

data import

qbase^{PLUS}



Easy . Fast . Reliable .

Subject

Summary

Detailed information

Step-by-step

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References



Subject

qbase^{PLUS} has extensive data import functionality. This chapter explains how run data from a wide variety of real-time PCR instruments can be directly imported in qbase^{PLUS}.

Summary

For most users there is no need to modify the data export files generated by their real-time PCR instrument software as qbase^{PLUS} supports export files from the majority of qPCR instruments and accompanying data analysis software.

Run data can be imported as follows:

1. Open an experiment ()
2. Click import () and select import run
3. Select the experiment in which the run needs to be imported, browse to your data file and select the proper file format (based on your instrument or analysis software)
4. Click import
5. While a run is added to your experiment, it is processed and analyzed at the same time.

Detailed information

For most users there is **no need to modify** the data file as qbase^{PLUS} supports export files from the majority of the real-time PCR instruments and accompanying data analysis software. qbase^{PLUS} cannot read the binary files that are used by the various instrument specific data collection softwares, nor can it analyze raw fluorescence data. Instead, a data file containing quantification cycle (Cq) values needs to be exported from the data collection software. 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 according to the Real-time PCR Data Markup Language (more info on <http://www.rdmf.org>). For most instruments, these tabular export files (Microsoft Excel or delimited text) can be directly imported in qbase^{PLUS}. More information on the supported instruments and a description of the file formats can be found in the support section of the Biogazelle website (<http://www.biogazelle.com/support/qbaseplus/formats>).

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




As of version 2.0, there are no longer any character restrictions on sample, target and run names. In contrast, there remain restrictions on experiment and project names whereby 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. Please note that for earlier version of the software, there were various character restrictions on sample, target and run names.

If your instrument is not supported, you are advised to modify your file so it matches the generic simple, (former) qBase or RDML (real-time PCR data mark-up language) [Lefever et al., Nucleic Acids Res, 2009; <http://www.rdml.org>] file format. RDML is a universal, open and XML based data exchange format recommended to be used according to the MIQE guidelines [Bustin et al., Clin Chem, 2009; <http://www.rdml.org/miqe/>]. qbase^{PLUS} is the only third party real-time PCR data-analysis software that is RDML compatible and MIQE compliant.

Runs are imported into experiments, and all information from one or multiple runs is stored in a qbase^{PLUS} experiment. The import procedure does not alter the original data file. Whereas imported Cq values cannot be modified, other types of information (such as target and sample names) can be changed after import.

Step-by-step

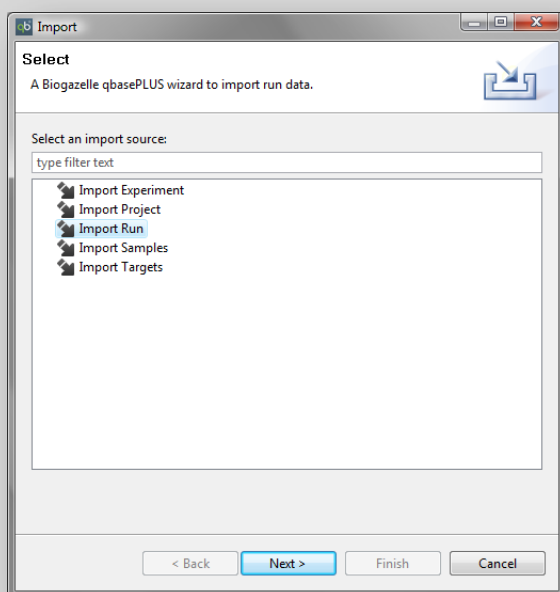
Step 1 - Open experiment

Open the project () and experiment () of interest in which the run(s) need(s) to be imported. If needed create a new experiment as follows: right click on the project () in the qbase^{PLUS} Project explorer tree in which you want to start a new experiment and click New experiment.

Step 2 - Launch import wizard

Start the import wizard by clicking the import button () in the command bar or by using the right-click context menu. Choose Import run and click Next (Figure 1).

▼ Figure 1 – import wizard



Step 3 - Import settings

Select the experiment in which the run needs to be imported in the top part of the import wizard.

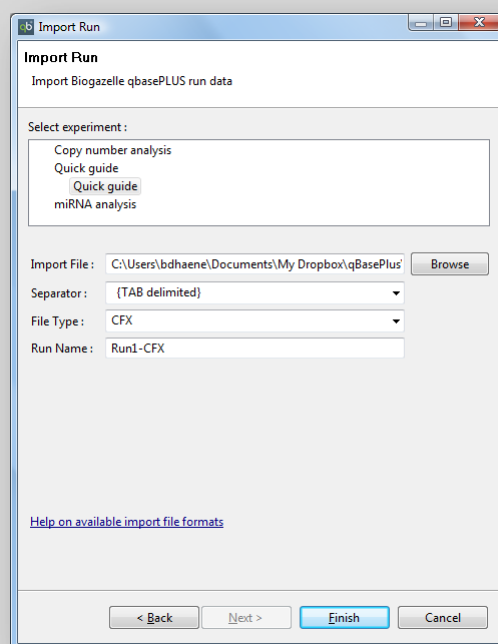
Browse for the file(s) to be imported (first text box) and select the file format corresponding to that of your import file (drop down list). Optionally, the run name can be altered in the third text box. Multiple runs can be imported at once using the 'Shift' or 'Ctrl' key while selecting the data files.

Do not forget to select the appropriate Separator (drop down list) if your import file format is a tab delimited text file (.txt), a comma or semicolon separated value file (.csv) (Figure 2).

Runs can only be imported into loaded (open) experiments. Experiments that are not open are not available for selection.

Users with a basic license can import ten 96-well runs at maximum within one experiment.

▼ Figure 2 – import settings



Step 4 - Extract sample and target information

If an annotated run (it contains Cq values, sample and target names, and quantities in case of standard samples) is imported, a sample and target list is automatically generated which can be accessed in the Project explorer (see chapter 'run annotation'). For instruments that provide only a single information field per well, there exists a functionality to extract a sample name, target name, or both based on a given delimiter (Figure 3). This allows you to provide the sample and target well annotation already in your real-time PCR instrument software.

For example, well information "SampleN|GeneZ" can be imported as sample name "SampleN" and target name "GeneZ" based on the delimiter "|" in this case. Other available delimiters are: '-', ':', ';', '_', and '@'. This functionality is only available for instruments that restrict export to a single information field per well: iCycler, MyiQ, iQ5, Opticon Monitor, Rotor gene, LightCycler, LightCycler 480, LightCycler 1536 and MX300xP.

Step 5 - Complete run import

Click 'import' to complete the import run wizard. You will see that the runs are added to your experiment and that they are by default analyzed at the same time. Please note that as of version 2.0, the option to stop automatic calculations has been introduced.

An individual window can be opened for each imported run by double clicking the run in the Project Explorer tree. This allows editing of sample and target information for each well. In addition, the sample and target list in the Project Explorer tree is automatically updated with the sample and target names provided they are included in the import files.

More information on the annotation of runs with target and sample names, sample types and quantities for the standard samples can be found in chapter 'Run annotation'.

▼ Figure 3 – Import settings

Select experiment:

Demo project 1
MAQC II
194 genes

Import File: C:\qBasePlus\qBasePlus demo data\Run files\24 seconds\Ru Browse

Separator: [v]

File Type: iCycler [v]

Run Name: Run1

Extract well information

Sample Target
 Sample - Target Target - Sample

Delimiter: | [v]

[Help on available import file formats](#)

Finish Cancel

References

Lefever S, Hellemans J, Pattyn F, Przybylski DR, Taylor C, Geurts R, Untergasser A, Vandesompele J; on behalf of the RDML consortium.

RDML: structured language and reporting guidelines for real-time quantitative PCR data. *Nucleic Acids Res.* 2009 Apr;37(7):2065-9.

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT.

The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem.* 2009 Apr;55(4):611-22.

