

AMPLIFICATION

Transferring Data From CFX Manager™ Software to qbase^{PLUS} Software — A Quick Guide

About This Guide

These instructions apply to CFX Manager software versions 1.0–1.5 and qbase^{PLUS} software versions 1.3 and later.

Getting Started

qbase^{PLUS} software analyzes threshold cycle values, or C(t)s, calculated by CFX Manager software. If you wish to adjust any settings which affect this calculation, such as C(t) Determination mode, Baseline Threshold mode, or Baseline Analysis mode, make these adjustments before exporting the data (see pp 73–75 in the CFX96™ and CFX384™ Real-Time PCR Detection Systems Instruction Manual).

Exporting Data From CFX Manager Software

1. Before starting, make sure that all samples, targets, dyes, and data files are named in English alphanumeric characters (a-z, A-Z, numbers, spaces, dashes, underscores, \$, #, :, ^, and µ). Other characters will not be recognized by qbase^{PLUS} software.
2. Open the data file (.pcrd file) in CFX Manager software.

Note: To export a Gene Study consisting of multiple plates, export each plate separately.

3. Click **Quantitation Data** (Figure 1).
4. Select all the data by clicking the header of the first column labeled “Well,” holding down Shift, scrolling to the right, and clicking the header of the last column labeled “SQ Std. Dev.”
5. Copy the data by right clicking and selecting **Copy**.
6. Open a new Excel spreadsheet.
7. Right click on cell **B1** in the spreadsheet and select **Paste**. This leaves the first column in the spreadsheet blank, which is the required format for import into qbase^{PLUS} software (Figure 2).
8. Save the file in .xls format by selecting **File > Save** on the menu bar, choosing **Microsoft Office Excel Workbook (*.xls)** from the drop-down menu, then clicking **Save** (Figure 3).

Note: Do not save the file in .xlsx format; this format is not recognized by qbase^{PLUS} software.

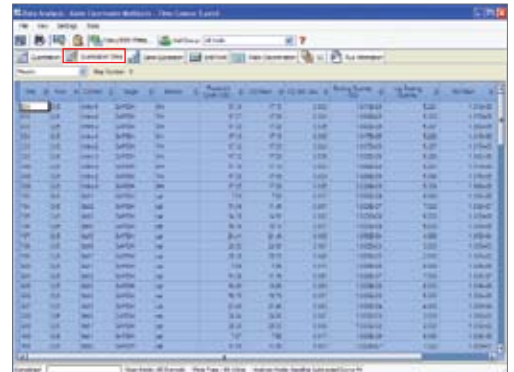


Fig. 1. Copying data from the Quantitation Data tab in the Data Analysis window of CFX Manager software.

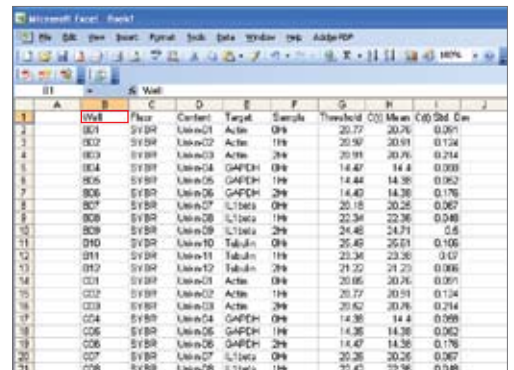


Fig. 2. Pasting data into a Microsoft Excel spreadsheet.

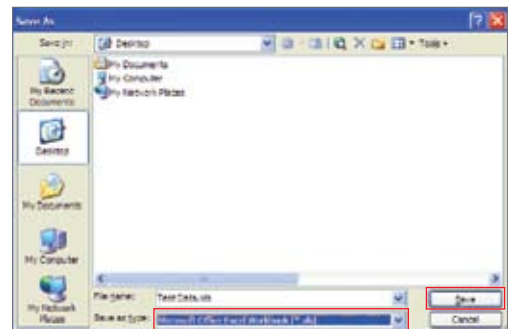


Fig. 3. Saving the file in .xls format.

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Importing Data Into qbase^{PLUS} Software

1. Open qbase^{PLUS} software. qbase^{PLUS} software uses a file tree structure to store qPCR data, as shown in the Project Explorer pane. You can expand or minimize the folders in the file tree by clicking the (+) and (-) signs next to each folder, or by double clicking on the name of the folder (Figure 4).
2. Create a new project folder by selecting **File > New > Project** in the main menu (Figure 5).
3. Click **Finish** in the confirmation window. Project folders store multiple experiment subfolders and default settings and fields to store your annotations and conclusions.
4. Create a new experiment subfolder by selecting **File > New > Experiment** in the main menu (Figure 6).
5. Select **Project 1** in the confirmation window.
6. Click **Finish**. qbase^{PLUS} creates a new experiment subfolder, named Experiment 1, located under Project 1 > Experiments. Experiment subfolders store multiple qPCR runs and tools for quality control, data analysis, and other settings.
7. To import a run, select **File > Import** in the main menu (Figure 7).
8. In the Import window, select **Import Run**, then click **Next** (Figure 8).

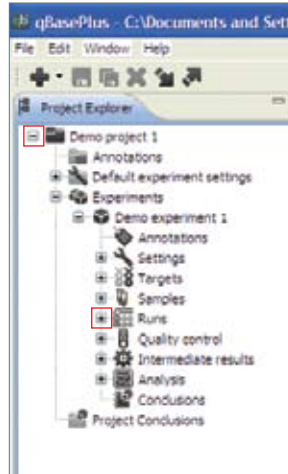


Fig. 4. The expanded file tree in Project Explorer.

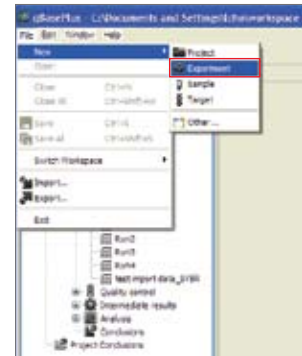
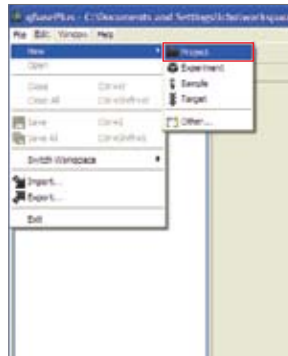


Fig. 5. Creating a new project folder. Fig. 6. Creating a new experiment subfolder.

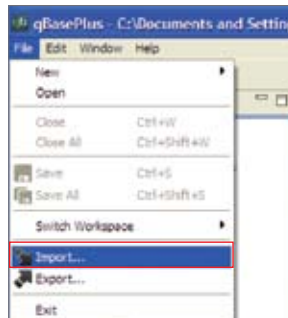


Fig. 7. Selecting Import from the File menu.

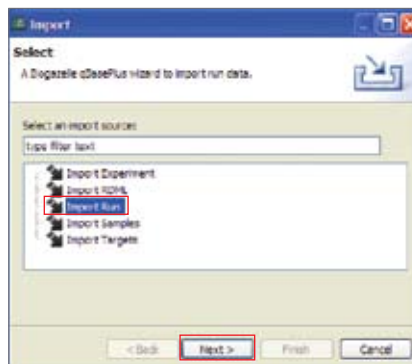


Fig. 8. Choosing to import a run.

9. The Import Run window opens (Figure 9). Perform the following steps:

- Select **Experiment 1**; this is where qbase^{PLUS} software places the data that you import
- Click **Browse**, select the saved Excel file with CFX Manager software data, then click **Open**; the Run Name field is automatically populated with the Excel file name

Note: These instructions are for importing a single Excel file. To import multiple Excel files simultaneously, click **Browse**, hold down Shift or Ctrl, select multiple Excel files, then click **Open**.

- Under the File Type drop-down menu, select **CFX**
- Click **Finish**

10. When the import is complete, the imported run or runs will be shown in the Project Explorer under Project 1 > Experiments > Experiment 1 > Runs (Figure 10). Each run contains data for one dye from one plate; multiplex plates are stored as several runs, one for each dye.

Note: For simplicity, this quick guide only shows data import through the file menu for new users. qbase^{PLUS} software also has shortcut icons and a context-sensitive right-click menu that provide alternative ways to accomplish the same tasks.

- For example, a second method to create a new experiment is to click the **+** icon and select **New Experiment**; a third method is to right click on the **Experiments** folder under Project 1 in the Project Explorer pane, then select **New Experiment**
- Similarly, a second method to initiate an import is to click the **📁** icon; a third method is to right click on **Runs** under Experiment 1 in the Project Explorer pane, then select **Import Run**

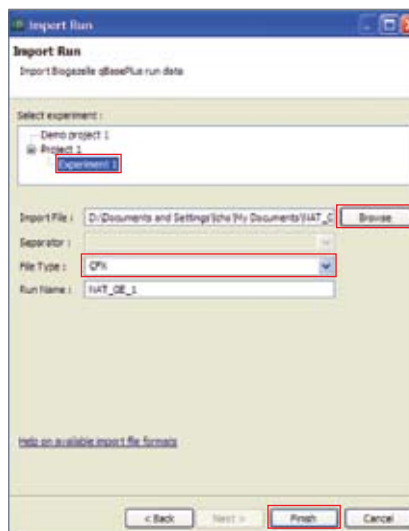


Fig. 9. The Import Run window.

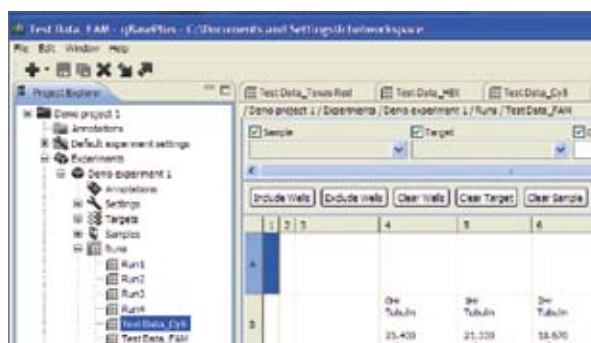


Fig. 10. Imported data displayed in the Project Explorer.

Analysis Options and Viewing Gene Expression Results

- As an option, qbase^{PLUS} software allows you to adjust the settings used to calculate gene expression results.
 - Under Experiment 1, expand the Settings folder
 - Double click on **Calculation parameters** or **Quality control settings**; each settings window opens as a tab in the main software pane to allow editing of the default values (optional)
- Under Experiment 1, expand the Targets folder. The Targets folder contains lists of Targets of Interest and Reference Targets. By default qbase^{PLUS} designates all targets as Targets of Interest upon import. In order to calculate gene expression results you must designate at least one Reference Target.
- Expand the list of Targets of Interest in the Targets folder.
- Right click on the name of the target that you want to use as a reference and select **Set Target Type > Reference Target** (Figure 11). qbase^{PLUS} software moves the target from the list of Targets of Interest to the list of Reference Targets.
- Double click on the name of a target in the list of Targets of Interest to view graphical results for the target expression (Figure 12).
- Expand the Analysis folder under Experiment 1 and double click on **Results table** to view the results for all targets in a spreadsheet view (Figure 13).

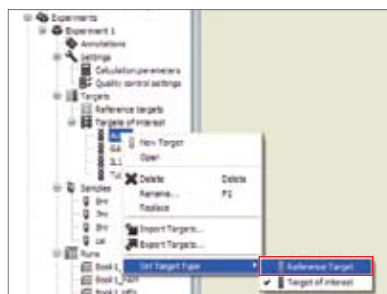


Fig. 11. Designating a Reference Target.

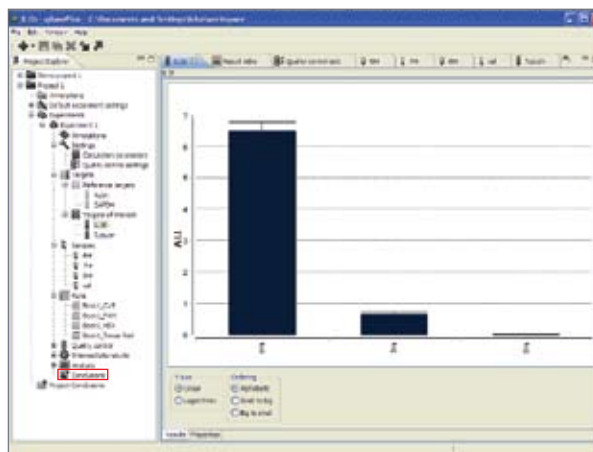


Fig. 12. Graph of gene expression data for a target of interest.

Target	ALI	SE	CI	SE	CI	SE	CI
ACT1	1.49E-2	0.17E-2	1.14E-2	1.84E-2	1.14E-2	2.54E-2	1.17E-2
GAPDH	5.49E-2	0.17E-2	4.82E-2	6.16E-2	4.82E-2	6.16E-2	6.16E-2
PTP	3.34E-2	0.17E-2	2.82E-2	4.16E-2	2.82E-2	4.16E-2	4.16E-2
HP	1.39E-2	0.17E-2	1.09E-2	1.49E-2	1.09E-2	1.49E-2	1.49E-2
IL2	5.49E-2	0.17E-2	4.82E-2	6.16E-2	4.82E-2	6.16E-2	6.16E-2

Fig. 13. Results table for all targets.

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