license.

If and insofar QIAGEN provides Third Party Software, the license terms for such Third Party Software shall additionally apply and prevail. If Open Software is provided, the license terms for such Open Software shall additionally apply and prevail. QIAGEN shall provide you with the corresponding source code of relevant Open Software, if the respective license terms of the Open Software include such obligation. QIAGEN shall inform if the SOFTWARE contains Third Party Software and/or Open Software and make available the corresponding license terms on request.

2. UPGRADES

If the SOFTWARE is an upgrade from a previous version, you are granted a single license to both copies, and you may not separately transfer the prior version(s) except as a one-time permanent transfer to another user of the latest upgrade and all prior versions as allowed in Section 4 below.

3. COPYRIGHT

The SOFTWARE, including any images, and text incorporated in the SOFTWARE, is copyrighted and is protected by German copyright laws and international treaty provisions. You may not copy any of the printed materials accompanying the SOFTWARE.

4. OTHER RESTRICTIONS

You may not rent or lease the SOFTWARE, but you may transfer the SOFTWARE and accompanying written materials on a permanent basis to another end user provided you delete the setup files from your computer, and the recipient agrees to the terms of this Agreement. You may not reverse engineer, decompile, or disassemble the SOFTWARE. Any transfer of the SOFTWARE must include the most recent upgrade and all prior versions.

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.

10. GOVERNING LAW, VENUE

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.

Third Party Software License Information

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 Copyright information

Trademarks

QIAGEN[®], Sample to Insight[®], Rotor-Gene[®] (QIAGEN Group). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

© 2017 QIAGEN, all rights reserved.

09/2017 HB-2468-001

www.qiagen.com

Technical Support

www.support.qiagen.com