

run annotation

qbase^{PLUS}



Easy . Fast . Reliable .

Subject

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Subject

This chapter explains how well information can be annotated in a PCR run (plate, rotor or chip). In addition, you learn how to create and rename samples & targets and how to copy run layouts.



Summary



Runs can be annotated before or after import in qbase^{PLUS}. If an annotated run (i.e. 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*. qbase^{PLUS} also supports runs without annotation: sample and target information can be added after importing the run into the program. Note that annotated runs can also be edited in qbase^{PLUS}. While imported Cq values cannot be modified, sample and target names can always be edited and additional sample and target information can be included. If necessary, single or multiple wells can be excluded from the analysis.


Detailed information

qbase^{PLUS} can handle both annotated and not yet or incompletely annotated runs. If an annotated run (which contains Cq values, sample and target names, and quantities in case of standards) is imported, a sample and target list is automatically generated which can be accessed in the **Project explorer**. If additional annotated runs are imported, the existing sample and target lists are automatically updated with the sample and target names that are included in the imported run(s). An annotated run can be easily edited in qbase^{PLUS}. Furthermore, qbase^{PLUS} also allows adding sample and target names after importing (not yet annotated) run(s).

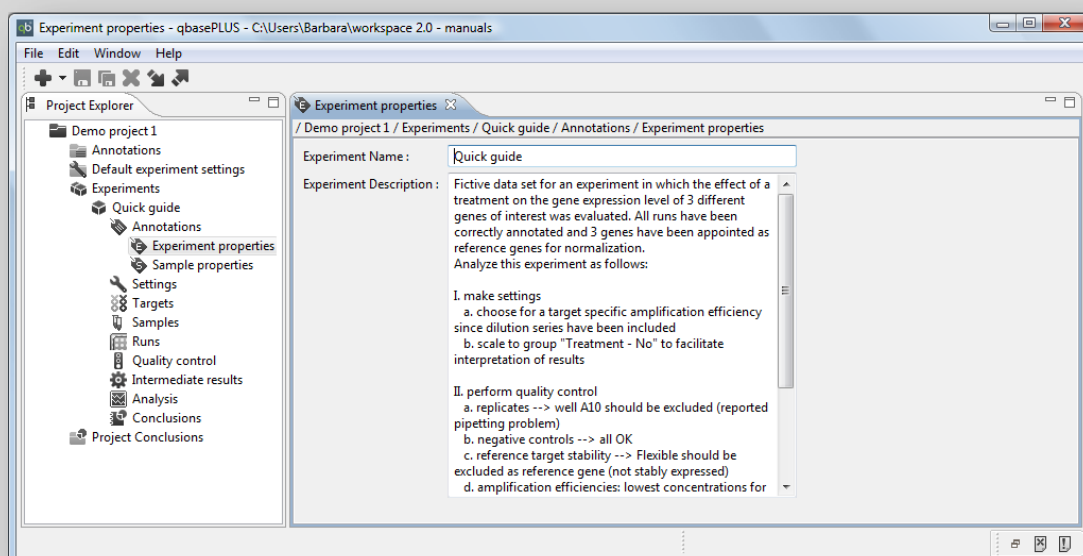
Experiment properties

General information or important notes related to an experiment can be saved. To access the **Experiment properties** window, double click on the **Annotations** icon  and then on the **Experiment properties** icon .

The **Experiment properties** window has two information fields, one holding the experiment name and the other containing additional information about the experiment (**Figure 1**). When changing the experiment name, the text will turn blue if it is a valid name (some special characters are not allowed). A red background indicates that an invalid experiment name has been entered. By pressing the Enter keyboard button or clicking the **Save** icon  the experiment name will be updated and the text will turn black again. An alternative way to change the experiment name is to select the experiment  and press the F2 keyboard button or to use the **Rename** option in the right-click context menu.

Experiment descriptions can be entered as free text. This field can hold multiple lines of information. Therefore the Enter keyboard button cannot be used to save the information. Use the **Save** icon  to save changes to the experiment description (blue text will turn black).



▼ Figure 1 - Experiment properties



Create new samples

This part describes how samples can be created individually. Information about how to import a list of sample names and accompanying information can be found elsewhere in this chapter.

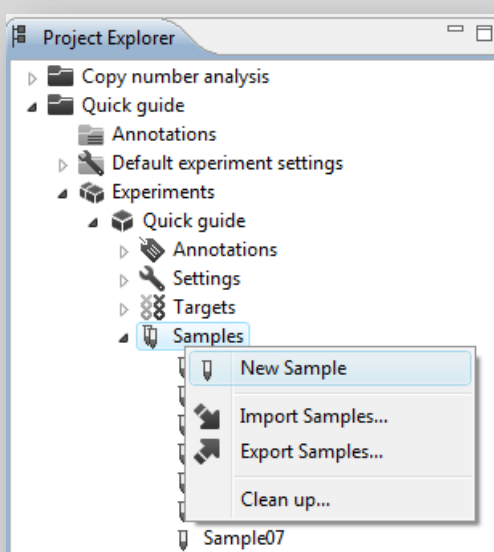
Step 1 - Add new sample

There are four ways to create new samples: (1) by making use of the right-click context menu in the project explorer (right click on the samples node  and select **New sample** - [Figure 2](#)), (2) by starting the new wizard by clicking  on the command bar, or (3) by using the menu bar (File > New). A fourth option is available in the run window only: select <New sample> from the sample drop down box.

Step 2 - New wizard

In case of option 2 and 3: choose **Sample** and subsequently select the experiment to which the sample belongs. Click **Finish** to complete the **New wizard**. Note that samples can only be added to loaded (open) experiments. Experiments that are not loaded are available for selection, but no sample can be added.

▼ Figure 2 – Sample right-click context menu



Step 3 - Sample properties

You will see that the new sample is added to the existing sample list. A Sample properties window opens where you can edit the sample name, the sample type (unknown sample, negative control, positive control, or standard), and the custom normalization factor.

Optionally, a sample description can be added. Blue text indicates unsaved entries. Modifications can be saved by clicking the disc icon, or by closing the sample window. In the latter case, a Save resource window opens that allows to save the modifications. A red background indicates that illegal characters have been used.

If you have more than 10 numbered samples we recommend using 'sample01' instead of 'sample1' because this results in a better alphabetical sorting in charts.

Prior to version 2.0, quantities for standard samples were to be added to wells in the run editor. Currently, the quantity value needs to be entered only once as a sample property. This means that the **different samples from a standard curve or dilution series should be given a different sample name**. If, during import, different quantities are provided for a single standard sample qbase^{PLUS} will only retain the last value that is imported.

The Custom normalization factor option (available since version 2.0) allows you to indicate a sample specific custom normalization factor. This option enables normalization based on a user provided value such as cell number count. In this method, a custom normalization factor must be entered for all samples, or be imported as a sample property during import of a sample list.

The Sample visibility in charts option allows you to indicate whether a specific sample or sample type has to be shown in the bar chart (Figure 3). By default, negative controls and standard curve samples will not be shown. This results in more intuitive charts and gives you more control on the content and look of your results.

Preferences can be modified in top menu (Window > Preferences). By default, only unknown samples and positive control samples are shown in the bar chart (Figure 4). Sample specific settings overrule the preferences.

Figure 3 – Sample properties

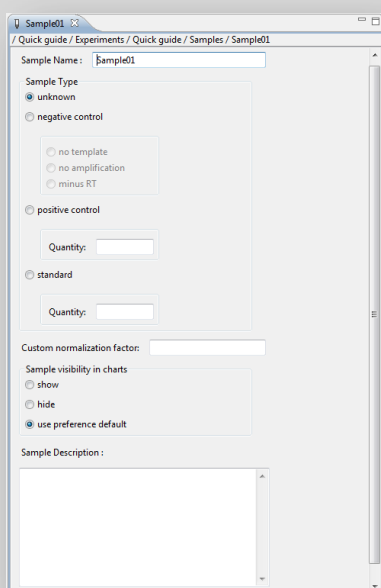
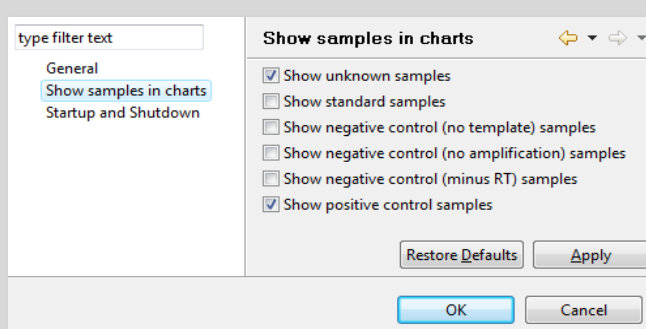


Figure 4 – Sample visibility options



Step 4 - Close Sample information windows

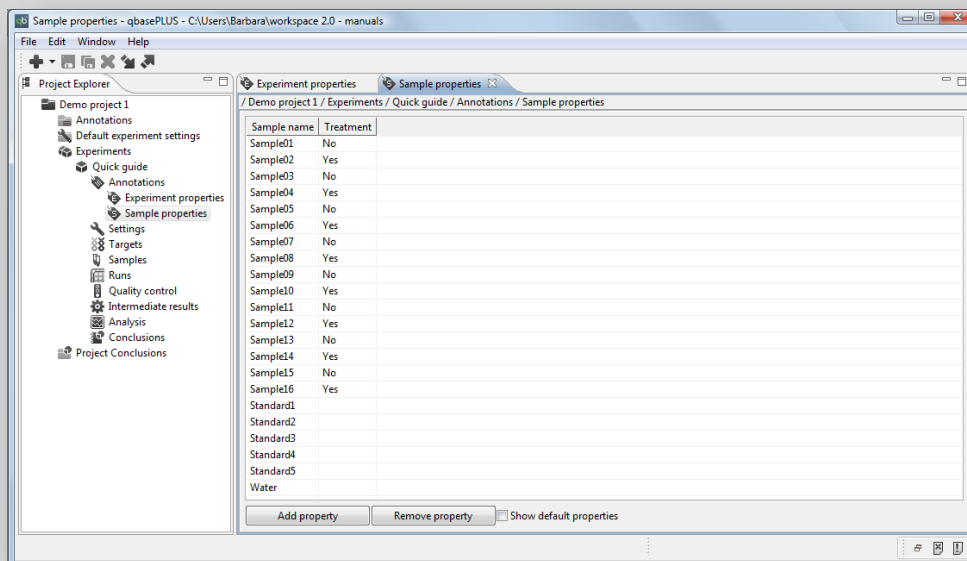
Several samples can be added in this way, one by one. Each time a sample is added, a Sample information window appears in the Main window. Close all tabs to clean up the Main window. Note that the right-click context menu contains a feature Close all for closing all windows at once (see 'User interface' chapter for more information on these functionalities).

Step 5 - Add custom sample properties (as of version 2.0)

Custom properties can be entered one by one in the Sample properties window (Figure 5). The Sample properties window can be accessed by double clicking the Annotations icon and then the Sample properties icon. Custom properties are used for visualization (sample grouping and rescaling) and statistics. To create a custom property, click Add property at the bottom and enter a name for the property. For each sample a value should be entered in this new property column.

Alternatively, custom sample properties can be imported during sample list import (see below).



▼ Figure 5 - Sample properties window



Create new targets

New targets are added in a similar way as described for the creation of new samples.

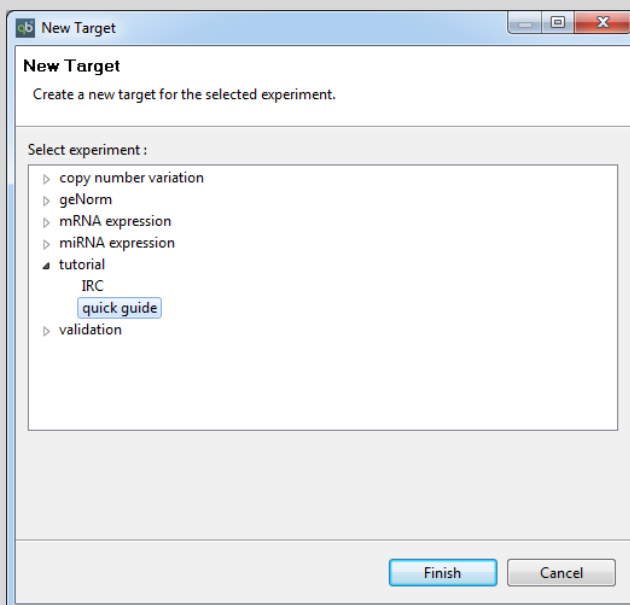
Step 1 - Add new target

There are four ways to create new targets: (1) by making use of the right-click context menu in the project explorer (right click on the targets node  and select New target), (2) by starting the new wizard by clicking  on the command bar, or (3) by using the menu bar (File > New). A fourth option is available in the run window only: select <New target> from the target drop down box.

Step 2 - New wizard

In case of option 2 and 3: choose Target and subsequently select the experiment to which the target needs to be added ([Figure 6](#)). Click Finish to complete the new wizard. Note that targets can only be added to loaded (open) experiments. Experiments that are not loaded are available for selection, but no targets can be added.

▼ Figure 6 – New target wizard



Step 3 - Target properties

You will see that the new target is added to the existing target list. A window opens in which you can edit the target name and enter useful information (such as gene ID & symbol, target type and sequence information) (Figure 7). Target information can be transferred through copy/paste from e.g. the RTPrimerDB database (available on <http://www.rtprimerdb.org/>). Blue text indicates unsaved entries. Modifications can be saved by clicking the disc icon, or by closing the target window. In the latter case, a save resource window opens that allows to save the modifications.

Note that this target window contains two tabs at the bottom. The properties tab contains the above-mentioned information, while the Bar Chart tab graphically displays the calibrated normalized relative quantities (CNRQ values, i.e. the fold changes relative to the reference level as defined in the Target scaling options) of the target across all samples.

Step 4 - Target type

Two types of targets are used in qbase^{PLUS}, Targets of interest and Reference targets. Reference targets (also referred to as housekeeping genes, a name deprecated by the MIQE guidelines, Bustin et al., 2009) are used for normalization. The target type can be defined via the target properties window (see step 3), or by using the right-click context menu in the Project explorer window (cursor needs to be on the target that has to be defined) and selecting Set Target Type from the drop down list.

Multiple targets can be simultaneously appointed as Reference targets or as Targets of interest by selecting all of them using the 'Shift' or the 'Ctrl' (Windows) or 'Command' (MacOSX) keyboard buttons.

Note that all targets labeled as Reference targets are used in the multiple reference gene normalization procedure (Vandesompele et al., 2002). To exclude one or more Reference targets, change their type into Target of interest, which causes the software to treat them as if they were Targets of interest.

Figure 7 – Target properties

The screenshot shows a window titled 'HPRT1' with a breadcrumb path: /geNorm / Experiments / pool / Targets / Reference targets / HPRT1. The window is divided into several sections:

- Properties:** Target Name: HPRT1, Official Gene Symbol: HPRT1, Entrez Gene ID: 3.251.
- Target Type:** Radio buttons for Reference Target (selected) and Target of Interest.
- Sequences:** Forward Primer: TGACACTGGCAAACAATG, Reverse Primer: GGTCCCTTTTCACCAGCAAGC, Probe 1: (empty), Probe 2: (empty), Amplicon: TGACACTGGCAAACAATGCAGACTTTGCTTTCCTTGGTCAGGCAGTATAAT.
- Commercial Assay:** Company: (empty), Order Number: (empty).

Import sample lists

Importing sample properties from additional columns in a sample import file (Figure 8) is a faster alternative to the individual creation of new samples. The format of these import files, as well as example files can be found at <http://www.biogazelle.com/support/qbaseplus/formats>.

Step 1 - Start Import samples wizard

Right-click in the project explorer on Samples (or a random sample) to start the Import samples wizard. The import button () in the toolbar will also lead to this wizard when selecting Samples.

Step 2 - Select sample list

Select the experiment in which the sample list needs to be imported. By default, the experiment selected in the project explorer is indicated as the target experiment. Browse for your sample list file and click the Next button.

Figure 8 – Sample import file

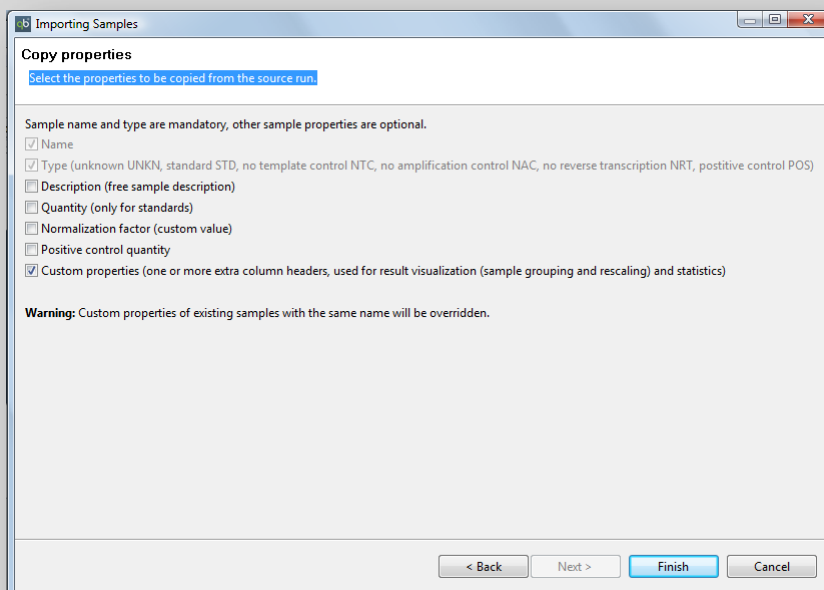
	A	B	C	D	E	F	G
1	name	type	description	quantity	normalization factor	positive control quantity	Treatment
2	sample01	UNKN					No
3	sample02	UNKN					Yes
4	sample03	UNKN					No
5	sample04	UNKN					Yes
6	sample05	UNKN					No
7	sample06	UNKN					Yes
8	sample07	UNKN					No
9	sample08	UNKN					Yes
10	sample09	UNKN					No
11	sample10	UNKN					Yes
12	sample11	UNKN					No
13	sample12	UNKN					Yes
14	sample13	UNKN					No
15	sample14	UNKN					Yes
16	sample15	UNKN					No
17	sample16	UNKN					Yes
18	water	NTC					
19	standard1	STANDARD					
20	standard2	STANDARD					
21	standard3	STANDARD					
22	standard4	STANDARD					
23	standard5	STANDARD					
24							
25							

Step 3 - Select properties to be copied (as of version 2.0)

Select the properties to be copied from the sample list. Sample name and type are mandatory; other properties are optional (Figure 9).

Click the Finish button to proceed.


Figure 9 – Import sample wizard



Import target lists

The procedure for the import of a target list is analogous to the procedure explained for the samples. The format of these import files, as well as example files can be found at <http://www.biogazelle.com/support/qbaseplus/formats>.

Step 1 - Start Import target wizard


Right-click in the project explorer on Targets (or a random sample) to start the Import target wizard. The import button () in the toolbar will also lead to this wizard when selecting Targets.

Step 2 - Select target list

Select the experiment in which the target list needs to be imported. By default, the experiment selected in the project explorer is indicated as the target experiment. Browse for your target list file and click the Finish button.

Edit samples and targets



Samples and targets can be edited in multiple ways: they can be deleted, renamed or replaced, or their properties and annotations can be altered.

Samples and targets can be **deleted** through the right-click context menu (cursor needs to be on the sample or target that should be deleted) or by selecting and clicking the Delete icon  in the command bar. Multiple samples or targets can be simultaneously deleted by selecting all of them.

As of version 2.0, there is an option to **clean up** sample and target lists (available through the context menu), resulting in automatic removal of all unused samples or targets (ie not appointed to any well).

Existing sample or target names can be modified (**rename**) by double clicking their name in the Project explorer. A window opens where you can change the name (and other properties). Alternatively, a name can be modified using the menu bar (Edit > Rename), through the right-click context menu or by using the F2 keyboard button (Figure 10).

It is not possible to rename a sample or target to a name that is already in use. E.g. if a given sample (or target) exists with two different names (e.g. due to typing error), the Replace option in the sample (target) right-click context menu should be used to **replace** the incorrect sample (target) name with the correct one (to be selected from a drop down list containing existing names).

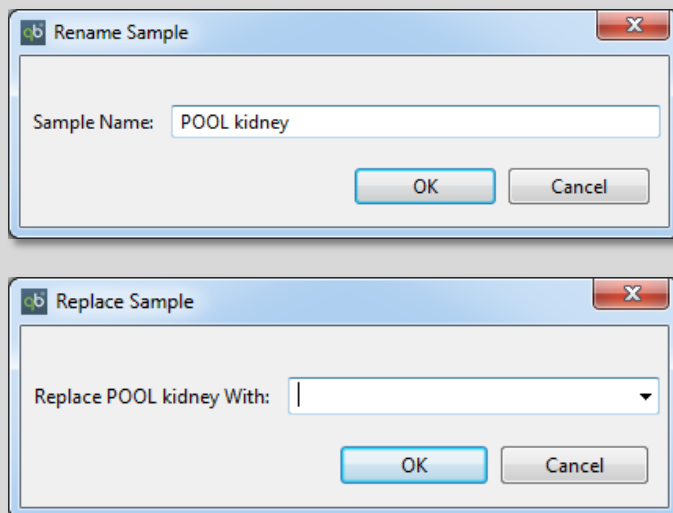
Sample/target settings and annotations are available by opening a Sample or Target window. Open such a window by double clicking the Sample  or Target  to be edited, or by using the Open option in the right-click context menu.

The Target window contains two tabs (at the bottom). When creating new targets, the Properties tab opens by default. Later, when reopening a Target for which results have already been calculated, the Results tab will be shown by default.

The Sample window also contains two tabs (at the bottom) as of version 2.0. When creating new samples, the Type tab opens by default. The Properties tab contains an overview of all custom properties for that particular sample.

An overview of all Custom sample properties of all samples is available in the Sample properties window (Figure 5).

▼ Figure 10 – Rename or replace



Well annotation

Following sample and target creation in the Project explorer, they have to be appointed to individual wells in a run. This part describes how to annotate single or multiple wells. See below for a description on how to copy the layout (position of samples or targets) of an entire run to another run.

Step 1 - Open run

Open the run by double-clicking its name in the Project explorer, or use the Open option in the run right-click context menu. The Run window contains a Properties tab at the bottom in which you can change the run name or alter the date on which the run was generated. It also contains information regarding the format of the run. Samples and targets can be appointed to wells in the Matrix tab that depicts the run layout with an annotation bar on top (Figure 11).

Step 2 - Appoint sample and target names

Select one or multiple wells in the run layout for which you want to add a sample, and use the Sample drop down list in the upper part of the window to search for the corresponding sample name. Multiple wells can be selected by dragging, by using the 'Shift' or 'Ctrl' keyboard buttons, or by selecting an entire row or column by clicking on the row or column header, respectively. As soon as a sample is appointed to a well, its name and type appear in the well (unknown, positive control, standard, or 3 types of negative control). Note that the sample type can be modified in the run annotation bar on top of the run layout itself (the same can be done through the Project explorer as mentioned before).

In a similar way, one or multiple wells can be assigned to a certain target. To this end, the Target drop down list is used.

Figure 11 – Run layout

The screenshot displays the 'Run1_SYBR' window in qbasePLUS. The interface includes a 'Project Explorer' on the left, a main grid for well annotation, and a 'Properties Matrix' at the bottom. The main grid has columns numbered 1-12 and rows labeled A, B, C, and D. Each cell in the grid contains a sample name and a numerical value. For example, in row A, column 1, it shows 'Sample01 Stable' with a value of 27,990. In row D, columns 11 and 12, it shows 'Water Stable' with a value of 'negative cont'. The 'Properties Matrix' at the bottom shows a table with columns for 'Message', 'Plug-in', and 'Date'.

Step 3 - Quantities

For 'standards', an input quantity must be defined in the corresponding field in the run annotation bar. In contrast to sample and target information, quantities are not selected from a drop down list but entered as a numerical value. Note that quantity values cannot be added to sample types other than Standard.

In the past, this was the only way to assign quantities for standard samples. As of version 2.0, this value can also be inputted as a sample property in the Sample window as described above (Figure 3).

Step 4 - Exclusion

In the run layout window, you can select the wells that need to be excluded from the analysis. This option does not erase the Cq value nor the sample/target annotation, which means that the excluded well can later be re-included in the analysis if needed.

Note that empty wells and wells without Cq value are automatically excluded by qbase^{PLUS} and cannot be included by the user.

Step 5 - Clear

The options Clear Sample and Clear Target can be used to remove sample and target names from selected wells. It does not remove any other well information.

The option clear wells completely erases the well specific information (including the Cq value), which means that it will be impossible to recover it later in the experiment. This option should be used cautiously, e.g. to remove a part of the run that is not relevant for the experiment.

Apply run layouts

To speed up run annotation, qbase^{PLUS} enables copying the layout of samples, targets or both from one run to another. When for instance the same set of samples is measured in different runs using the same layout (this is, the samples are measured in the same position in all runs), sample annotation of a run only needs to be performed once, saving you valuable time. This functionality can also be used across different (open) experiments.

Step 1 - Start Apply run layout wizard

Right-click in project explorer on the run (or selection of multiple runs) for which you want to edit the run information (sample name and type, target name, quantities of standard wells) and select the Apply run layout option.

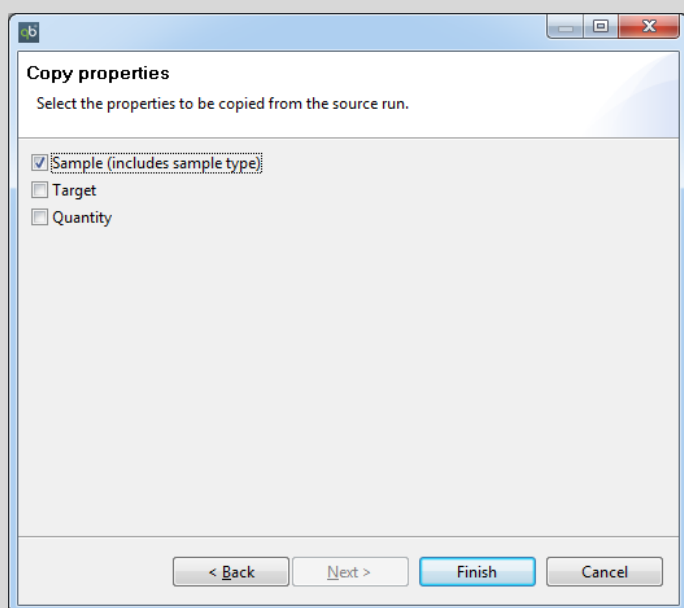
Step 2 - Select template run

Select the run that serves as a template. Note that only runs can be selected from open (loaded) experiments. If needed, cancel the current procedure and right-click on closed (unloaded) experiment and select Load Experiment.

Step 3 - Apply run layout options

Select properties that need to be copied (Figure 12). If annotation information is already present in the destination run, a warning will be displayed to notify you that information will be overwritten.

▼ Figure 12 – Apply run layout options



References

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F.

Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* 2002 Jun 18;3(7):RESEARCH0034

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

