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 1.6 and later and qbase^{PLUS} software versions 1.5 and later.

Getting Started

qbase^{PLUS} software analyzes quantification cycle (Cq) values calculated by CFX Manager software. If you wish to adjust any settings that affect this calculation, such as Cq Determination mode, Baseline Threshold mode, or Baseline Analysis mode, make these adjustments before exporting the data (see the CFX96 Touch™, CFX96 Touch Deep Well™, CFX Connect™, and CFX384 Touch™ Real-Time PCR Detection Systems Instruction Manual for details).

Exporting Data from CFX Manager Software

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.

Data can be exported as either a real-time PCR data markup language (RDML) file (.rdml) or an Excel file (.csv). RDML is a universal qPCR data export/import format that facilitates data sharing from diverse instrument platforms without the need for a copy of the instrument software. The .html format of RDML files can contain not only Cq and target information, but also user details, full run parameters, sample descriptions, primer and probe sequences, etc.

To export an RDML file (with CFX Manager software version 3.0 or later):

1. Select **Export > Export RDML File > RDML v1.1** (if using qbase^{PLUS} software version 2.3 or later; Figure 1) or **RDML v1.0** (if using earlier versions of qbase^{PLUS} software).
2. Save the file in an appropriate location.

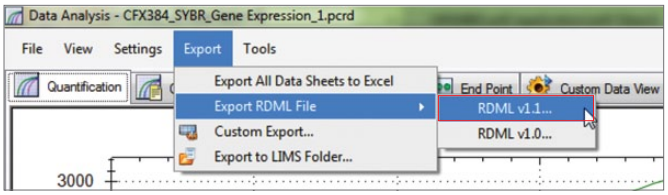

Fig. 1. Exporting an RDML file.

To export data using Excel:

1. Open the data file (.pcrd file) in CFX Manager software.

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

2. Click **Quantification Data**.
3. Select all the data by clicking the upper-left-most cell (Figure 2).
4. Copy the data by right clicking and selecting **Copy**.
5. Open a new Excel spreadsheet.

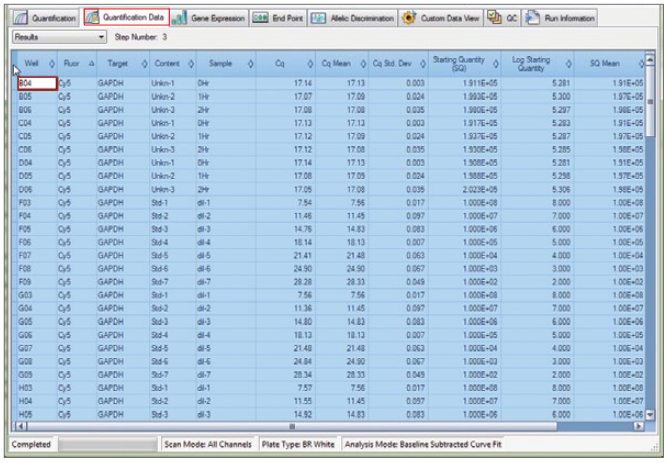

Fig. 2. Copying data from the Quantification Data tab in the Data Analysis window of CFX Manager software.

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6. Right click cell **A1** in the spreadsheet and select **Paste**. The first column in the spreadsheet will be blank, which is the required format for import into qbase^{PLUS} software (Figure 3).
7. Save the file in .xls format by selecting **File > Save As** on the menu bar, choosing **Microsoft Office Excel Workbook (*.xls)** from the dropdown menu, then clicking **Save** (Figure 4).

Note: Do not save the file in .xlsx format unless you are using qbase^{PLUS} software version 2.0 or later.

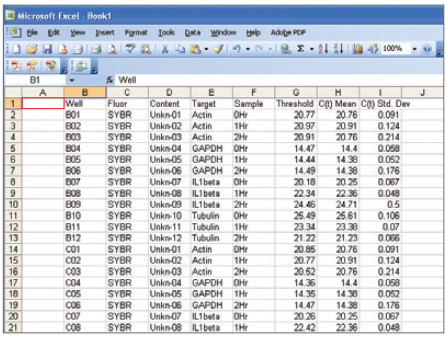

Fig. 3. Pasting data into a Microsoft Excel spreadsheet.

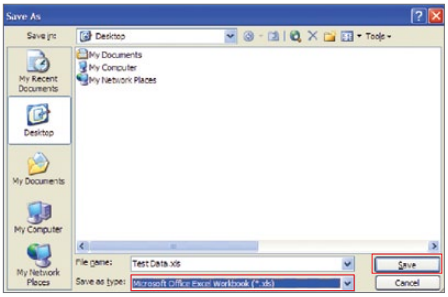

Fig. 4. Saving the file in .xls format.

Importing Data into qbase^{PLUS} Software

Note: If using CFX Manager software version 2.0 or later, qbase^{PLUS} software version 2.0 or later is required for importing Excel files.

To import an RDML file:

1. In qbase^{PLUS} software, select **Import > Import Project**.
2. Browse for and open the saved RDML file.
3. Click **Finish**.
4. A new project will be created containing a run for each fluorophore used.

To import an Excel file:

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 the name of the folder (Figure 5).
2. Create a new project folder by selecting **File > New > Project** in the main menu (Figure 6).
3. Click **Finish** in the confirmation window. Project folders store multiple experiment subfolders and default settings and fields to store your annotations and conclusions.

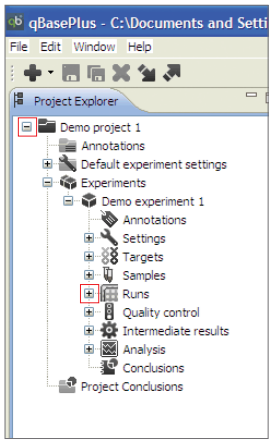

Fig. 5. The expanded file tree in Project Explorer.

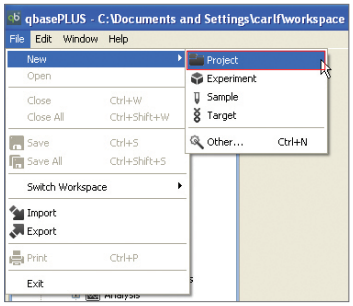

Fig. 6. Creating a new project folder.

4. Create a new experiment subfolder by selecting **File > New > Experiment** in the main menu (Figure 7).

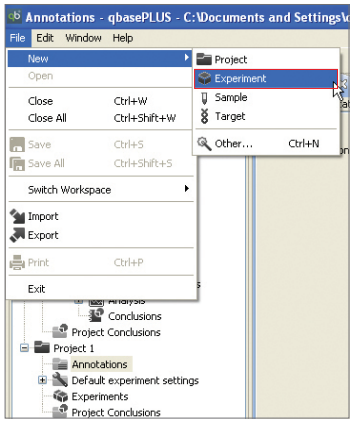

Fig. 7. Creating a new experiment subfolder.

5. Select **Project 1** in the confirmation window.
6. Click **Finish**. qbase^{PLUS} software 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 8).
8. In the Import window, select **Import Run**, then click **Next** (Figure 9).

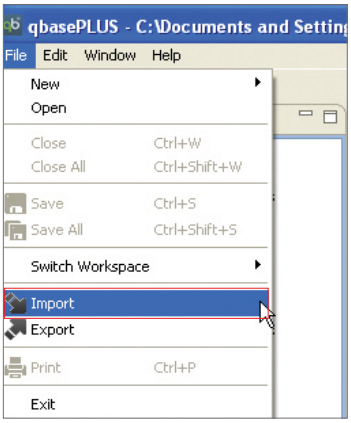

Fig. 8. Selecting Import from the File menu.

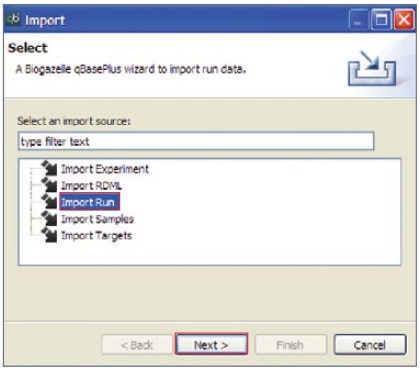

Fig. 9. Choosing to import a run.

9. The Import Run window opens (Figure 10).
   - 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: To import multiple Excel files simultaneously, click **Browse**, hold down Shift or Ctrl, select multiple Excel files, then click **Open**.

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

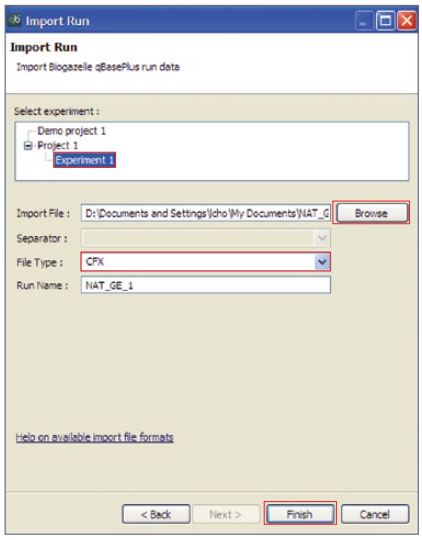

Fig. 10. The Import Run window.

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 11). Each run contains data for one dye from one plate; multiplex plate data are stored as several runs, one for each dye.

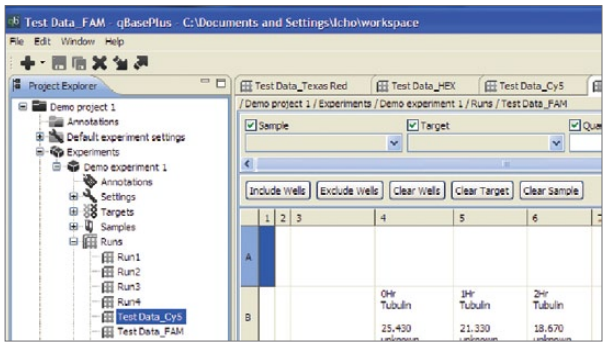

Fig. 11. Imported data displayed in the Project Explorer.

Note: For simplicity, this quick guide shows data import only 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 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 **Runs** under Experiment 1 in the Project Explorer pane, then select **Import Run**

Analysis Options and Viewing Gene Expression Results

1. 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 **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)
2. Under Experiment 1, expand the Targets folder. The Targets folder contains lists of Targets of Interest and Reference Targets. By default, qbase^{PLUS} software designates all targets as Targets of Interest upon import (unless RDML import is used). In order to calculate gene expression results you must designate at least one Reference Target.
3. Expand the list of Targets of Interest in the Targets folder.
4. Right click the name of the target that you want to use as a reference and select **Set Target Type > Reference Target** (Figure 12). qbase^{PLUS} software moves the target from the list of Targets of Interest to the list of Reference Targets.
5. Double click the name of a target in the list of Targets of Interest to view graphical results for the target expression (Figure 13).
6. Expand the Analysis folder under Experiment 1 and double click **Result table** to view the results for all targets in a spreadsheet view (Figure 14).

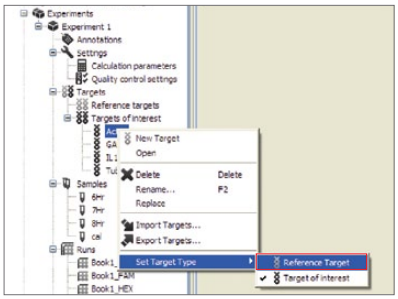

Fig. 12. Designating a Reference Target.

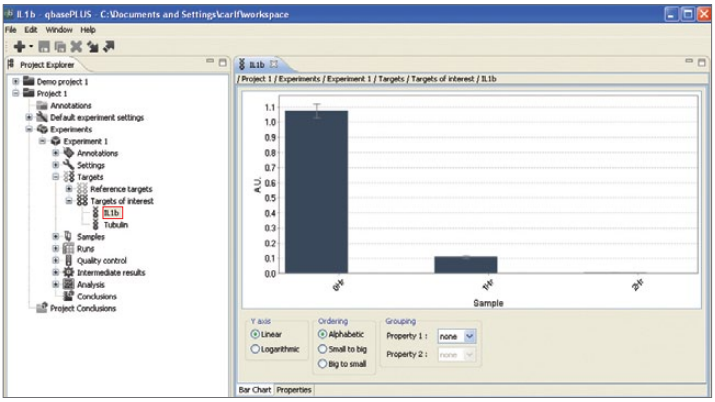

Fig. 13. Graph of gene expression data for a Target of Interest.

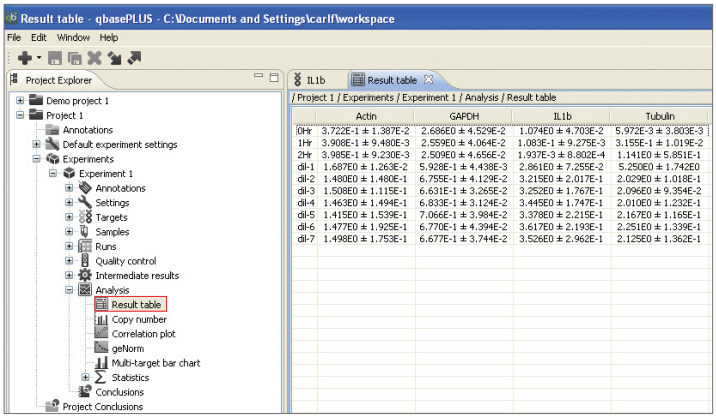

Fig. 14. Result table for all targets.

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