

QuantStudio™ Design and Analysis Software

USER GUIDE

Command-Line Application for QuantStudio™ Design and Analysis desktop Software v1.2.x

for use with:

QuantStudio™ 3 and 5 Real-Time PCR Systems

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Revision A.0



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About this guide

Revision history

Revision	Date	Description
A.0	December 2015	Initial release

About this guide

Purpose

This guide provides technical information for engineering software that can be used to integrate the QuantStudio™ 3 and 5 Real-Time PCR Systems into a laboratory information system (LIS).

The document describes the:

- Command-line interface, which can be used to automate the creation of new experiment files and the export of data from existing files.
- Specifications of importable and exportable file formats, which are compatible with and generated by the QuantStudio™ Design and Analysis desktop Software.

Assumptions

This guide assumes that:

- All software elements (QuantStudio™ Design and Analysis desktop Software and other applications) reside and execute on the same local area network (LAN), and possibly on the same physical computer.
- The QuantStudio™ Design and Analysis desktop Software has been correctly installed and configured, including the separately licensed security, auditing, and electronic signature (SAE) module.
- The QuantStudio™ Design and Analysis desktop Software and the API, when running in headless mode, do not present user interface elements, relying on the invoking application to do so.

1

Command-line application

This chapter covers:

- About the command-line application 8
- Supporting files for experiment creation 9
- Precedence rules for experiment file generation 10
- Run the command-line application from a command prompt 11
- Example commands 14

About the command-line application

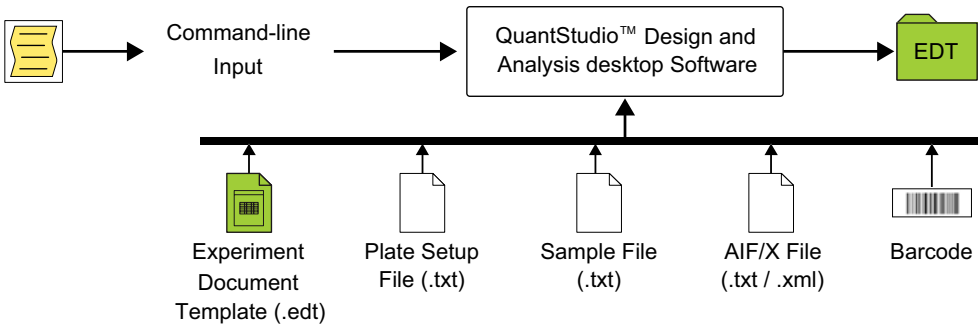
The QuantStudio™ desktop Software includes a command-line application that allows you to generate and export batches of experiment files from an MS DOS prompt or a batch file. The application is intended for advanced users who choose to create or export experiments using a scripting language.

IMPORTANT! After you use the command-line application to generate experiment files, validate the contents of the files by opening them in the QuantStudio™ desktop Software.

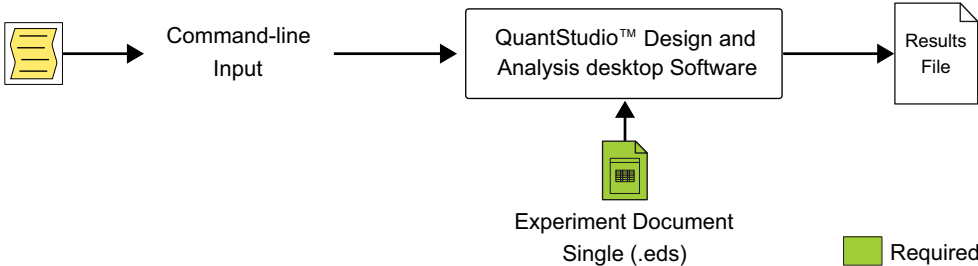
Command-line workflows

The command-line interface supports the workflows in the following figure. For each workflow, the figure shows both the required and optional supporting files.

Single Experiment File Creation Workflow



Export Workflow



Supporting files for experiment creation

The file generation function (`cmdlineutil.exe -expngen`) can use the files shown below. The command does not require all input files.

File	Description
assay information file (.aif or .aix)	A tab-delimited or XML data file that is shipped on a CD with each assay ordered from Thermo Fisher Scientific. (For some products, assay information files are available for download from the Thermo Fisher Scientific website following delivery.) The file, which contains data describing the assay, can be imported into the QuantStudio™ desktop Software for use in related experiments. See “Assay information file (AIF)” on page 23 for more information.
barcode file (.txt)	A user-created, line-separated text file that contains the barcode of each consumable for which you want to create an experiment file. See “Barcode file format” on page 23 for more information.
experiment document single file (.edt)	A QuantStudio™ desktop Software file that contains all information about a particular plate consumable, including metadata (name, barcode, comments), plate setup (well contents, assay definitions), run method (thermal cycling protocol), run results, analysis protocol, analysis results, audit records, and other plate-specific data.
experiment document template file (.edt)	A QuantStudio™ desktop Software file used as a template to create experiment files. The file can contain plate setup (well contents, assay definitions), run method (thermal cycling protocol), run results, analysis protocol, and other plate-specific data.
plate setup file (.txt)	A user-created, tab-delimited text file that describes the layout of a consumable for an experiment to be run on the QuantStudio™ 3 and 5 Systems. The file defines the arrangement of assays and samples on the consumable. See “Plate setup file format” on page 17 for more information.
sample file (.txt)	A user-created, tab-delimited text file containing sample data that can be imported into the QuantStudio™ desktop Software for use in related experiments. See “Sample file format” on page 22 for more information.

Precedence rules for experiment file generation

When generating experiment files (.eds), the QuantStudio™ desktop Software command-line interface relies on a set of precedence rules to resolve conflicts that arise from the data supplied by the input files. Assay information files (.aif or .aix), plate setup files (.txt), and template files (.edt) can contain data used to populate the same fields of new experiment files. For example, both template and plate setup files can contain location data for samples and assays. The following table describes the precedence rules for the data supplied by each input file.

Files used for experiment file (.eds) creation	Precedence rule												
Template file (.edt)	The values in the template take precedence except for: • Experiment Name – Determined by the File Name Convention preference. • Bar Code – Determined by the barcode, if present. Otherwise, the value is null. • Experiment File Name – Determined by the File Name Convention preference.												
• Template file (.edt) • Assay information file (.aif/.aix)	All values in the template file take precedence, except for: • Gene Expression Targets/Assay Definition • Genotyping Assay/SNP Definition • Passive Reference If any conflicts exist between the assay information file and the template for the attributes above, then the assay information file values always take precedence.												
• Template file (.edt) • Plate setup file (.txt)	All values in the template file take precedence, except for: <table border="0"> <tr> <td>• Target/Assay/SNP to Well Assignment</td><td>• Biological Group Color</td></tr> <tr> <td>• Sample to Well Assignment</td><td>• Target Color</td></tr> <tr> <td>• Task to Well Assignment</td><td>• Gene Expression Targets Definition</td></tr> <tr> <td>• Biological Group to Well Assignment</td><td>• Genotyping Assay Definition</td></tr> <tr> <td>• Well Quantity to Well Assignment</td><td>• Passive Reference</td></tr> <tr> <td>• Sample Color</td><td></td></tr> </table>	• Target/Assay/SNP to Well Assignment	• Biological Group Color	• Sample to Well Assignment	• Target Color	• Task to Well Assignment	• Gene Expression Targets Definition	• Biological Group to Well Assignment	• Genotyping Assay Definition	• Well Quantity to Well Assignment	• Passive Reference	• Sample Color	
• Target/Assay/SNP to Well Assignment	• Biological Group Color												
• Sample to Well Assignment	• Target Color												
• Task to Well Assignment	• Gene Expression Targets Definition												
• Biological Group to Well Assignment	• Genotyping Assay Definition												
• Well Quantity to Well Assignment	• Passive Reference												
• Sample Color													
• Template file (.edt) • Plate setup file (.txt) • Assay information file (.aif/.aix)	All values in the template take precedence, except for the following. The following assay information file values take precedence over Plate Setup and Template: • Gene Expression Targets/Detectors Definition • GT Assay/Marker Definition • Passive Reference The following Plate Setup values take precedence over the template: <table border="0"> <tr> <td>• Block Type</td><td>• Well Quantity to Well Assignment</td></tr> <tr> <td>• Target/Assay/Marker to Well Assignment</td><td>• Sample Color</td></tr> <tr> <td>• Sample to Well Assignment</td><td>• Biological Group Color</td></tr> <tr> <td>• Task to Well Assignment</td><td>• Target Color</td></tr> <tr> <td>• Biological Group to Well Assignment</td><td></td></tr> </table>	• Block Type	• Well Quantity to Well Assignment	• Target/Assay/Marker to Well Assignment	• Sample Color	• Sample to Well Assignment	• Biological Group Color	• Task to Well Assignment	• Target Color	• Biological Group to Well Assignment			
• Block Type	• Well Quantity to Well Assignment												
• Target/Assay/Marker to Well Assignment	• Sample Color												
• Sample to Well Assignment	• Biological Group Color												
• Task to Well Assignment	• Target Color												
• Biological Group to Well Assignment													

Run the command-line application from a command prompt

- Run the application
1. In the QuantStudio™ desktop Software, select **Start ▶ Run**.
 2. In the Run dialog box, enter **cmd** in the Open field, then click **OK**.
 3. In the DOS prompt, change to the installation directory and enter the command:
 - a. Enter **cd C:\Program Files\Applied Biosystems\QuantStudio Design and Analysis Software\bin**, then press **Enter**.
 - b. Enter **cmdlineutil.exe**, followed by **-expgen** or **-export**, then all applicable parameters and arguments. See “Command syntax and arguments” on page 11 for a complete list of command-line parameters.

- View the command-line help
- The command-line application includes a help function that provides the information in this chapter. To view help for:
- The entire application, enter **cmdlineutil.exe -help**
 - A particular function, enter **cmdlineutil.exe -expgen -help** to view the file generation help, or **cmdlineutil.exe -export -help** to view the file export help.

- Command syntax and arguments
- Syntax and arguments used for batch file creation
- The command used to create batches of files uses the following syntax:
- ```
cmdlineutil.exe -expgen [parameters]
```
- The following is a list of the acceptable parameters that can be included in any order. See “Example commands” on page 14 for an example of the experiment creation command.

---

**IMPORTANT!** Enclose file paths in double quotes to allow spaces in the string.

---

- a *<filepath>*  
(Optional) Specifies the path and name (*<filepath>*) of the assay information file (.aif or .aix) that the software uses to create new experiment files.  
Example: -a "C:\assayfiles\assayfile.aif"
- b *<filepath>*  
(Optional) Specifies the path and name (*<filepath>*) of the barcode file that the software uses to create new files. If the -b parameter is not used, then the software creates the number of experiments specified by the -n parameter.  
Example: -b "C:\barcodefiles\barcodefile.txt"
- c *<string>*  
(Optional) When the -f parameter is included, specifies the alphanumeric string that the software includes in the file names of the new experiments. If no value is supplied, “custom” is used as the default value.  
Example: -c "Batch001\_"

**-f** *<option>*

(Optional) Specifies the convention that the software uses to name the new files. The convention can consist of all or some of the following interchangeable arguments, in any order:

Custom Name Field – The alphanumeric string specified by the -c parameter.

ID – The barcode of the plate specified in the barcode file specified by the -b parameter.

Example: -f "Custom Name Field\_ID"

If the -f parameter is used without arguments, then the software names files according to the following convention: "Custom Name Field\_ID"

**-l** *<dirpath>*

(Required) Specifies the path of the directory (*<dirpath>*) to which the software saves the new files.

Example: -l "C:\Applied Biosystems\QuantStudio Design and Analysis Software\User Files\experiments"

Before creating experiment files, the software confirms whether the export location exists and aborts if the location does not exist.

**-m** *<filepath>*

(Optional) Specifies the path and name (*<filepath>*) of the sample file that the software uses to create new files.

Example: -m "C:\samplefiles\samplefile.txt"

**-n** *<integer>*

(Optional) If the -b parameter is not included, specifies number of experiments (*<integer>*) that the software will create. If no value is supplied, the software creates 25 experiments by default.

Example: -n 31

**-s** *<filepath>*

(Optional) Specifies the path and name (*<filepath>*) of the setup file that the software uses to create new files.

Example: -s "C:\setupfiles\setupfile.txt"

**-t** *<filepath>*

(Required) Specifies the path and name (*<filepath>*) of the QuantStudio™ desktop Software template file that the software uses to create new files.

Example: -t "C:\Applied Biosystems\QuantStudio Design and Analysis Software\User Files\experiments"

**-v**

(Optional) Configures the software to operate in verbose mode, where the software displays each operation as it is performed.

## Syntax and arguments used for results export

The command used to export the results from experiment files uses the following syntax:

```
cmdlineutil.exe -export [parameters]
```

The following is a list of the acceptable parameters that can be included in any order. See “Example commands” on page 14 for examples of the experiment export command.

---

**IMPORTANT!** Enclose file paths in double quotes to allow spaces in the string.

---

**-e <dirpath>**

(*Required*) Specifies the path to the directory (<dirpath>) that contains the experiment files (.eds) for which the software exports data.

Example: -e "C:\Applied Biosystems\QuantStudio Design and Analysis Software\User Files\experiments"

**-f <option>**

(*Required*) Specifies the format of the exported data (see page 26 for the export file specifications):

QuantStudio – Exports data in a native format compatible with the QuantStudio™ 3 and 5 Real-Time PCR Systems, or in a legacy format compatible with the QuantStudio™ 6 and 7 Flex Real-Time PCR Systems, QuantStudio™ Dx Real-Time PCR Instrument, and QuantStudio™ Real-Time PCR System.

RDML – Exports data in the real-time data markup language (RDML) format.

Example: -f "QuantStudio"

**-l <path>**

(*Optional*) Specifies the path (<path>) of the directory to which the software saves the exported files.

Example: -l "C:\exports\"

**-s <option>**

(*Optional*) Specifies the data spanning option (<option>) that determines how the software exports data from multiple experiments:

single – Exports data for all experiments into one contiguous data file.

multiple – Exports data for each experiment to a separate data file.

Example: -s "multiple"

**-x <filepath>**

(*Required*) Specifies the file format of the exported file:

QuantStudio export format – .txt, .xls, or .xlsx

RDML export format – .rdml

Example: -x "xlsx"

## Example commands

### Batch file creation

The following example uses all parameters described in “Command syntax and arguments” on page 11 (required and optional) to generate a set of experiment files.

```
cmdlineutil.exe -expngen -t "C:\Applied Biosystems\QuantStudio Design and
Analysis Software\User Files\experiments\templates\standard_curve.edt" -s
"C:\Applied Biosystems\QuantStudio Design and Analysis Software\User
Files\experiments\examples\Plate Setup
Files\QuantStudio_820629.txt" -m "C:\Applied Biosystems\QuantStudio
Design and Analysis Software\User Files\experiments\examples\SampleNames\
SampleFileNames.txt" -c "alloptionsused" -f "Plate Barcode_Custom Name
Field" -b "C:\barcodes - v12.txt" -l "C:\Applied Biosystems\QuantStudio
Design and Analysis Software\User Files\experiments"
```

For this example, the command-line application:

- Imports assay definitions from the AIF\_820629.txt assay information file.
- Imports sample names from the SampleFileNames.txt sample file.
- Generates an experiment for each barcode in the barcodes - v12.txt barcode file, where each new experiment uses the settings found in the standard\_curve.edt template file and the QuantStudio\_820629.txt setup file.

Note: The setup file links the information from the AIF\_820629.txt and SampleFileNames.txt to each new experiment file.

- Saves all generated files using the following naming convention:

*<barcode>\_alloptionsused*

- Saves all generated files to:

C:\Applied Biosystems\QuantStudio Design and Analysis  
Software\User Files\experiments\<date\time>

Note: The command-line application automatically creates a time-stamped folder at the export location for each batch operation. For example, the folder created for files generated on April 7, 2010 at 12:48:35 would be: 2010-04-07 124835

### Results export

The following example performs a real-time data markup language (RDML) export of experiments in the QuantStudio™ desktop Software experiments directory to the exports directory of the C drive. The software generates an RDML file for each individual experiment file.

```
cmdlineutil.exe -export -e "C:\Applied Biosystems\QuantStudio Design and
Analysis Software\User Files\experiments\" -f "QuantStudio"
-l "C:\exports\" -s "single" -x "rdml"
```

```
cmdlineutil.exe -export -e "C:\Applied Biosystems\QuantStudio Design and
Analysis Software\User Files\Tests\" -f "QuantStudio" -l "C:\exports\"
-s "single" -x "rdml"
```

# Import file formats and specifications

This chapter covers:

|                                       |    |
|---------------------------------------|----|
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| ■ Plate setup file format . ....      | 17 |
| ■ Sample file format . ....           | 22 |
| ■ Barcode file format . ....          | 23 |
| ■ Assay information file (AIF) . .... | 23 |

## About the importable files

The QuantStudio™ desktop Software supports several import file formats that can be used to automate experiment creation and assay and sample data import. The files can be used with the command-line application (see page 7) or the QuantStudio™ desktop Software application programming interface (API) to integrate the QuantStudio™ 3 and 5 Systems into a laboratory information system (LIS).

Note: The file specifications listed in this appendix are subject to change. For updated information, review the QuantStudio™ desktop Software Release Notes.

### About the import file formats

| File format                           | Description                                                                                                                                                                                                                                                                                                                | See...  |
|---------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| Plate setup file (.txt)               | A user-created, tab-delimited text file that describes the layout of a consumable for an experiment to be run on the QuantStudio™ 3 and 5 Systems. The file defines the arrangement of assays and samples on the consumable, and provides other experiment data, such as the thermal profile and data collection settings. | page 17 |
| Sample file (.txt)                    | A user-created, tab-delimited text file containing sample data that can be imported into the QuantStudio™ desktop Software for use in related experiments.                                                                                                                                                                 | page 22 |
| Assay information file (.aif or .aix) | A tab-delimited or XML data file that is shipped on a CD with each assay ordered from Thermo Fisher Scientific. The file, which contains data describing the assay, can be imported into the QuantStudio™ desktop Software for use in related experiments.                                                                 | page 23 |
| Barcode file (.txt)                   | A user-created, text file containing the barcodes of consumables for which you want to create experiment files using the command-line utility.                                                                                                                                                                             | page 23 |

### Conventions

The following conventions are used in the rest of this section:

- `normal` – Normal text must be entered exactly as it appears.
- `<italic>` – Italicized text between brackets must be substituted with custom values.
- `[ required text ]` – Text appearing between brackets is required information. All information inside the brackets must be present for the QuantStudio™ desktop Software to import it.
- `{ optional text }` – Text appearing between braces is optional.
- Unless noted otherwise, separate all fields in a row using a tab character (U+0009).
- Unless noted otherwise, end all rows using a carriage-return character (U+000D).

## Plate setup file format

You can use plate setup files to automatically populate setup information into an open experiment in the QuantStudio™ desktop Software or into new experiments created by the command-line application (see page 7). A plate setup file is a tab-delimited ASCII text file (.txt) that contains data that describes the location experiment data information. The files can be created manually using a text processor or generated automatically by third-party applications.

---

**IMPORTANT!** To guarantee successful import of the plate setup file into an experiment, the file must contain all the elements described in the following section and in the order that they appear.

---

### File structure

The plate setup file consists of a header, which specifies the instrument model for which the experiment is designed, and a sample setup section.

| Section                 | Description                                                                                                  | See...  |
|-------------------------|--------------------------------------------------------------------------------------------------------------|---------|
| Plate setup file header | Defines the instrument model for which the experiment is designed and the dye used as the passive reference. | page 17 |
| Plate setup file body   | Defines the contents of an instrument consumable, including target, SNP assay, sample, and task assignments. | page 18 |

### Plate setup file header

The plate setup file begins with a header that consists of two lines. Each line starts with an asterisk (\*) and ends with a carriage return in the following pattern:

`* <field name> = <field value>`

The header must contain the lines shown in the following table.

| Field             | Description                                                                     | Valid Values                                                                                                                                                                                                 |
|-------------------|---------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Instrument Type   | The model of QuantStudio™ 3 and 5 Systems for which the experiment is designed. | QuantStudio 3 or QuantStudio 5                                                                                                                                                                               |
| Passive Reference | The dye that the experiment will use as a passive reference.                    | <ul style="list-style-type: none"><li>The name of a dye in the Dye Library of the QuantStudio™ desktop Software†, or</li><li>&lt;blank&gt; if the consumable does not contain a passive reference.</li></ul> |

† Custom dyes are allowed as long as they are in the Dye Library.

**Note:** The QuantStudio™ desktop Software automatically removes any leading and trailing white space around the field name and field value.

**Example:**

```
* Instrument Type = QuantStudio 3
* Passive Reference = ROX
```

## Plate setup file body

The body of a plate setup file contains either target information, which can be imported into all experiments except Genotyping, or SNP assay information. This information can be imported into Genotyping experiments only. The body consists of three required elements (the header, the column header, and the body) that describe the contents of an instrument consumable. The sample setup column header and body can appear in any order.

---

**IMPORTANT!** Observe the following guidelines when creating a plate setup file:

- Do not insert blank lines between the sample setup header and the column header.
  - Do not use illegal characters, including backslash (\), tab, asterisk (\*), hard return, soft return, brackets([ or ]), or comma (,).
- 

### Sample setup header

The header contains the label that defines the beginning of the sample setup data.

Example:

```
[Sample Setup]
```

### Sample setup column header

The column header contains the headings that define the positions of the data columns in the sample setup body. The headings are separated by tab characters. See “Plate setup data columns” on page 19 for a list of the data column headers.

Example:

```
Well Sample Name Sample Color Biogroup Name Biogroup Color Target Name...
```

### Sample setup body

Contains the sample setup data where each row defines the contents of a single well on the consumable, including the: well contents (sample, target, or SNP assay added to the well), task assignments, and comments. If a well contains multiple assays (multiplex PCR), the data for the additional assays are defined on separate lines by repeating the well designation. See “Plate setup data columns” on page 19 for a list of the data column headers.

Note: The sample setup data rows can occur in any order.

Example:

```
Well Sample Name Sample Color Biogroup Name Biogroup Color Target Name...
1 Liver cDNA "RGB(25,0,0)"
2 Liver cDNA "RGB(25,0,0)"
3 Liver cDNA "RGB(25,0,0)"
4 Heart cDNA "RGB(0,25,0)"
5 Heart cDNA "RGB(0,25,0)"
...
```

Plate setup data  
columns

The following table lists the headings and columns that are present in the plate setup file body of all experiment types followed by the columns that are specific to Genotyping experiments and non-Genotyping experiments.

|                       | Column name      | Description                                                                                                                                                                       | Valid values                                                 |
|-----------------------|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|
| All experiments       | Well             | The number of the well on the consumable, where the well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.               | <Positive integer (1 to 96/384)> <sup>‡</sup>                |
|                       | Sample Name      | The name of the sample contained by the associated well.                                                                                                                          | <100-character string>                                       |
|                       | Sample Color     | (Optional) The RGB color of the associated sample.                                                                                                                                | "RGB (<r>, <g>, <b>) "§                                      |
|                       | Biogroup Name    | (Optional) The name of the associated biological group.                                                                                                                           | <100-character string>                                       |
|                       | Biogroup Color   | (Optional) The RGB color of the biological group.                                                                                                                                 | "RGB (<r>, <g>, <b>) "§                                      |
|                       | Comments         | (Optional) Additional text that describes the well.                                                                                                                               | "<1024-character string>"                                    |
| All except Genotyping | Target Name      | The name of the target detected or amplified by the assay in the associated well.                                                                                                 | <100-character string> <sup>††</sup>                         |
|                       | Target Color     | (Optional) The RGB color of the target.                                                                                                                                           | "RGB (<r>, <g>, <b>) "§                                      |
|                       | Task             | The task assignment of the target assay at the well. <sup>†</sup>                                                                                                                 | <UNKNOWN   STANDARD   NTC   ENDOGENOUS   IPC   BlockedIPC>   |
|                       | Reporter         | The reporter dye used by the associated target assay.                                                                                                                             | <dye name> <sup>††††</sup>                                   |
|                       | Quencher         | The quencher dye used by the associated target assay.                                                                                                                             | <dye name> <sup>††</sup>                                     |
|                       | Quantity         | (Optional) The quantity of standard present in the given well expressed as a float or integer. If the associated well is not assigned the STANDARD task, then the field is blank. | <float or Integer>                                           |
| Genotyping only       | SNP Assay Name   | The name of the SNP assay detected or amplified by the assay in the associated well.                                                                                              | <100-character string> <sup>††</sup>                         |
|                       | SNP Assay Color  | (Optional) SNP assay color in RGB                                                                                                                                                 | "RGB (<r>, <g>, <b>) "§                                      |
|                       | Task             | The task assignment of the SNP assay at the well. <sup>†</sup>                                                                                                                    | <UNKNOWN   NTC   PC_ALLELE_1   PC_ALLELE_2   PC_ALLELE_BOTH> |
|                       | Allele1 Name     | The name of the first allele detected by the SNP assay.                                                                                                                           | <100-character string> <sup>††</sup>                         |
|                       | Allele1 Color    | The RGB color used to represent data for the first allele.                                                                                                                        | "RGB (<r>, <g>, <b>) "§                                      |
|                       | Allele1 Reporter | The reporter dye used to label the probe for the first allele.                                                                                                                    | <dye name> <sup>††††</sup>                                   |
|                       | Allele1 Quencher | The quencher dye used to label the probe for the first allele.                                                                                                                    | <dye name> <sup>††</sup>                                     |
|                       | Allele2 Name     | The name of the second allele detected by the SNP assay.                                                                                                                          | <100-character string> <sup>††</sup>                         |
|                       | Allele2 Color    | The RGB color used to represent data for the second allele.                                                                                                                       | "RGB (<r>, <g>, <b>) "§                                      |
|                       | Allele2 Reporter | The reporter dye used to label the probe for the second allele.                                                                                                                   | <dye name> <sup>††††</sup>                                   |
|                       | Allele2 Quencher | The quencher dye used to label the probe for the second allele.                                                                                                                   | <dye name> <sup>††</sup>                                     |

<sup>†</sup> See the *QuantStudio™ Design and Analysis desktop Software Getting Started Guide* to determine the tasks applicable to the experiment.

<sup>‡</sup> Cannot be blank.

§ Contains (r)ed, (b)lue, and (g)reen color values between 0 to 255. The field must be set within double quotes with no spaces between the values.

<sup>††</sup> Can be empty if the Task field is empty. Otherwise, the field must contain a value.

<sup>††††</sup> The dye must already exist in the QuantStudio™ desktop Software Dye Library. The dye name must be 100 characters or less.

The following example shows a plate setup file created for a quantitative PCR experiment to be run on the QuantStudio™ 3 and 5 Systems. The experiment evaluates the expression of two targets (CCKAR and GH1) in three samples (cDNA from the liver, heart, and brain). For both assays, the probes are labeled with the FAM™ reporter dye and the non-fluorescent quencher (NFQ-MGB). Biological groups are not used in this experiment.

```
* Instrument Type = QuantStudio 3 or QuantStudio 5
* Passive Reference = ROX
[Sample Setup]
Well Sample Name Sample Color Biogroup Name Biogroup Color Target Name Target Color Task Reporter Quencher Quantity Comments
1 Liver cDNA "RGB(25,0,0)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
2 Liver cDNA "RGB(25,0,0)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
3 Liver cDNA "RGB(25,0,0)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
4 Heart cDNA "RGB(0,25,0)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
5 Heart cDNA "RGB(0,25,0)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
6 Heart cDNA "RGB(0,25,0)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
7 Brain cDNA "RGB(0,0,25)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
8 Brain cDNA "RGB(0,0,25)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
9 Brain cDNA "RGB(0,0,25)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
...
376 Liver cDNA "RGB(25,0,0)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
377 Liver cDNA "RGB(25,0,0)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
378 Liver cDNA "RGB(25,0,0)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
379 Heart cDNA "RGB(0,25,0)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
380 Heart cDNA "RGB(0,25,0)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
381 Heart cDNA "RGB(0,25,0)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
382 Brain cDNA "RGB(0,0,25)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
383 Brain cDNA "RGB(0,0,25)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
384 Brain cDNA "RGB(0,0,25)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
```

The following example shows a plate setup file for a multiplex version of the experiment above, where the assays for the two targets (CCKAR and GH1 targets) are added to the same well. For both assays, the probes are labeled with the FAM™ reporter dye and the non-fluorescent quencher (NFQ-MGB).

```
* Instrument Type = QuantStudio 3 or QuantStudio 5
* Passive Reference = ROX
[Sample Setup]
Well Sample Name Sample Color Biogroup Name Biogroup Color Target Name Target Color Task Reporter Quencher Quantity Comments
1 Liver cDNA "RGB(25,0,0)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
1 Liver cDNA "RGB(25,0,0)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
2 Liver cDNA "RGB(25,0,0)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
2 Liver cDNA "RGB(25,0,0)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
3 Liver cDNA "RGB(25,0,0)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
3 Liver cDNA "RGB(25,0,0)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
4 Heart cDNA "RGB(0,25,0)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
4 Heart cDNA "RGB(0,25,0)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
5 Heart cDNA "RGB(0,25,0)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
5 Heart cDNA "RGB(0,25,0)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
6 Heart cDNA "RGB(0,25,0)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
6 Heart cDNA "RGB(0,25,0)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
7 Brain cDNA "RGB(0,0,25)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
7 Brain cDNA "RGB(0,0,25)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
8 Brain cDNA "RGB(0,0,25)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
8 Brain cDNA "RGB(0,0,25)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
9 Brain cDNA "RGB(0,0,25)" CCKAR "RGB(98,25,0)" ENDOGENOUS FAM NFQ-MGB
9 Brain cDNA "RGB(0,0,25)" GH1 "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
...
```

## Presence/Absence experiments

The following example shows a plate setup file created for a Presence/Absence experiment to be run on a QuantStudio™ 3 and 5 Systems. The experiment screens samples for the presence of a pathogen (*E. coli* O157:H7). The detection assay uses FAM™ and VIC® dye-labeled probes to amplify a unique genomic sequence and an internal positive control (IPC).

```
* Instrument Type = QuantStudio 3 or QuantStudio 5
* Passive Reference = ROX
[Sample Setup]
Well Sample Name Sample Color Biogroup Name Biogroup Color Target Name Target Color Task Reporter Quencher Quantity Comments
1 Control "RGB(25,0,0)" E.coli "RGB(98,25,0)" NTC FAM NFQ-MGB
1 Control "RGB(25,0,0)" IPC "RGB(98,25,0)" NTC VIC NFQ-MGB
2 Control "RGB(25,0,0)" E.coli "RGB(98,25,0)" NTC FAM NFQ-MGB
2 Control "RGB(25,0,0)" IPC "RGB(98,25,0)" NTC VIC NFQ-MGB
3 Control "RGB(25,0,0)" E.coli "RGB(98,25,0)" NTC FAM NFQ-MGB
3 Control "RGB(25,0,0)" IPC "RGB(98,25,0)" NTC VIC NFQ-MGB
4 Pos Control "RGB(0,25,0)" E.coli "RGB(98,25,0)" IPC FAM NFQ-MGB
4 Pos Control "RGB(0,25,0)" IPC "RGB(98,25,0)" IPC VIC NFQ-MGB
5 Pos Control "RGB(0,25,0)" E.coli "RGB(98,25,0)" IPC FAM NFQ-MGB
5 Pos Control "RGB(0,25,0)" IPC "RGB(98,25,0)" IPC VIC NFQ-MGB
6 Pos Control "RGB(0,25,0)" E.coli "RGB(98,25,0)" IPC FAM NFQ-MGB
6 Pos Control "RGB(0,25,0)" IPC "RGB(98,25,0)" IPC VIC NFQ-MGB
7 Blocked IPC "RGB(0,0,25)" E.coli "RGB(98,25,0)" BlockedIPC FAM NFQ-MGB
7 Blocked IPC "RGB(0,0,25)" IPC "RGB(0,0,105)" BlockedIPC VIC NFQ-MGB
8 Blocked IPC "RGB(0,0,25)" E.coli "RGB(0,0,105)" BlockedIPC FAM NFQ-MGB
8 Blocked IPC "RGB(0,0,25)" IPC "RGB(0,0,105)" BlockedIPC VIC NFQ-MGB
9 Blocked IPC "RGB(0,0,25)" E.coli "RGB(0,0,105)" BlockedIPC FAM NFQ-MGB
9 Blocked IPC "RGB(0,0,25)" IPC "RGB(0,0,105)" BlockedIPC VIC NFQ-MGB
10 Sample01 "RGB(90,0,0)" E.coli "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
10 Sample01 "RGB(90,0,0)" IPC "RGB(0,0,105)" UNKNOWN VIC NFQ-MGB
11 Sample01 "RGB(90,0,0)" E.coli "RGB(0,0,105)" UNKNOWN FAM NFQ-MGB
11 Sample01 "RGB(90,0,0)" IPC "RGB(0,0,105)" UNKNOWN VIC NFQ-MGB
...
```

## Genotyping experiments

The following example shows a plate setup file created for a Genotyping experiment to be run on a QuantStudio™ 3 and 5 Systems. The experiment screens samples for one SNP target (rs15934), using a set of allele-specific probes labeled with the FAM™ and VIC® reporter dyes and the non-fluorescent quencher (NFQ-MGB).

```
* Instrument Type = QuantStudio 3 or QuantStudio 5
* Passive Reference = ROX
[Sample Setup]
Well Sample Name Sample Color SNP Assay Name SNP Assay Color Task Allele1 Name Allele1 Color Allele1 Reporter Allele1 Quencher Allele2 Name Allele2 Color Allele2 Reporter Allele2 Quencher Comments
1 Neg Control "RGB(25,0,0)" SNP rs15934 "RGB(0,75,0)" NTC G "RGB(0,0,50)" VIC NFQ-MGB A "RGB(0,50,0)" FAM NFQ-MGB
2 Neg Control "RGB(25,0,0)" SNP rs15934 "RGB(0,75,0)" NTC G "RGB(0,0,50)" VIC NFQ-MGB A "RGB(0,50,0)" FAM NFQ-MGB
3 Neg Control "RGB(25,0,0)" SNP rs15934 "RGB(0,75,0)" NTC G "RGB(0,0,50)" VIC NFQ-MGB A "RGB(0,50,0)" FAM NFQ-MGB
4 A11 Control "RGB(25,0,0)" SNP rs15934 "RGB(0,75,0)" PC_ALLELE_1 G "RGB(0,0,50)" VIC NFQ-MGB A "RGB(0,50,0)" FAM NFQ-MGB
5 A11 Control "RGB(25,0,0)" SNP rs15934 "RGB(0,75,0)" PC_ALLELE_1 G "RGB(0,0,50)" VIC NFQ-MGB A "RGB(0,50,0)" FAM NFQ-MGB
6 A11 Control "RGB(25,0,0)" SNP rs15934 "RGB(0,75,0)" PC_ALLELE_1 G "RGB(0,0,50)" VIC NFQ-MGB A "RGB(0,50,0)" FAM NFQ-MGB
7 A12 Control "RGB(25,0,0)" SNP rs15934 "RGB(0,75,0)" PC_ALLELE_2 G "RGB(0,0,50)" VIC NFQ-MGB A "RGB(0,50,0)" FAM NFQ-MGB
8 A12 Control "RGB(25,0,0)" SNP rs15934 "RGB(0,75,0)" PC_ALLELE_2 G "RGB(0,0,50)" VIC NFQ-MGB A "RGB(0,50,0)" FAM NFQ-MGB
9 A12 Control "RGB(25,0,0)" SNP rs15934 "RGB(0,75,0)" PC_ALLELE_2 G "RGB(0,0,50)" VIC NFQ-MGB A "RGB(0,50,0)" FAM NFQ-MGB
10 Sample01 "RGB(25,0,0)" SNP rs15934 "RGB(0,75,0)" UNKNOWN G "RGB(0,0,50)" VIC NFQ-MGB A "RGB(0,50,0)" FAM NFQ-MGB
11 Sample01 "RGB(25,0,0)" SNP rs15934 "RGB(0,75,0)" UNKNOWN G "RGB(0,0,50)" VIC NFQ-MGB A "RGB(0,50,0)" FAM NFQ-MGB
12 Sample01 "RGB(25,0,0)" SNP rs15934 "RGB(0,75,0)" UNKNOWN G "RGB(0,0,50)" VIC NFQ-MGB A "RGB(0,50,0)" FAM NFQ-MGB
13 Sample02 "RGB(25,0,0)" SNP rs15934 "RGB(0,75,0)" UNKNOWN G "RGB(0,0,50)" VIC NFQ-MGB A "RGB(0,50,0)" FAM NFQ-MGB
14 Sample02 "RGB(25,0,0)" SNP rs15934 "RGB(0,75,0)" UNKNOWN G "RGB(0,0,50)" VIC NFQ-MGB A "RGB(0,50,0)" FAM NFQ-MGB
15 Sample02 "RGB(25,0,0)" SNP rs15934 "RGB(0,75,0)" UNKNOWN G "RGB(0,0,50)" VIC NFQ-MGB A "RGB(0,50,0)" FAM NFQ-MGB
...
```

## Sample file format

The QuantStudio™ desktop Software can import sample files to populate sample information into an open experiment. A sample file is a tab-delimited ASCII text file (.txt) that contains sample/well designations, and custom sample properties. The files can be created manually using a text processor or generated automatically by third-party applications.

---

**IMPORTANT!** To guarantee successful import, the file must contain all the elements described in the following section and in the order that they appear.

---

Note: The command-line application (see page 7) does not import sample files. If you are using the application to create experiments, use plate setup files to import sample information into the new experiments (see “Plate setup file format” on page 17).

### File structure

#### Sample file header row

The sample file begins with an optional header row that contains column headers for well number (“Well”), sample name (“Sample Name”), and optional custom properties names. The order of the columns is important and cannot be changed.

#### Sample file body

A body of rows, containing the sample data, follows the optional header row. Each body row defines the sample information for a single well on the consumable, including: well number, sample name, and any applicable custom fields. The body can contain data for a subset of wells on the consumable, so the rows for empty wells can be omitted from the file. The sample body rows can occur in any order.

| Column name        | Description                                                                                                                                                         | Valid values                     |
|--------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|
| Well               | The number of the well on the consumable, where the well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom. | <Positive integer (1 to 96/384)> |
| Sample Name        | The name of the sample contained by the associated well.                                                                                                            | <100-character string>           |
| Custom1... Custom6 | (Optional) Additional text that describes the sample in the well.                                                                                                   | <1024-character string>          |

### Example

| Well | Sample Name | Custom1 | Custom2 | Custom3 | Custom4 | Custom5 | Custom6 |
|------|-------------|---------|---------|---------|---------|---------|---------|
| 21   | Sample 1    | test1   | test2   | test3   | test4   | test5   | test6   |
| 22   | Sample 2    | test1   | test2   | test3   | test4   | test5   | test6   |
| 23   | Sample 3    | test1   | test3   | test4   | test5   | test6   |         |
| 1    | Sample 5    | test1   | test2   | test3   | test4   | test5   | test6   |
| 2    | Sample 6    | test1   | test2   | test3   | test4   | test5   | test6   |
| 3    | Sample 7    | test1   | test2   | test3   | test4   | test5   | test6   |
| 4    | Sample 8    | test1   | test2   | test3   | test4   | test5   | test6   |
| ...  |             |         |         |         |         |         |         |

## Barcode file format

The QuantStudio™ desktop Software command-line application can import barcode files to populate experiment files (.eds) it generates with barcode information. A barcode file is a tab-delimited ASCII text file (.txt) that contains a list of barcodes. The files can be created manually using a text processor or generated automatically by third-party applications.

---

**IMPORTANT!** To guarantee successful import, the file must contain all the elements described in the following section and in the order that they appear.

---

### File structure

The barcode file contains a list of barcodes, where each line defines a single barcode terminated by a carriage return. The barcodes can occur in any order and cannot contain starting or trailing white space.

**Note:** The QuantStudio™ desktop Software command-line application does not validate the barcodes.

### Example

```
HA996346102
IB894812348
DD834814679
EK209825848
AF092387348
FF225676243
```

## Assay information file (AIF)

The QuantStudio™ desktop Software command-line application can import data for Thermo Fisher Scientific assays from assay information files (.aif), which are shipped on a CD with each assay order. The .aif contains technical details about all assays in the shipment. It includes information about assay concentrations; reporters and quenchers used; part and lot numbers; and assay, vial, and plate ID numbers. The file name includes the number from the barcode on the plate.



# 3

## Export file formats and specifications

This chapter covers:

- About the exportable files . . . . . 26
- QuantStudio™ export formats . . . . . 27
- RDML export format . . . . . 44

## About the exportable files

This chapter describes the export formats supported by the QuantStudio™ Design and Analysis desktop Software. The information provided is intended for users who want to integrate the QuantStudio™ Design and Analysis desktop Software with third-party applications, including downstream analysis software and laboratory information system (LIS) tools.

Note: The file specifications listed in this appendix are subject to change. For updated information, review the QuantStudio™ Design and Analysis desktop Software Release Notes.

### Export formats

The QuantStudio™ Design and Analysis desktop Software can export setup and results data from experiment files (.eds) in several file formats that allow further downstream analysis. The export formats feature standardized data structures and markup to maximize accessibility by downstream applications.

The QuantStudio™ Design and Analysis desktop Software supports the following export formats:

| File format                     | Description                                                                                                                                                                                                                            | See...  |
|---------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| QuantStudio™ 3 or 5 export file | A QuantStudio™ 3 or 5 formatted text file that contains setup and/or results data exported from an experiment file (.eds).                                                                                                             | page 27 |
| RDML export file                | A compressed XML file that contains setup and/or results data exported from an experiment file (.eds) and parsed in Real-time PCR Data Markup Language (RDML). The file is stored as a compressed file using the PKZIP archive format. | page 44 |

## QuantStudio™ export formats

The QuantStudio™ Design and Analysis desktop Software can export setup and results data from experiment files (.eds) to tab-delimited text files (.txt) in a native QuantStudio™ 3 or 5 System export format (QuantStudio™ 3 or 5) and a legacy QuantStudio™ Dx Instrument format (QuantStudio™ Dx). Data exported in the QuantStudio™ export format can be opened by common spreadsheet applications, such as Microsoft® Excel®, or imported by laboratory information management system (LIS) applications or databases that have been configured to parse the file format.

### File structure

The following table shows the data structure common to data exported in the QuantStudio™ export format, regardless of experiment type. Each row represents one or more lines of data in the exported file corresponding to a common functional group. The QuantStudio™ export format allow the user to customize and/or omit columns. The columns and orders described below are the default configuration (all columns in their natural order). Actual files may contain fewer columns if the user modified the configuration.

| Section             | Description                                                                                                                                                                                                                                                                                                                    | See...  |
|---------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| File header         | Describes the qualities of the QuantStudio™ 3 or 5 System used to run the experiment and several general experiment properties, such as the date and time of the run and the dye used as the passive reference.                                                                                                                | page 28 |
| Sample setup data   | Describes the configuration of samples on the experiment consumable, including sample location, target or SNP assay properties, and task assignments.                                                                                                                                                                          | page 30 |
| Raw data            | Contains the raw data collected by the QuantStudio™ 3 or 5 System during the experiment run.                                                                                                                                                                                                                                   | page 32 |
| Amplification data  | Contains the normalized data collected during the cycling stage of PCR amplification, which the QuantStudio™ Design and Analysis desktop Software uses to generate the amplification plot.<br><br>Note: Not applicable for Presence/Absence, Genotyping, or Melt Curve experiments that are run without a PCR (cycling) stage. | page 33 |
| Multicomponent data | Contains the spectral data used by the QuantStudio™ Design and Analysis desktop Software to generate the multicomponent plot that displays the contribution of each dye over the duration of the PCR run.                                                                                                                      | page 33 |
| Results data        | Contains the normalized, processed, and analyzed data generated by the QuantStudio™ Design and Analysis desktop Software.                                                                                                                                                                                                      | page 34 |

## File header

The plate setup file begins with a header that describes the qualities of the QuantStudio™ 3 or 5 System used to run the experiment and several other general experiment properties. Each line starts with an asterisk (\*) and ends with a carriage return in the following pattern:

```
* <field name> = <field value>
```

Note: The QuantStudio™ Design and Analysis desktop Software automatically removes any leading and trailing white space around the field name and field value.

The header contains the lines listed in the following table.

| Field                                    | Description                                                                                                                                                    | Output                                                                            |
|------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| Block Type                               | The model of the sample block installed to the QuantStudio™ 3 or 5 System at the time the experiment was run.                                                  | 96-well or 384-well                                                               |
| Calibration Date <sup>†</sup>            | The date and time that the current background, ROI, uniformity, normalization, or dye calibration was performed and when it will expire.                       | <date and time>                                                                   |
| Calibration Expiration Date <sup>†</sup> |                                                                                                                                                                |                                                                                   |
| Calibration Expired <sup>†</sup>         | Expiration status of the calibration. Indicates whether the calibration of the QuantStudio™ 3 or 5 System was current at the time that the experiment was run. | Yes or No                                                                         |
| Chemistry                                | The chemistry of the experiment.                                                                                                                               | <100-character string>                                                            |
| Date Created                             | The date and time that the QuantStudio™ 3 or 5 System started exporting the experiment.                                                                        | <date and time>                                                                   |
| Experiment Barcode                       | The barcode of the PCR reaction plate entered into the optional Plate Barcode field.                                                                           | <100-character string>                                                            |
| Experiment Comment                       | The comment to associate with the experiment entered into the optional Comment field.                                                                          | <2000-character string>                                                           |
| Experiment File Name                     | The path to the experiment file on the local computer hard drive.                                                                                              | <filepath>                                                                        |
| Experiment Name                          | The name of experiment entered into the Experiment Name field.                                                                                                 | <100-character string>                                                            |
| Experiment Run End Time <sup>†</sup>     | The date and time that the QuantStudio™ 3 or 5 System finished running the experiment.                                                                         | <date and time>                                                                   |
| Experiment Type                          | The type of chemistry application for which the experiment is designed.                                                                                        | Standard Curve, Presence/Absence, Relative Standard Curve, or DDCT Quantification |
| Instrument Name <sup>†</sup>             | The name of the QuantStudio™ 3 or 5 System that ran the experiment.                                                                                            | <100-character string>                                                            |
| Instrument Serial Number <sup>†</sup>    | The serial number of the QuantStudio™ 3 or 5 System that ran the experiment.                                                                                   | <100-character string>                                                            |
| Instrument Type                          | The model of the QuantStudio™ 3 or 5 System that ran the experiment.                                                                                           | QuantStudio 3 or QuantStudio 5                                                    |
| Passive Reference                        | The dye used as a passive reference (or blank if the consumable did not contain one).                                                                          | <100-character string>                                                            |
| Quantification cycle method              | The method of quantification for the associated experiment.                                                                                                    | <100-character string>                                                            |

| Field                                   | Description                                                                                                   | Output                          |
|-----------------------------------------|---------------------------------------------------------------------------------------------------------------|---------------------------------|
| Signal Smoothing On                     | The smoothing setting status for the experiment. Indicates whether smoothing is turned on for the experiment. | true or false                   |
| Stage\Cycle where Analysis is performed | The stage and cycle during the thermal cycling protocol when the QuantStudio™ 3 or 5 System collected data.   | Stage <integer>, Step <integer> |
| User Name                               | Name of the user entered into the User Name field.                                                            | <100-character string>          |

† This field present only after the experiment has been run.

## Sample setup data

When selected as an export option, the QuantStudio™ Design and Analysis desktop Software exports sample setup data after the file header. The sample setup data describes the sample configuration on the experiment consumable, including positions, sample names, task assignments, assay information, and color coding.

The data consists of a column header followed by the sample data fields, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the QuantStudio™ Design and Analysis desktop Software lists the data for each additional assay on separate rows, repeating the well number and sample information. The data included in the sample setup data export varies depending on experiment type.

This section describes the following sample setup data formats:

- Quantification and Presence/Absence experiments . . . . . 30
- Genotyping experiments . . . . . 31

## Quantification and Presence/Absence experiments

The table below describes the sample setup data that can be exported from Absolute Quantification, Relative Quantification, or Presence/Absence experiments. The body can contain all or some of the data columns below depending on the export configuration.

Note: For Genotyping experiments, see “Genotyping experiments” on page 31.

| Column       | Description                                                                                                        | Output                                     |
|--------------|--------------------------------------------------------------------------------------------------------------------|--------------------------------------------|
| Well         | The number of the well on the consumable.                                                                          | Integer (1 to 96/384) <sup>†</sup>         |
| Sample Name  | The name of the sample contained by the well.                                                                      | 100-character string                       |
| Sample Color | The RGB color of the associated sample.                                                                            | "RGB (<r>, <g>, <b>) " <sup>‡</sup>        |
| Target Name  | The name of one target in the well, if applicable. If a well contains multiple targets one row is used per target. | 100-character string                       |
| Target Color | The RGB color of the associated assay.                                                                             | "RGB (<r>, <g>, <b>) " <sup>‡</sup>        |
| Task         | The task the target is used for in this well.                                                                      | UNKNOWN, STANDARD, IPC, NTC, or BlockedIPC |
| Reporter     | The reporter dye that labels the probe for the target assay.                                                       | 100-character string                       |
| Quencher     | The quencher dye that labels the probe for the target assay.                                                       | 100-character string                       |
| Quantity     | Standard quantity (if applicable). This column only appears for Standard Curve experiments.                        | Float or Integer                           |
| Comments     | Additional text that describes the well.                                                                           | 1024-character string                      |

<sup>†</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

<sup>‡</sup> Contains (r)ed, (b)lue, and (g)reen color values, each between 0 to 255. The field is enclosed in double quotes with no spaces between the values.

## Genotyping experiments

The table below describes the sample setup data that can be exported from a Genotyping experiment. The body can contain all or some of the data columns below depending on the export configuration.

Note: For all other experiments, see “Quantification and Presence/Absence experiments” on page 30.

| Column           | Description                                                                                                                                         | Output                              |
|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|
| Well             | The number of the well on the consumable.                                                                                                           | Integer (1 to 96/384) <sup>†</sup>  |
| Sample Name      | The name of the sample contained by the well.                                                                                                       | 100-character string                |
| Sample Color     | The RGB color of the associated sample.                                                                                                             | "RGB (<r>, <g>, <b>) " <sup>‡</sup> |
| SNP Assay Name   | The name of the SNP assay applied to the well. If the well contains multiple assays, the data for each SNP assay are exported in an additional row. | 100-character string                |
| SNP Assay Color  | The RGB color of the associated SNP assay.                                                                                                          | "RGB (<r>, <g>, <b>) " <sup>‡</sup> |
| Task             | The task assignment of the SNP assay at the well.                                                                                                   | UNKNOWN or NTC                      |
| Allele1 Name     | The name of the first allele for the associated SNP assay.                                                                                          | 100-character string                |
| Allele1 Color    | The RGB color of the first allele for the associated SNP assay.                                                                                     | "RGB (<r>, <g>, <b>) " <sup>‡</sup> |
| Allele1 Reporter | The reporter dye that labels the probe for the first allele.                                                                                        | 100-character string                |
| Allele1 Quencher | The quencher dye that labels the probe for the first allele.                                                                                        | 100-character string                |
| Allele2 Name     | The name of the second allele for the associated SNP assay.                                                                                         | 100-character string                |
| Allele2 Color    | The RGB color of the second allele for the associated SNP assay.                                                                                    | "RGB (<r>, <g>, <b>) " <sup>‡</sup> |
| Allele2 Reporter | The reporter dye that labels the probe for the second allele.                                                                                       | 100-character string                |
| Allele2 Quencher | The quencher dye that labels the probe for the second allele.                                                                                       | 100-character string                |
| Comments         | Additional text that describes the well                                                                                                             | 1024-character string               |

<sup>†</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

<sup>‡</sup> Contains (r)ed, (b)lue, and (g)reen color values, each between 0 to 255. The field is enclosed in double quotes with no spaces between the values.

## Raw data

The QuantStudio™ Design and Analysis desktop Software can export the unprocessed raw data (R) collected by the QuantStudio™ 3 or 5 System during the experiment run. The raw data consists of fluorescence readings collected by the QuantStudio™ 3 or 5 System that have not been normalized to the passive reference.

The section begins with a column header followed by the raw data, where each row contains the data for a single well separated by tab characters. Each line of raw data consists of readings sorted by filter set, where each filter set represents an excitation/emission filter pair that was selected during experiment setup. The bins are named for the corresponding filter combination according to the following convention:

*<excitation filter name>-<emission filter name>*

| Column       | Description                                                                                                                  | Output                             |
|--------------|------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| Well         | The number of the well on the consumable.                                                                                    | Integer [1 to 96/384] <sup>†</sup> |
| Cycle        | The cycle of the run during which the QuantStudio™ 3 or 5 System recorded the fluorescence.                                  | Integer                            |
| <Filter Set> | The raw fluorescence for the well measured by the QuantStudio™ 3 or 5 System for the associated bin at the designated cycle. | Float                              |

<sup>†</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## Amplification data

The QuantStudio™ Design and Analysis desktop Software can export the processed amplification data used to generate the amplification plot of a real-time PCR experiment. The amplification data ( $R_n$ ) are the raw fluorescence readings collected by the QuantStudio™ 3 or 5 System normalized to the fluorescence from the passive reference. If available, the exported amplification data also exports the baseline-compensated normalized fluorescence data ( $\Delta R_n$ ) calculated by the software.

The section begins with a column header followed by the amplification data, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the QuantStudio™ Design and Analysis desktop Software lists the data for each additional assay on separate rows, repeating the well number and sample information.

| Column      | Description                                                                                                                                             | Output                             |
|-------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| Well        | The number of the well on the consumable.                                                                                                               | Integer [1 to 96/384] <sup>†</sup> |
| Cycle       | The cycle of the run during which the instrument recorded the fluorescence.                                                                             | Integer                            |
| Target Name | For Genotyping experiments, the name of the SNP assay assigned to the well and the allele name.                                                         | <SNP assay name><br>-<allele name> |
|             | For all other experiments, the name of the target assigned to the well.                                                                                 | Name of the target                 |
| $R_n$       | The raw fluorescence for the associated well normalized to the fluorescence of the passive reference dye (reporter signal or passive reference signal). | Float                              |
| Delta $R_n$ | The baseline compensated $R_n$ value for the associated well                                                                                            | Float                              |

<sup>†</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## Multicomponent data

The QuantStudio™ Design and Analysis desktop Software can export the data used to generate the multicomponent plot of a real-time PCR experiment. The multicomponent data tracks the raw fluorescence of all reporter dyes present on the reaction consumable throughout the duration of the experiment run.

The section begins with a column header followed by the multicomponent data, where each row contains the data for a single well separated by tab characters. The multicomponent data contains a dye column for each dye present on the reaction consumable, including reporter dyes, quencher dyes (except non-fluorescent dyes), and the passive reference.

| Column     | Description                                                                                                             | Output                             |
|------------|-------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| Well       | The number of the well on the consumable.                                                                               | Integer [1 to 96/384] <sup>†</sup> |
| Cycle      | The cycle of the run during which the QuantStudio™ 3 or 5 System recorded the fluorescence data.                        | Integer                            |
| <Dye name> | The raw fluorescence for the designated dye measured by the QuantStudio™ 3 or 5 System at the specified well and cycle. | Float                              |

<sup>†</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## Results data

The QuantStudio™ Design and Analysis desktop Software can export the results data from an analyzed experiment file. The format and content of the results data depends on the experiment type and the analysis settings.

The section begins with a column header followed by the results data, where each row contains the data for a single well separated by tab characters. If a well contains more than one assay (target), the QuantStudio™ Design and Analysis desktop Software lists the data for each additional assay on separate rows, repeating the well number and sample information.

This section describes the following results data formats:

|                                               |    |
|-----------------------------------------------|----|
| ■ BioGroup analysis results (study) . . . . . | 35 |
| ■ Biological replicate results . . . . .      | 36 |
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## BioGroup analysis results (study)

The following table describes the biological replicate data exported from a study of relative quantification experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

| Column                     | Description                                                                                                                                                                                                  | Output               |
|----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| Biogroup Name              | The name of the biological replicate group.                                                                                                                                                                  | 100-character string |
| Target                     | The name of the target assay assigned to the well.                                                                                                                                                           | 100-character string |
| Task                       | The task assigned to the target in the well.                                                                                                                                                                 | UNKNOWN or NTC       |
| Omitted                    | Indicates whether the well was omitted from the analysis (true) or included (false).                                                                                                                         | true or false        |
| # Tech Replicates          | The number of technical replicates in the associated biological replicate group.                                                                                                                             | Integer              |
| RQ                         | The relative quantity calculated for the replicate wells of the target/sample combination.                                                                                                                   | Float                |
| RQ Min                     | The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval.                                                               | Float                |
| RQ Max                     | The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval.                                                               | Float                |
| C <sub>T</sub> Mean        | The average C <sub>T</sub> of the replicate wells for the specified target/sample combination.                                                                                                               | Float                |
| Delta C <sub>T</sub> Mean  | The average $\Delta C_T$ of the replicate wells for the specified target/sample combination.                                                                                                                 | Float                |
| Delta C <sub>T</sub> SD    | The standard deviation of the $\Delta C_T$ for the replicate well. Depending on the analysis settings, this column may be replaced with "Delta C <sub>T</sub> SE" (the standard error of the $\Delta C_T$ ). | Float                |
| Delta Delta C <sub>T</sub> | The $\Delta\Delta C_T$ value of the replicate wells for the specified target/sample combination.                                                                                                             | Float                |

## Biological replicate results

The following table describes the biological replicate data exported from an experiment. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

| Column                     | Description                                                                                                                                                                                                  | Output               |
|----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| Biogroup Name              | The name of the biological replicate group.                                                                                                                                                                  | 100-character string |
| Target Name                | The name of the target assay assigned to the well.                                                                                                                                                           | 100-character string |
| Task                       | The task assigned to the target in the well.                                                                                                                                                                 | UNKNOWN or NTC       |
| RQ                         | The relative quantity calculated for the replicate wells of the target/sample combination.                                                                                                                   | Float                |
| RQ Min                     | The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval.                                                               | Float                |
| RQ Max                     | The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval.                                                               | Float                |
| C <sub>T</sub> Mean        | The average C <sub>T</sub> of the replicate wells for the specified target/sample combination.                                                                                                               | Float                |
| Delta C <sub>T</sub> Mean  | The average $\Delta C_T$ of the replicate wells for the specified target/sample combination.                                                                                                                 | Float                |
| Delta C <sub>T</sub> SD    | The standard deviation of the $\Delta C_T$ for the replicate well. Depending on the analysis settings, this column may be replaced with "Delta C <sub>T</sub> SE" (the standard error of the $\Delta C_T$ ). | Float                |
| Delta Delta C <sub>T</sub> | The $\Delta\Delta C_T$ value of the replicate wells for the specified target/sample combination.                                                                                                             | Float                |

## Genotyping

The following table describes the results data exported from Genotyping experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

| Column                                        | Description                                                                                                                                       | Output                                                                                                    |
|-----------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|
| Well                                          | The number of the well on the consumable.                                                                                                         | Integer (1 to 96/384) <sup>†</sup>                                                                        |
| Sample Name                                   | The name of the sample contained by the well.                                                                                                     | 100-character string                                                                                      |
| SNP Assay Name                                | The name of the SNP assay added to the well.                                                                                                      | 100-character string                                                                                      |
| Task                                          | The task assigned to the target in the well.                                                                                                      | UNKNOWN or NTC                                                                                            |
| Allele1 Rn                                    | The raw fluorescence associated with the allele 1 probe of the SNP assay at the well normalized to the fluorescence of the passive reference dye. | Float                                                                                                     |
| Allele2 Rn                                    | The raw fluorescence associated with the allele 2 probe of the SNP assay at the well normalized to the fluorescence of the passive reference dye. | Float                                                                                                     |
| Pass. Ref                                     | The raw fluorescence of the passive reference at the well.                                                                                        | Float                                                                                                     |
| Quality(%)                                    | The confidence of the automatic allele call.                                                                                                      | Float (1 to 100)                                                                                          |
| Call                                          | The allele call assigned to the sample at the specified well.                                                                                     | Homozygous<br><allele x/allele x>,<br>Heterozygous<br><allele x/allele y>, or<br>Negative Control<br>(NC) |
| Method                                        | The method used to call alleles.                                                                                                                  | Auto or Manual                                                                                            |
| Allele1 Automatic<br>C <sub>T</sub> Threshold | Whether the allele 1 threshold was determined automatically (true) or manually (false).                                                           | true or false                                                                                             |
| Allele1 Baseline<br>Start                     | The start cycle used to calculate the baseline section of allele 1.                                                                               | Float                                                                                                     |
| Allele1 Baseline<br>End                       | The end cycle used to calculate the baseline section of allele 1.                                                                                 | Float                                                                                                     |
| Allele2 Automatic<br>C <sub>T</sub> Threshold | Whether the allele 2 threshold was determined automatically (true) or manually (false).                                                           | true or false                                                                                             |
| Allele2 Baseline<br>Start                     | The first cycle used to calculate the baseline for allele 2.                                                                                      | Float                                                                                                     |
| Allele2 Baseline<br>End                       | The last cycle used to calculate the baseline for allele 2.                                                                                       | Float                                                                                                     |
| Custom1...<br>Custom6                         | The contents of the custom text fields found in the Results table of the experiment.                                                              | 1024-character string<br>(per field)                                                                      |

<sup>†</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## Melt Curve

The following table describes the results data exported from Melt Curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

| Column                             | Description                                                                                                                                                                                                                        | Output                             |
|------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| Well                               | The number of the well on the consumable.                                                                                                                                                                                          | Integer (1 to 96/384) <sup>†</sup> |
| Sample Name                        | The name of the sample contained by the well.                                                                                                                                                                                      | 100-character string               |
| Target Name                        | The name of the target assay assigned to the well.                                                                                                                                                                                 | 100-character string               |
| Task                               | The task assigned to the target in the well.                                                                                                                                                                                       | UNKNOWN or NTC                     |
| Reporter                           | The reporter dye that labels the probe for the target assay.                                                                                                                                                                       | 100-character string               |
| Quencher                           | The quencher dye that labels the probe for the target assay.                                                                                                                                                                       | 100-character string               |
| C <sub>T</sub>                     | The calculated threshold cycle (C <sub>T</sub> ) for the target at the specified well.                                                                                                                                             | Float                              |
| C <sub>T</sub> Mean                | The average C <sub>T</sub> of the replicate wells for the specified target/sample combination.                                                                                                                                     | Float                              |
| C <sub>T</sub> SD                  | The standard deviation of the average C <sub>T</sub> of the replicate wells for the specified target.                                                                                                                              | Float                              |
| Quantity                           | <ul style="list-style-type: none"> <li>Unknown wells – The calculated quantity for the sample at the well.</li> <li>Standard wells – The quantity assigned to the standard at the well.</li> </ul>                                 | Float                              |
| Quantity Mean                      | <ul style="list-style-type: none"> <li>Unknown wells – The average quantity of the replicate wells for the target/sample.</li> <li>Standard wells – The quantity assigned to the replicate wells for the target/sample.</li> </ul> | Float                              |
| Quantity SD                        | The standard deviation of the average quantity of the replicate wells for the target/sample.                                                                                                                                       | Float                              |
| Automatic C <sub>T</sub> Threshold | Whether the threshold was determined automatically (true) or manually (false).                                                                                                                                                     | true or false                      |
| C <sub>T</sub> Threshold           | The threshold cycle (C <sub>T</sub> ) for the sample at the well.                                                                                                                                                                  | Float                              |
| Automatic C <sub>T</sub> Baseline  | Whether the baseline was determined automatically (true) or manually (false).                                                                                                                                                      | true or false                      |
| Baseline Start                     | The first cycle used to calculate the baseline.                                                                                                                                                                                    | Integer                            |
| Baseline End                       | The last cycle used to calculate the baseline.                                                                                                                                                                                     | Integer                            |
| Tm1... Tm3                         | The first, second, and third melting temperatures (T <sub>m</sub> ) calculated in degrees Celsius.                                                                                                                                 | Float                              |
| Comments                           | Additional text that describes the well.                                                                                                                                                                                           | 1024-character string              |
| Custom1... Custom6                 | The contents of the custom text fields found in the Results table of the experiment.                                                                                                                                               | 1024-character string (per field)  |

<sup>†</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## Presence/Absence

The following table describes the results data exported from Presence/Absence experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

| Column                    | Description                                                                                                                                         | Output                                                                   |
|---------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Well                      | The number of the well on the consumable.                                                                                                           | Integer [1 to 96/384] <sup>†</sup>                                       |
| Sample Name               | The name of the sample contained by the well.                                                                                                       | 100-character string                                                     |
| Target Name               | The name of the target assay assigned to the well.                                                                                                  | 100-character string                                                     |
| Task                      | The task assigned to the target in the well.                                                                                                        | UNKNOWN or NTC                                                           |
| Reporter                  | The reporter dye that labels the probe for the target assay.                                                                                        | 100-character string                                                     |
| Quencher                  | The quencher dye that labels the probe for the target assay.                                                                                        | 100-character string                                                     |
| Rn                        | The raw fluorescence for the associated well normalized to the fluorescence of the passive reference dye.                                           | Float                                                                    |
| Rn Mean                   | The averaged normalized fluorescence ( $R_n$ ) for the associated replicate wells that contain the same target/sample combination.                  | Float                                                                    |
| Rn SD                     | The standard deviation of the normalized fluorescence ( $R_n$ ) for the associated replicate wells that contain the same target/sample combination. | Float                                                                    |
| Threshold Value           | The calculated value of the threshold for a positive call.                                                                                          | Float                                                                    |
| Call                      | The presence/absence call assigned to the sample at the specified well.                                                                             | Negative Control, Blocked IPC Control, IPC Failed, Positive, or Negative |
| Comments                  | Additional text that describes the well                                                                                                             | 1024-character string                                                    |
| Automatic $C_T$ Threshold | Indicates whether the threshold was determined automatically (true) or manually (false).                                                            | true or false                                                            |
| $C_T$ Threshold           | The threshold cycle ( $C_T$ ) for the sample at the well.                                                                                           | Float                                                                    |
| Automatic $C_T$ Baseline  | Indicates whether the baseline was determined automatically (true) or manually (false).                                                             | true or false                                                            |
| Baseline Start            | The first cycle used to calculate the baseline.                                                                                                     | Float                                                                    |
| Baseline End              | The last cycle used to calculate the baseline.                                                                                                      | Float                                                                    |
| Custom1... Custom6        | The contents of the custom text fields found in the Results table of the experiment.                                                                | 1024-character string (per field)                                        |

<sup>†</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

Standard Curve and Comparative C<sub>T</sub>

The following table describes the results data exported from Standard Curve and Comparative C<sub>T</sub> experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

| Column                                                                                                       | Description                                                                                                                                                                                                                        | Output                             |
|--------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| Well                                                                                                         | The number of the well on the consumable.                                                                                                                                                                                          | Integer (1 to 96/384) <sup>†</sup> |
| Sample Name                                                                                                  | The name of the sample contained by the well.                                                                                                                                                                                      | 100-character string               |
| Target Name                                                                                                  | The name of the target assay added to the well.                                                                                                                                                                                    | 100-character string               |
| Task                                                                                                         | The task assigned to the target in the well.                                                                                                                                                                                       | UNKNOWN, NTC, or STANDARD          |
| Reporter                                                                                                     | The reporter dye that labels the probe for the target assay.                                                                                                                                                                       | 100-character string               |
| Quencher                                                                                                     | The quencher dye that labels the probe for the target assay.                                                                                                                                                                       | 100-character string               |
| C <sub>T</sub>                                                                                               | The calculated threshold cycle (C <sub>T</sub> ) for the target at the specified well.                                                                                                                                             | Float                              |
| C <sub>T</sub> Mean                                                                                          | The average C <sub>T</sub> of the replicate wells for the specified target/sample combination.                                                                                                                                     | Float                              |
| C <sub>T</sub> SD                                                                                            | The standard deviation of the average C <sub>T</sub> of the replicate wells for the specified target.                                                                                                                              | Float                              |
| Quantity                                                                                                     | <ul style="list-style-type: none"> <li>Unknown wells – The calculated quantity for the sample at the well.</li> <li>Standard wells – The quantity assigned to the standard at the well.</li> </ul>                                 | Float                              |
| Quantity Mean                                                                                                | <ul style="list-style-type: none"> <li>Unknown wells – The average quantity of the replicate wells for the target/sample.</li> <li>Standard wells – The quantity assigned to the replicate wells for the target/sample.</li> </ul> | Float                              |
| Quantity SD                                                                                                  | The standard deviation of the average quantity of the replicate wells for the target/sample combination                                                                                                                            | Float                              |
| Automatic C <sub>T</sub> Threshold                                                                           | Whether the threshold was determined automatically (true) or manually (false).                                                                                                                                                     | true or false                      |
| C <sub>T</sub> Threshold                                                                                     | The threshold cycle (C <sub>T</sub> ) for the sample at the well                                                                                                                                                                   | Float                              |
| Automatic C <sub>T</sub> Baseline                                                                            | Whether the baseline was determined automatically (true) or manually (false).                                                                                                                                                      | true or false                      |
| Baseline Start                                                                                               | The first cycle used to calculate the baseline.                                                                                                                                                                                    | Integer                            |
| Baseline End                                                                                                 | The last cycle used to calculate the baseline.                                                                                                                                                                                     | Integer                            |
| Custom1... Custom6                                                                                           | The contents of the custom text fields found in the Results table of the experiment.                                                                                                                                               | 1024-character string (per field)  |
| If analysis flags are present, results data is present in additional columns named for the associated flags. |                                                                                                                                                                                                                                    | true or false                      |

<sup>†</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## Study data

The following table describes the results data exported from a study of relative quantification experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

| Column                             | Description                                                                                                                                                                                                  | Output                             |
|------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------|
| Experiment Name                    | The name of the of the experiment.                                                                                                                                                                           | 100-character string               |
| Well                               | The number of the well on the consumable.                                                                                                                                                                    | Integer (1 to 96/384) <sup>†</sup> |
| Omitted                            | Whether the well was omitted from the analysis (true) or included (false).                                                                                                                                   | true or false                      |
| Sample                             | The name of the sample contained by the well.                                                                                                                                                                | 100-character string               |
| Target                             | The name of the target assay assigned to the well.                                                                                                                                                           | 100-character string               |
| Task                               | The task assigned to the target in the well.                                                                                                                                                                 | UNKNOWN or NTC                     |
| Reporter                           | The reporter dye that labels the probe for the target assay.                                                                                                                                                 | 100-character string               |
| Quencher                           | The quencher dye that labels the probe for the target assay.                                                                                                                                                 | 100-character string               |
| RQ                                 | The relative quantity calculated for the replicate wells of the target/sample combination.                                                                                                                   | Float                              |
| RQ Min                             | The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval.                                                               | Float                              |
| RQ Max                             | The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval.                                                               | Float                              |
| C <sub>T</sub>                     | The calculated threshold cycle (C <sub>T</sub> ) for the target at the specified well.                                                                                                                       | Float                              |
| C <sub>T</sub> Mean                | The average C <sub>T</sub> of the replicate wells for the specified target/sample combination.                                                                                                               | Float                              |
| Delta C <sub>T</sub>               | The $\Delta C_T$ value of the replicate wells for the specified target/sample combination.                                                                                                                   | Float                              |
| Delta C <sub>T</sub> Mean          | The average $\Delta C_T$ of the replicate wells for the specified target/sample combination.                                                                                                                 | Float                              |
| Delta C <sub>T</sub> SD            | The standard deviation of the $\Delta C_T$ for the replicate well. Depending on the analysis settings, this column may be replaced with "Delta C <sub>T</sub> SE" (the standard error of the $\Delta C_T$ ). | Float                              |
| Delta Delta C <sub>T</sub>         | The $\Delta\Delta C_T$ value of the replicate wells for the specified target/sample combination.                                                                                                             | Float                              |
| Automatic C <sub>T</sub> Threshold | Indicates whether the threshold was determined automatically (true) or manually (false).                                                                                                                     | true or false                      |
| C <sub>T</sub> Threshold           | The C <sub>T</sub> for the sample at the well.                                                                                                                                                               | Float                              |
| Automatic C <sub>T</sub> Baseline  | Whether the baseline was determined automatically (true) or manually (false).                                                                                                                                | true or false                      |
| Baseline Start                     | The first cycle used to calculate the baseline.                                                                                                                                                              | Float                              |
| Baseline End                       | The last cycle used to calculate the baseline.                                                                                                                                                               | Float                              |
| Efficiency                         | The calculated efficiency of the target assay for the specified target/sample combination.                                                                                                                   | Float (1 to 100)                   |

<sup>†</sup> Well numbers start at 1 for well A1 (upper-left corner) and increase from left to right and from top to bottom.

## Technical analysis result (study)

The following table describes the technical replicate data exported from a study of relative quantification experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

| Column                     | Description                                                                                                                                                                                                  | Output               |
|----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| Sample Name                | The name of the sample contained by the well.                                                                                                                                                                | 100-character string |
| Target Name                | The name of the target assay assigned to the well.                                                                                                                                                           | 100-character string |
| Task                       | The task assigned to the target in the well.                                                                                                                                                                 | UNKNOWN or NTC       |
| Omitted                    | Indicates whether the well was omitted from the analysis (true) or included (false).                                                                                                                         | true or false        |
| RQ                         | The relative quantity calculated for the replicate wells of the target/sample combination.                                                                                                                   | Float                |
| RQ Min                     | The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval.                                                               | Float                |
| RQ Max                     | The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval.                                                               | Float                |
| C <sub>T</sub> Mean        | The average C <sub>T</sub> of the replicate wells for the specified target/sample combination.                                                                                                               | Float                |
| Delta C <sub>T</sub> Mean  | The average $\Delta C_T$ of the replicate wells for the specified target/sample combination.                                                                                                                 | Float                |
| Delta C <sub>T</sub> SD    | The standard deviation of the $\Delta C_T$ for the replicate well. Depending on the analysis settings, this column may be replaced with "Delta C <sub>T</sub> SE" (the standard error of the $\Delta C_T$ ). | Float                |
| Delta Delta C <sub>T</sub> | The $\Delta\Delta C_T$ value of the replicate wells for the specified target/sample combination.                                                                                                             | Float                |

## Technical replicate results

The following table describes the results data exported from Melt Curve experiments. Because columns can be omitted from the results, the exported file may contain a subset of the data columns below.

| Column                     | Description                                                                                                                                                                                                  | Output               |
|----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| Sample Name                | The name of the sample contained by the well.                                                                                                                                                                | 100-character string |
| Target Name                | The name of the target assay assigned to the well.                                                                                                                                                           | 100-character string |
| Task                       | The task assigned to the target in the well.                                                                                                                                                                 | UNKNOWN or NTC       |
| RQ                         | The relative quantity calculated for the replicate wells of the target/sample combination.                                                                                                                   | Float                |
| RQ Min                     | The minimum relative quantity calculated for the replicate wells of the target/sample combination. The lower limit of the confidence interval.                                                               | Float                |
| RQ Max                     | The maximum relative quantity calculated for the replicate wells of the target/sample combination. The upper limit of the confidence interval.                                                               | Float                |
| C <sub>T</sub> Mean        | The average C <sub>T</sub> of the replicate wells for the specified target/sample combination.                                                                                                               | Float                |
| Delta C <sub>T</sub> Mean  | The average $\Delta C_T$ of the replicate wells for the specified target/sample combination.                                                                                                                 | Float                |
| Delta C <sub>T</sub> SD    | The standard deviation of the $\Delta C_T$ for the replicate well. Depending on the analysis settings, this column may be replaced with "Delta C <sub>T</sub> SE" (the standard error of the $\Delta C_T$ ). | Float                |
| Delta Delta C <sub>T</sub> | The $\Delta\Delta C_T$ value of the replicate wells for the specified target/sample combination.                                                                                                             | Float                |

## RDML export format

The QuantStudio™ Design and Analysis desktop Software can export data from real-time quantitative PCR experiments as well-formed Real-time PCR Data Markup Language (RDML), a structured extensible markup language (XML) standard for quantitative PCR data. In combination with the Minimal Information (MIQPCR) guidelines, the RDML element structure describes all aspects of a qPCR experiment, including setup, analysis, and data interpretation. The exported RDML data is saved as a flat text file that can be used to transfer qPCR data between the QuantStudio™ Design and Analysis desktop Software and third-party applications.

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**IMPORTANT!** The RDML export format is available only for Standard Curve, Gene Expression, and Relative Standard Curve experiments.

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For more  
information

The RDML standard is maintained by the RDML consortium, an organization that consists of key developer groups and a member community. For more information on the RDML format, visit the RDM organization website ([www.rdml.org](http://www.rdml.org)). The website features free data management tools, including an on-line RDML file generator and RDML software libraries.

# Documentation and support

## Related documentation

| Document                                                                                   | Publication number | Description                                                                                                                                                                                                               |
|--------------------------------------------------------------------------------------------|--------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide</i> | MAN0010407         | Describes the QuantStudio™ 3 and 5 Real-Time PCR Systems hardware and software and provides information on preparing, using, maintaining, and troubleshooting the system.                                                 |
| <i>QuantStudio™ Real-Time PCR System Help</i>                                              | MAN0010422         | Describes the QuantStudio™ 3 and 5 Real-Time PCR Systems touchscreen and provides procedures for configuration, calibration, and performing a run.                                                                        |
| <i>QuantStudio™ Design and Analysis desktop Software Command-Line Application Guide</i>    | MAN0010409         | Describes how to use the command-line interface of the QuantStudio™ Design and Analysis desktop Software and provides the procedure to automate the creation of new experiment files and export data from existing files. |
| <i>QuantStudio™ Design and Analysis desktop Software User Guide</i>                        | MAN0010408         | Describes how to perform the six different experiments on the QuantStudio™ Design and Analysis desktop Software                                                                                                           |
| <i>QuantStudio™ Design and Analysis desktop Software Help</i>                              | MAN0010415         | Describes the QuantStudio™ Design and Analysis desktop Software and provides procedures for common tasks.                                                                                                                 |
| <i>SAE Admin Console Help</i>                                                              | MAN0010417         | Describes the Security, Audit, and e-Signature (SAE) Administrator Console and provides procedures for common tasks.                                                                                                      |
| <i>SAE Admin Console User Guide</i>                                                        | MAN0010410         | Describes how to use the Security, Audit, and e-Signature (SAE) Administrator Console.                                                                                                                                    |

| Document                                                                 | Publication number | Description                                                                                                                                                                                                                                                                  |
|--------------------------------------------------------------------------|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>QuantStudio™ Design and Analysis cloud Software Help</i>              | MAN0010414         | Describes the QuantStudio™ Design and Analysis cloud Software and provides procedures for common tasks.                                                                                                                                                                      |
| <i>QuantStudio™ 3 and 5 Real-Time PCR Systems Site Preparation Guide</i> | MAN0010405         | Explains how to prepare your site to receive and install the QuantStudio™ 3 and 5 Real-Time PCR Systems. Intended for personnel who schedule, manage, and perform the tasks required to prepare the site for installation of the QuantStudio™ 3 and 5 Real-Time PCR Systems. |

**Note:** For additional documentation, see “Customer and technical support” on page 9.

## Customer and technical support

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

**Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

## Limited product warranty

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