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

For use with the Q-Rex Software v2.0 for Rotor-Gene® Q and QIAquant® to classify the genotypes of unknown samples
# Contents

1 Introduction

1.1 About this user manual

1.2 General information

1.2.1 Technical assistance

1.2.2 Policy statement

1.2.3 Version management

1.2.4 Intended use

1.3 Getting help

2 Working with end-point experiments

2.1 Set up an experiment

2.2 Run an experiment

2.3 Analyze an experiment

2.3.1 Add an analysis

2.3.1.1 Define target pair

2.3.2 View plots

2.3.2.1 View scatter plot

2.3.3 Define analysis parameters

2.3.4 Define genotyping parameters

2.3.5 Compensating crosstalk

2.3.5.1 Crosstalk compensation for QIAquant

2.3.5.2 Crosstalk compensation for Rotor-Gene Q

2.3.5.2.1 Performing crosstalk compensation

2.3.5.2.2 Defining crosstalk compensation settings

2.3.5.2.3 Saving compensation factors

2.3.5.2.4 Loading compensation factors

2.3.6 Copy analysis parameter

2.3.7 View results

2.3.7.1 Tubes view
2.4 Report and export results ................................................................. 2-23
2.5 Troubleshooting .............................................................................. 2-24
2.6 Glossary .......................................................................................... 2-24
3 Appendices .......................................................................................... 1
  3.1 Appendix A – Limited License Agreement ..................................... 3-2
  3.2 Appendix B – Liability clause ......................................................... 3-6
  3.3 Appendix C – Revision history ....................................................... 3-7
  3.4 Copyright information .................................................................... 3-8
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 EndPoint 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 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 EndPoint 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 the analysis aspects of the Q-Rex Software only. 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 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 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, QIAquant, Q-Rex Software, 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.

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 2.0.0.36.

1.2.4 Intended use

The Q-Rex EndPoint 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 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 or well 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 Plugin 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.

![Image of a scatter plot]

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 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 Q-Rex Software User Manual for details.
Normalization  See the Q-Rex Software User Manual for details.
Genotyping  See "Define genotyping parameters" for details.

Optionally, the following additional analysis features can be used:
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/wells or sample types, the regions are not automatically updated. Click Auto again to have the software calculate new regions based on the updated tube/well selection.

**Note:** Automatic region detection requires at least four tubes/wells for the active target. The sample type of that tube or well 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 ranges 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.

**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.
2.3.5.1 Crosstalk compensation for QIAquant

For the QIAquant, a generic crosstalk compensation can be applied that is suitable for most commonly used fluorophores. To set up crosstalk compensation, expand the Crosstalk compensation section in the Analysis tab of the drawer. Check the Compensate crosstalk checkbox to apply crosstalk compensation. The compensation appears on the normalized data of the channels.

Note: For a customer-specific crosstalk compensation, please contact QIAGEN Technical Services.

2.3.5.2 Crosstalk compensation for Rotor-Gene Q

Crosstalk compensation for Rotor-Gene Q allows you to remove signal bleed from one channel into another by:

Performing crosstalk compensation
Defining crosstalk compensation settings
Saving compensation factors
Loading compensation factors
2.3.5.2.1 Performing crosstalk compensation

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

To access the **Crosstalk compensation** settings dialog, click **Settings**. 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 that 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.
2.3.5.2.2 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 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 limit is set
- automatically
- based on NTCs

Select tubes for compensation
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.

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 and 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.

### 2.3.5.2.3 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.

2.3.5.2.4 Loading compensation factors

To load compensation factors saved on your system, click **Load factors** in the Define Crosstalk Compensation Settings dialog.
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 **X**, 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, select at least one compensation in the file to be loaded. Compensations that are not applicable to your experiment appear 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.
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 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.

2.3.7.1 Tubes view

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

<table>
<thead>
<tr>
<th>Tube</th>
<th>Sample name</th>
<th>Sample type</th>
<th>Genotype</th>
<th>Green</th>
<th>Yellow</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Heterozygous Yellow</td>
<td>Sample</td>
<td>Heterozygous Yellow 0.58 0.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>Heterozygous Yellow</td>
<td>Sample</td>
<td>Heterozygous Yellow 0.96 0.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Heterozygous Yellow</td>
<td>Sample</td>
<td>Heterozygous Yellow 0.41 0.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Heterozygous Yellow</td>
<td>Sample</td>
<td>Heterozygous Yellow 0.67 0.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>Heterozygous Green/Yellow</td>
<td>Sample</td>
<td>Heterozygous Green/Yellow 0.58 0.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>58</td>
<td>Heterozygous Green/Yellow</td>
<td>Sample</td>
<td>Heterozygous Green/Yellow 0.58 0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>Heterozygous Green/Yellow</td>
<td>Sample</td>
<td>Heterozygous Green/Yellow 0.58 0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Heterozygous Green/Yellow</td>
<td>Sample</td>
<td>Heterozygous Green/Yellow 0.58 0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>Heterozygous Green/Yellow</td>
<td>Sample</td>
<td>Heterozygous Green/Yellow 0.58 0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>Heterozygous Green/Yellow</td>
<td>Sample</td>
<td>Heterozygous Green/Yellow 0.58 0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>Heterozygous Green/Yellow</td>
<td>Sample</td>
<td>Heterozygous Green/Yellow 0.58 0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>Heterozygous Green/Yellow</td>
<td>Sample</td>
<td>Heterozygous Green/Yellow 0.58 0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>65</td>
<td>Heterozygous Green/Yellow</td>
<td>Sample</td>
<td>Heterozygous Green/Yellow 0.58 0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>Heterozygous Green/Yellow</td>
<td>Sample</td>
<td>Heterozygous Green/Yellow 0.58 0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>67</td>
<td>Heterozygous Green/Yellow</td>
<td>Sample</td>
<td>Heterozygous Green/Yellow 0.58 0.55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are organized into the following columns:

**Column label** | **Description**
--- | ---
Tube/Well | Indicates the tube position in the rotor or well position on the plate.
Sample name | Lists the sample name.
Sample type

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

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

<table>
<thead>
<tr>
<th>Column label</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>Lists the genotypes determined for each tube or well and each target pair.</td>
</tr>
<tr>
<td>Raw/Norm. end-point fluorescence</td>
<td>Displays the end-point fluorescence as raw or normalized values for each target depending on the chosen analysis setting.</td>
</tr>
</tbody>
</table>
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 behaviors 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:

<table>
<thead>
<tr>
<th>Message ID</th>
<th>Error text</th>
<th>Comments and suggestions</th>
</tr>
</thead>
<tbody>
<tr>
<td>20175000</td>
<td>Minimal number of selected tubes/wells required.</td>
<td>Automatic threshold calculation was attempted with an incorrect tubes selection.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>To use the automatic threshold calculation, at least one tube or well for the active target with a sample type other than Not in use must be selected.</td>
</tr>
</tbody>
</table>

Warning messages:

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

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

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The above limitation shall apply accordingly in case of:
- delay,
- compensation due to defect,
- compensation for wasted expenses.

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3.3 Appendix C – Revision history

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<tr>
<th>Document Revision History</th>
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<tbody>
<tr>
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3.4 Copyright information

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