

Import file guidelines - LC480

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). Please note that Microsoft Excel 2007 (.xlsx), OpenOffice.org Calc (.ods) and proprietary or binary instrument files are not (yet) 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 - LC480

The following guidelines are based on a specific instrument and 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: LC480

Software: LightCycler 480 Software 1.5

3 - Generating LC480 export files (method 1)

Step 1: Perform an *absolute quantification analysis*

Step 2: Display all samples in the *amplification curves graph* and *results table* by clicking on the upper left corner of the plate (Figure 1).

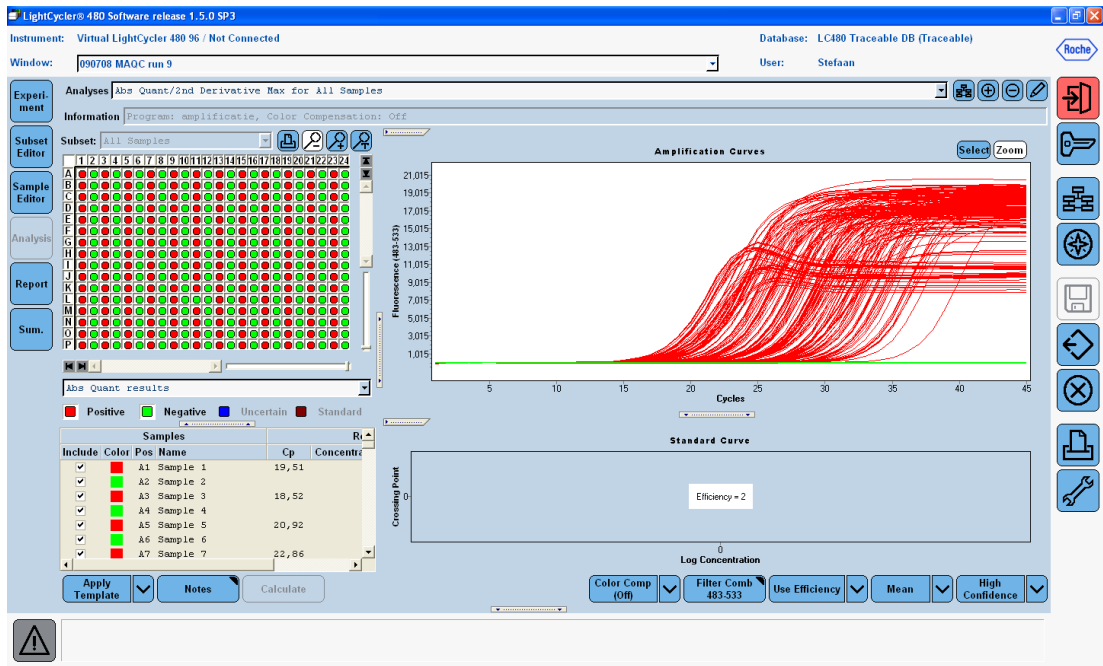


Figure 1

- Step 3: Select the entire results table by clicking the upper left corner. Alternatively, select the first row of the table and then SHIFT-select the last row of the table.
- Step 4: Once the table is selected (blue color appears), copy it (Ctrl + C).
- Step 5: Open Excel (or compatible alternatives such as Calc) and paste the table (Ctrl + V). The table should look like the one shown in Figure 2.

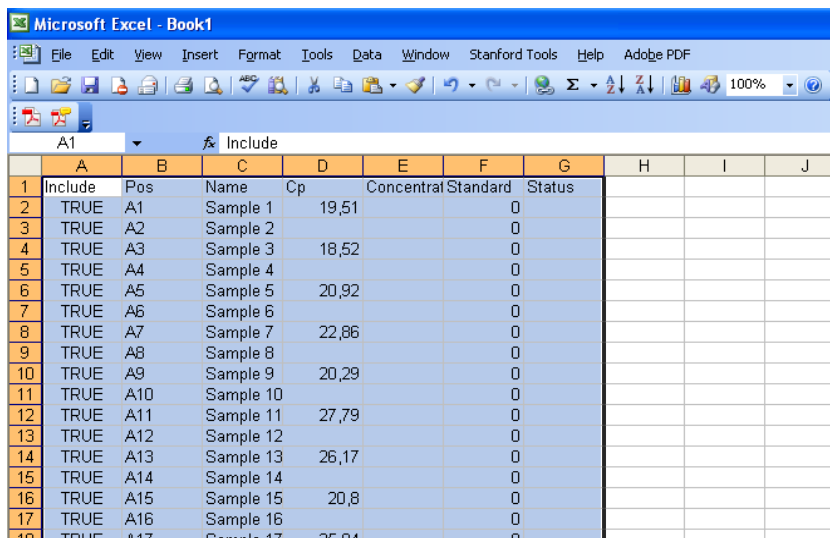


Figure 2

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

4 - Generating LC480 export files (method 2)

- Step 1: Perform an *absolute quantification analysis*

- Step 2: Display all samples in the *amplification curves graph* and *results table* by clicking on the upper left corner of the plate (Figure 1).
- Step 3: Click the right mouse button on the table and select *Export Table* (Figure 3).

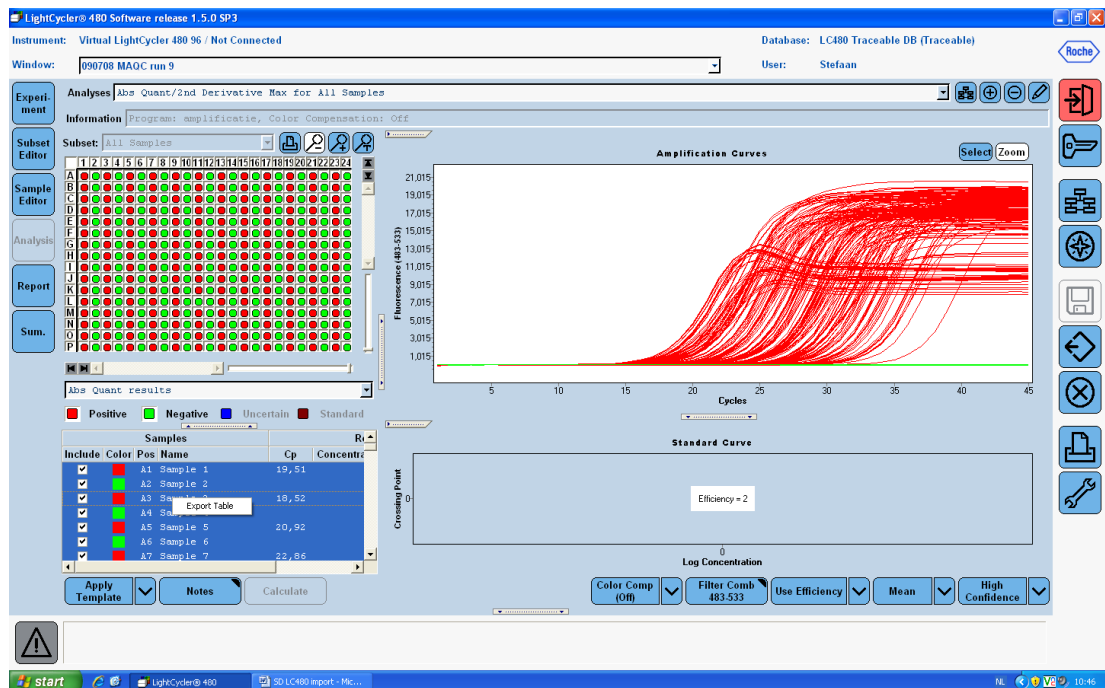


Figure 3

- Step 4: Save the .txt file (e.g. resulting.txt).
- Step 5: Open the resulting.txt file, remove the first line from this document and save it again. The content of this file should now start with *Include*.

5 - LC480 data table format

LC480 run files contain a header row with the following columns:

- Exclusion status > Include
- Well ID > Pos
- Sample name > Name
- Cq value > CP
- Quantity > Standard

Two different example files can be found in the support section of the Biogazelle website. [LC480-ExampleFile1.xls](#) is an example of an export file generated by method 1, [LC480-ExampleFile2.xls](#) is an example of an export file generated according to the second method.

6 - Import LC480 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 4).

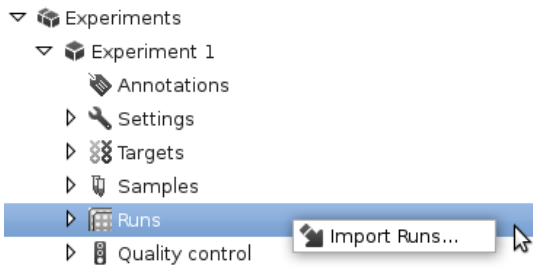


Figure 4

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 LC480 file format from the *File type* drop down list (Figure 5). Optionally, the run name can be altered in the *Run name* text box.

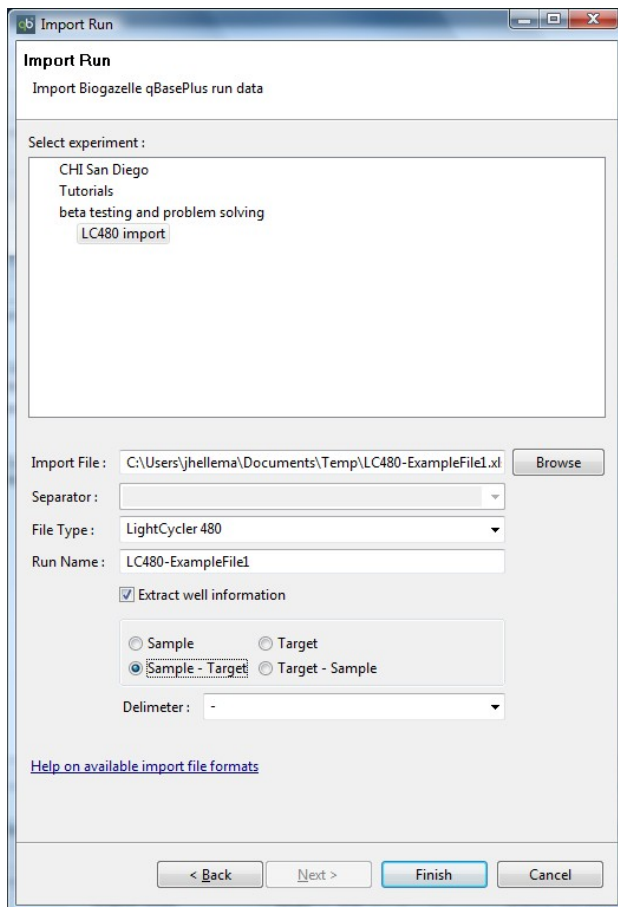


Figure 5

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 30-days demo license. Users with a free license can only import runs one by one.

Step 4: The LC480 export files contain only one name per well. By default, this name is used as sample name by qbase^{PLUS}. As of version 1.3, qbase^{PLUS} offers the option to use this name for target (gene) information as well. Activate *Extract well information* and choose the

way you want to use the information contained within the name field. This can be *Sample* or *Target*, or a combination of both if the run has been annotated to contain both values separated by delimiter of choice.

- Step 5:** 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.