

Import file guidelines - CFX

1 - General information

Import files should contain a list of Quantification Cycle (**Cq**) values. Depending on the instrument software that generates the data, Cq values may have alternative names such as Ct, Cp, or TOP. Cq is the official abbreviation in the Real-time PCR Data Markup Language (more info on <http://www.rdml.org>).

The qbase^{PLUS} calculation engine is based on Cq values. Sigmoidal amplification curve data are not supported.

Import file formats can be either a tab delimited text file (.txt), a comma or semicolon separated value file (.csv) or a Microsoft Excel file (.xls). Microsoft Excel 2007 (.xlsx), OpenOffice.org Calc (.ods) and proprietary or binary instrument files are not supported.

In qbase^{PLUS}, there are character restrictions on sample, target and run names. Only the following characters are allowed: all alphanumerical characters (0-9, a-z, A-Z), space, _, -, \$, #, :, ^, . and the greek letter mu (μ). **Illegal characters** such as brackets and slashes should be removed or replaced.

2 - CFX

These guidelines are based on a specific instrument - software (version) combination. The described procedures may not be suitable for other software versions (e.g. new upgrades) but the described file format allows you to verify compliance of your data files with qbase^{PLUS}. See below for a list of supported instruments and software (versions).

Instruments: Bio-Rad CFX96 and Bio-Rad CFX384

Software: Bio-Rad's CFX1.1 software

3 - Generating CFX export files

Step 1: Open data file and (optionally) adjust *Analysis Mode*, *C(t) Determination* and *Baseline Threshold*.

Step 2: Go to the *Quantification Data* tab (Figure 1)

Well	Fluor	Content	Target	Sample	Threshold Cycle (Ct)	Cq Mean	Cq Std. Dev
B01	SYBR	Unkn-01	Actin	0Hr	22,19	22,17	0,086
B02	SYBR	Unkn-02	Actin	1Hr	22,33	22,25	0,119
B03	SYBR	Unkn-03	Actin	2Hr	22,32	22,13	0,224
B04	SYBR	Unkn-04	GAPDH	0Hr	15,79	15,70	0,079
B05	SYBR	Unkn-05	GAPDH	1Hr	15,78	15,69	0,079
B06	SYBR	Unkn-06	GAPDH	2Hr	15,86	15,68	0,248
B07	SYBR	Unkn-07	IL1beta	0Hr	22,08	22,12	0,047
B08	SYBR	Unkn-08	IL1beta	1Hr	24,42	24,40	0,052
B09	SYBR	Unkn-09	IL1beta	2Hr	26,69	27,02	0,785
B10	SYBR	Unkn-10	Tubulin	0Hr	27,01	27,13	0,109
B11	SYBR	Unkn-11	Tubulin	1Hr	24,88	24,90	0,100
B12	SYBR	Unkn-12	Tubulin	2Hr	22,76	22,76	0,085
C01	SYBR	Unkn-01	Actin	0Hr	22,24	22,17	0,086
C02	SYBR	Unkn-02	Actin	1Hr	22,11	22,25	0,119
C03	SYBR	Unkn-03	Actin	2Hr	21,88	22,13	0,224
C04	SYBR	Unkn-04	GAPDH	0Hr	15,64	15,70	0,079

Figure 1

Step 3: Click on top left cell of the data table, copy the quantification data into memory (by pressing CTRL+C) and paste in new Microsoft Excel sheet (CTRL+V). Only one plate (run) should be copied to an Excel file.

Step 4: Save file as binary Excel file (XLS, not XML based XLSX).

Notes: The number of columns can vary, depending on the plate layout (e.g. either or not inclusion of 'standard' sample types).

The Excel file should always have an empty first column.

Make sure the same decimal separator is used in Excel as in the CFX program.

4 - CFX data table format

CFX run files contain a header row with the following columns:

- Well ID > Well
- Fluor column to extract multi-color run files into separate runs
- Sample type > Content
- Target name > Target
- Sample name > Sample
- Cq value > Threshold Cycle (C(t))
- Quantity > Starting Quantity (SQ)

Two different example files can be [found](#) in the support section of the Biogazelle website. [CFX-ExampleFile1.xls](#) is an example of an export file from an experiment containing standard samples, [CFX-ExampleFile2.xls](#) is an example of an export file from an experiment without standard samples.

5 - Import CFX run files into qbase^{PLUS}

Step 1: Open the experiment () in which the run(s) need to be imported by double clicking it in the Project Explorer tree or by using the *Load experiment* option in the experiment context menu.

Step 2: Start the import wizard by clicking the *Import* button () in the command bar, select *import run* and click *next*. Alternatively, right click on runs () and select *Import runs ...* from the context menu (Figure 2).

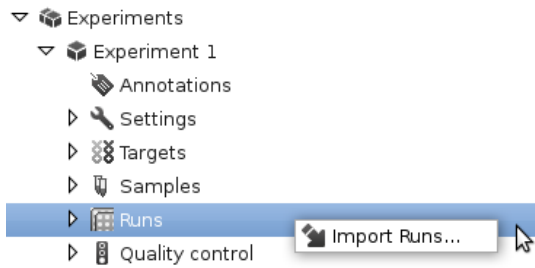


Figure 2

Step 3: Select the experiment in which the run needs to be imported in the top part of the import wizard window. By default the active experiment is already selected. Browse for the file(s) to be imported by clicking the *Browse* button and select the CFX file format from the *File type* drop down list (Figure 3). Optionally, the run name can be altered in the *Run name* text box.

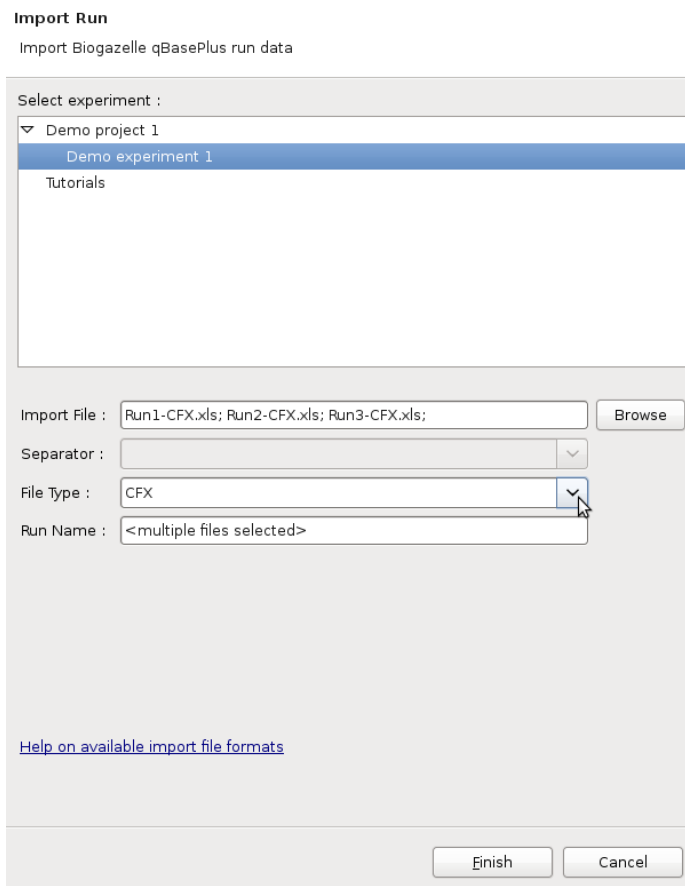


Figure 3

Note - Runs can only be imported into loaded (open) experiments. Closed (unloaded) experiments are not available for selection.

Note - Simultaneous (batch) import of multiple files, as selected in the *Browse* window, is only available for fully licensed customers or users with a temporary demo license. Users with a free license can only import runs one by one.

Step 4: Click *Finish* to complete the import run wizard. You will see that the imported runs are added to your experiment. An individual window is opened for every imported run to allow editing of sample and target information. In addition, the sample and target list is automatically updated with the sample and target names that are included in the imported runs.