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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TRADEMARKS

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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.

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Command-line application

This chapter covers:

About the command-line application

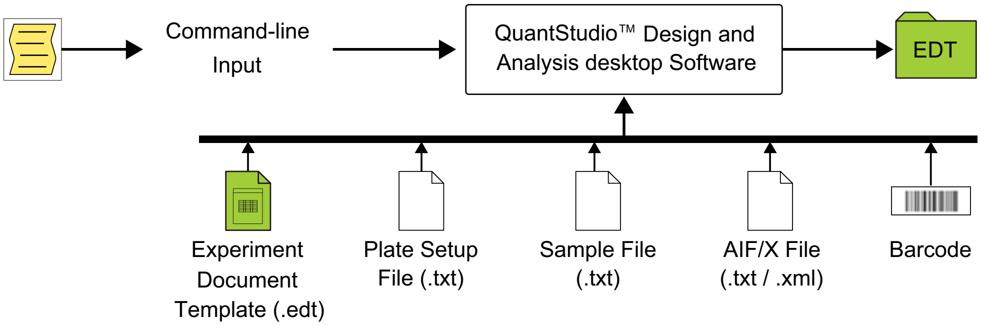
The QuantStudio™ desktop Software includes a command-line application that allows you to generate and export batches of experiment files from an MS DOS prompt or a batch file. The application is intended for advanced users who choose to create or export experiments using a scripting language.

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.

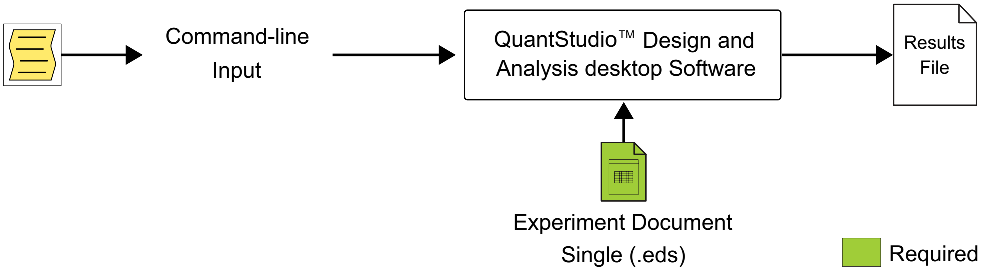
Command-line workflows

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 –expgen`) 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 Scientifc. (For some products, assay information files are available for download from the Thermo Fisher Scientifc 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: • Target/Assay/SNP to Well Assignment • Sample to Well Assignment • Task to Well Assignment • Biological Group to Well Assignment • Well Quantity to Well Assignment • Sample Color • Biological Group Color • Target Color • Gene Expression Targets Definition • Genotyping Assay Definition • Passive Reference
• 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: • Block Type • Target/Assay/Marker to Well Assignment • Sample to Well Assignment • Task to Well Assignment • Biological Group to Well Assignment • Well Quantity to Well Assignment • Sample Color • Biological Group Color • Target Color

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 -expgen -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”
```

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Import file formats and specifications

This chapter covers:

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 Scientifc. 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.	• The name of a dye in the Dye Library of the QuantStudio™ desktop Software†, or • <*blank*> if the consumable does not contain a passive reference.

† 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)>*[‡]
	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>*[††]
	Target Color	(*Optional*) The RGB color of the target.	"RGB(*<r>*,*<g>*,*<b>*)"[§]
	Task	The task assignment of the target assay at the well.[†]	<UNKNOWN | STANDARD | NTC | ENDOGENOUS | IPC | BlockedIPC>
	Reporter	The reporter dye used by the associated target assay.	*<dye name>*[††‡‡]
	Quencher	The quencher dye used by the associated target assay.	*<dye name>*[‡‡]
	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>*[††]
	SNP Assay Color	(*Optional*) SNP assay color in RGB	"RGB(*<r>*,*<g>*,*<b>*)”[§]
	Task	The task assignment of the SNP assay at the well.[†]	<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>*[††]
	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>*[††‡‡]
	Allele1 Quencher	The quencher dye used to label the probe for the first allele.	*<dye name>*[‡‡]
	Allele2 Name	The name of the second allele detected by the SNP assay.	*<100-character string>*[††]
	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>*[††‡‡]
	Allele2 Quencher	The quencher dye used to label the probe for the second allele.	*<dye name>*[‡‡]

[†] See the *QuantStudio™ Design and Analysis desktop Software Getting Started Guide* to determine the tasks applicable to the experiment.

[‡] 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.

[††] Can be empty if the Task field is empty. Otherwise, the field must contain a value.

[‡‡] The dye must already exist in the QuantStudio™ desktop Software Dye Library. The dye name must be 100 characters or less.

Examples

Quantitative PCR experiments

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    Al1 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    Al1 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    Al1 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    Al2 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    Al2 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    Al2 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 Scientifc 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

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. 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†	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†		
Calibration Expired†	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†	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†	The name of the QuantStudio™ 3 or 5 System that ran the experiment.	*<100-character string>*
Instrument Serial Number†	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

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)†
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>)"`‡
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>)"`‡
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

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

‡ 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)†
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>*`)"`‡
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>*`)"`‡
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>*`)"`‡
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>*`)"`‡
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

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

‡ 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)†
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

† 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 (ΔR_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)[†]
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>-<allele name>`
	For all other experiments, the name of the target assigned to the well.	Name of the target
Rn	The raw fluorescence for the associated well normalized to the fluorescence of the passive reference dye (reporter signal or passive reference signal).	Float
Delta Rn	The baseline compensated R_n value for the associated well	Float

[†] 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)[†]
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

[†] 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)

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_T Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Delta C_T Mean	The average ΔC_T of the replicate wells for the specified target/sample combination.	Float
Delta C_T SD	The standard deviation of the ΔC_T for the replicate well. Depending on the analysis settings, this column may by replaced with "Delta C_T SE" (the standard error of the ΔC_T).	Float
Delta Delta C_T	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_T Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Delta C_T Mean	The average ΔC_T of the replicate wells for the specified target/sample combination.	Float
Delta C_T SD	The standard deviation of the ΔC_T for the replicate well. Depending on the analysis settings, this column may by replaced with "Delta C_T SE" (the standard error of the ΔC_T).	Float
Delta Delta C_T	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)[†]
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` <*allele x*/*allele x*>, `Heterozygous` <*allele x*/*allele y*>, or `Negative Control (NC)`
Method	The method used to call alleles.	`Auto` or `Manual`
Allele1 Automatic C_T Threshold	Whether the allele 1 threshold was determined automatically (true) or manually (false).	`true` or `false`
Allele1 Baseline Start	The start cycle used to calculate the baseline section of allele 1.	Float
Allele1 Baseline End	The end cycle used to calculate the baseline section of allele 1.	Float
Allele2 Automatic C_T Threshold	Whether the allele 2 threshold was determined automatically (true) or manually (false).	`true` or `false`
Allele2 Baseline Start	The first cycle used to calculate the baseline for allele 2.	Float
Allele2 Baseline End	The last cycle used to calculate the baseline for allele 2.	Float
Custom1... Custom6	The contents of the custom text fields found in the Results table of the experiment.	1024-character string (per field)

[†] 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)[†]
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_T	The calculated threshold cycle (C_T) for the target at the specified well.	Float
C_T Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
C_T SD	The standard deviation of the average C_T of the replicate wells for the specified target.	Float
Quantity	• Unknown wells – The calculated quantity for the sample at the well. • Standard wells – The quantity assigned to the standard at the well.	Float
Quantity Mean	• Unknown wells – The average quantity of the replicate wells for the target/sample. • Standard wells – The quantity assigned to the replicate wells for the target/sample.	Float
Quantity SD	The standard deviation of the average quantity of the replicate wells for the target/sample.	Float
Automatic C_T Threshold	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	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_m) 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)

[†] 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)†
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)

† 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_T

The following table describes the results data exported from Standard Curve and Comparative C_T 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)[†]
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_T	The calculated threshold cycle (C_T) for the target at the specified well.	Float
C_T Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
C_T SD	The standard deviation of the average C_T of the replicate wells for the specified target.	Float
Quantity	• Unknown wells – The calculated quantity for the sample at the well. • Standard wells – The quantity assigned to the standard at the well.	Float
Quantity Mean	• Unknown wells – The average quantity of the replicate wells for the target/sample. • Standard wells – The quantity assigned to the replicate wells for the target/sample.	Float
Quantity SD	The standard deviation of the average quantity of the replicate wells for the target/sample combination	Float
Automatic C_T Threshold	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	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`

[†] 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)[†]
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_T	The calculated threshold cycle (C_T) for the target at the specified well.	Float
C_T Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Delta C_T	The ΔC_T value of the replicate wells for the specified target/sample combination.	Float
Delta C_T Mean	The average ΔC_T of the replicate wells for the specified target/sample combination.	Float
Delta C_T SD	The standard deviation of the ΔC_T for the replicate well. Depending on the analysis settings, this column may by replaced with “Delta C_T SE” (the standard error of the ΔC_T).	Float
Delta Delta C_T	The $\Delta\Delta C_T$ value of the replicate wells for the specified target/sample combination.	Float
Automatic C_T Threshold	Indicates whether the threshold was determined automatically (true) or manually (false).	`true` or `false`
C_T Threshold	The C_T for the sample at the well.	Float
Automatic C_T 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)

[†] 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_T Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Delta C_T Mean	The average ΔC_T of the replicate wells for the specified target/sample combination.	Float
Delta C_T SD	The standard deviation of the ΔC_T for the replicate well. Depending on the analysis settings, this column may by replaced with "Delta C_T SE" (the standard error of the ΔC_T).	Float
Delta Delta C_T	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_T Mean	The average C_T of the replicate wells for the specified target/sample combination.	Float
Delta C_T Mean	The average ΔC_T of the replicate wells for the specified target/sample combination.	Float
Delta C_T SD	The standard deviation of the ΔC_T for the replicate well. Depending on the analysis settings, this column may by replaced with "Delta C_T SE" (the standard error of the ΔC_T).	Float
Delta Delta C_T	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.

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

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**). 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
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	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.
QuantStudio™ Real-Time PCR System Help	MAN0010422	Describes the QuantStudio™ 3 and 5 Real-Time PCR Systems touchscreen and provides procedures for configuration, calibration, and performing a run.
QuantStudio™ Design and Analysis desktop Software Command-Line Application Guide	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.
QuantStudio™ Design and Analysis desktop Software User Guide	MAN0010408	Describes how to perform the six different experiments on the QuantStudio™ Design and Analysis desktop Software
QuantStudio™ Design and Analysis desktop Software Help	MAN0010415	Describes the QuantStudio™ Design and Analysis desktop Software and provides procedures for common tasks.
SAE Admin Console Help	MAN0010417	Describes the Security, Audit, and e-Signature (SAE) Administrator Console and provides procedures for common tasks.
SAE Admin Console User Guide	MAN0010410	Describes how to use the Security, Audit, and e-Signature (SAE) Administrator Console.

Document	Publication number	Description
QuantStudio™ Design and Analysis cloud Software Help	MAN0010414	Describes the QuantStudio™ Design and Analysis cloud Software and provides procedures for common tasks.
QuantStudio™ 3 and 5 Real-Time PCR Systems Site Preparation Guide	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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    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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