

# copy number analysis

qbase<sup>PLUS</sup>



Easy . Fast . Reliable .

## Subject

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### References

## Subject

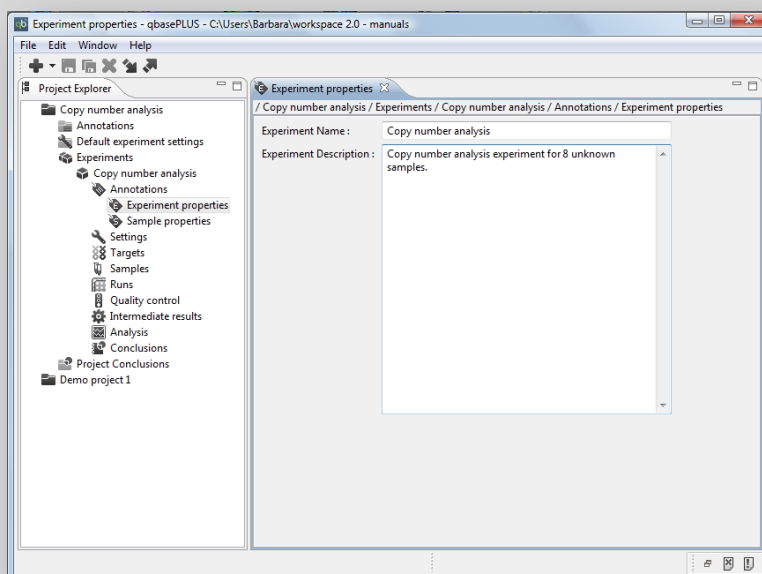
This chapter describes how you can use qbase<sup>PLUS</sup> for advanced copy number variant analysis [premium license required].

## Step-by-step



### Step 1 - Create a new experiment

In qbase<sup>PLUS</sup> qPCR run data are organized in experiments. To start data analysis, a new empty experiment needs to be created. Right click on the project (📁) in the qbase<sup>PLUS</sup> project explorer tree in which you want to start a new experiment and click *New experiment*. If needed, create a new project first in a similar way (*New > Project*). An experiment name and description can be provided in the *Experiment properties* window (Figure 1).

▼ Figure 1 - Annotating experiments



## Step 2 - Import run data

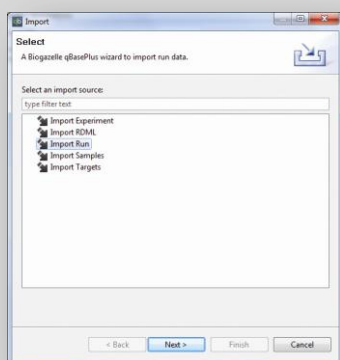
qbase<sup>PLUS</sup> experiments contain data from one or more runs. Run data can be imported by clicking the downward pointing arrow (  ) in the qbase<sup>PLUS</sup> toolbar followed by the selection of the Import Run option (Figure 2). Alternatively, right click on the project explorer Run element (  ) of the experiment in which you want to import the run and select Import Runs ... .

### Make the appropriate settings and selections in the next window

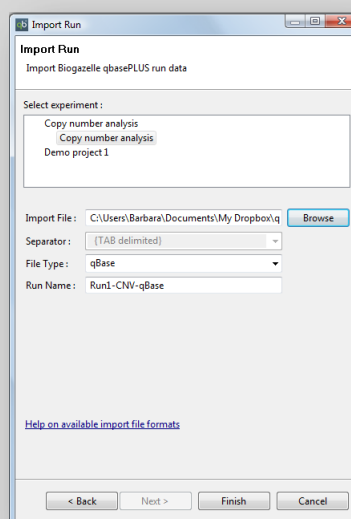
1. Select the experiment in which you want to import the run data (Copy number analysis in this example).
2. Browse for your run files. If multiple runs need to be imported in the selected experiment and all runs have the same file type, i.e. are derived from the same instrument using the same data collection software, you can make use of the batch import function (CTRL + click in Windows, command + click in MacOSX). In this Copy number analysis experiment, we will import the following file: 'Run1-CNV-qbase.xls' (Figure 3).
3. Select the file type that corresponds to the instrument and data collection software version that has been used for the creation of your run files ('qBase' for this example).

Click Finish to start the run import procedure. If needed, well annotation such as sample and target name can be added or modified in the run editor window that can be opened by double clicking the run name in the Project explorer tree. As in this example a fully annotated run file (containing a target and sample name for each well) is used, it does not require further editing.

▼ Figure 2



▼ Figure 3



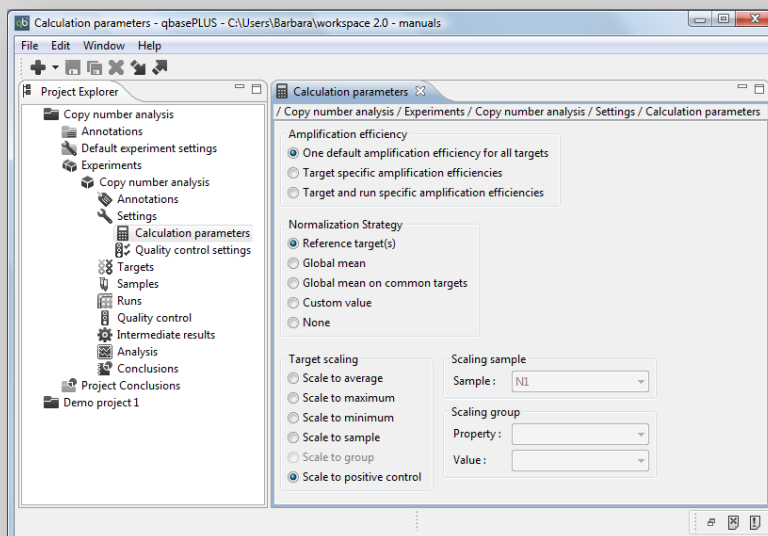
### Step 3 - Define calculation parameters

The parameters for the calculations can be defined in the Calculation parameters window (Project Explorer > Settings > Calculation parameters). This is the place to select the appropriate normalization strategy and target scaling option.

Please note that the multiple reference target normalization strategy is used by default. Users with a trial or premium license have the option to choose different normalization procedures to suit their specific needs in different types of experiments. In this example, we use the Reference targets normalization strategy, which allows the use of more than one reference sample for more accurate copy number calling.

The default option for target scaling is Scale to average. For copy number analysis you should select the Scale to positive control option (Figure 4).

▼ Figure 4 – Calculation parameters



## Step 4 - Define control samples

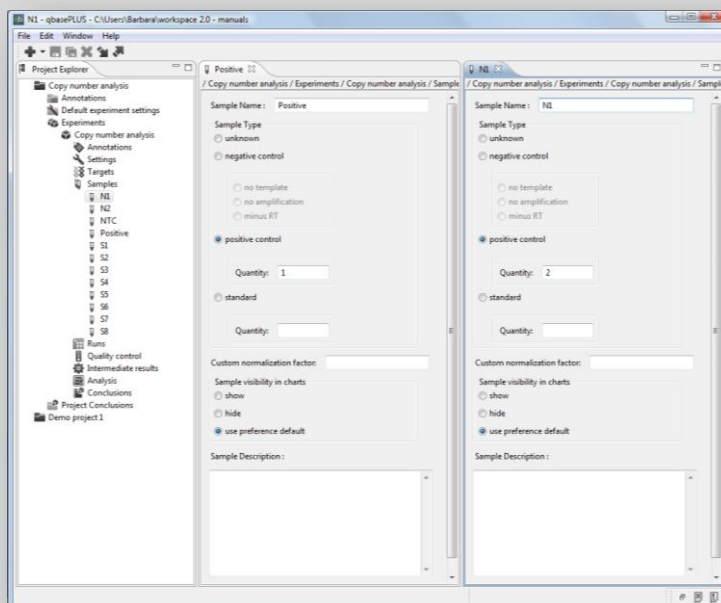
At least two types of control samples should be included in every qPCR-based copy number analysis [D'haene et al., 2010]. As for all PCR based assays, a no template control should be included to detect the presence of contaminating DNA. Specific for qPCR-based copy number analysis, is the inclusion of reference samples (positive controls) with a known copy number. These control samples are used as a reference point (or calibrator) for determination of the true copy numbers. The inclusion of multiple reference samples will result in more accurate results (analogous to using multiple reference targets for more accurate normalization).

Positive and negative control samples should be appropriately labeled in the sample list. Open the Sample properties window for the control samples and indicate the sample type (negative control or positive control). The positive control option allows you to indicate a sample specific copy number (e.g. 2 for a normal control or 1 for a deletion control) (Figure 5).

Please note that reference/calibrator samples with varying copy numbers can be used to provide greater flexibility in reference sample choice and to allow known deletions or duplications to be used both as a reference and as a point for quality control on the ability of the assay to accurately call deletions or duplications.

In this Copy number analysis experiment, we included three positive control samples with a known copy number: 2 normal controls (N1 and N2) and a deletion control (Positive).


▼ Figure 5 – Positive control sample







## Step 5 - Select reference genes



qbase<sup>PLUS</sup> supports the use of one single or multiple reference genes for normalization when choosing the Reference target(s) normalization strategy. You only need to indicate which targets should be used for normalization by selecting them, followed by a right click on any of these targets and Set target type to Reference target. This procedure can be done for multiple targets simultaneously by holding the CTRL key (Windows) or Command key (Mac) while selecting the targets. As soon as reference targets (ZNF15 and GPR80 in the Copy number analysis experiment) are defined as reference targets, the results for your target of interest will be available.

## Step 6 - Quality control

The parameters for quality control can be defined in the Quality control setting window (Project Explorer > Settings > Quality control settings ) (Figure 6). These parameters do not affect the result of the analysis, but define the required precision and accuracy of the analysis.

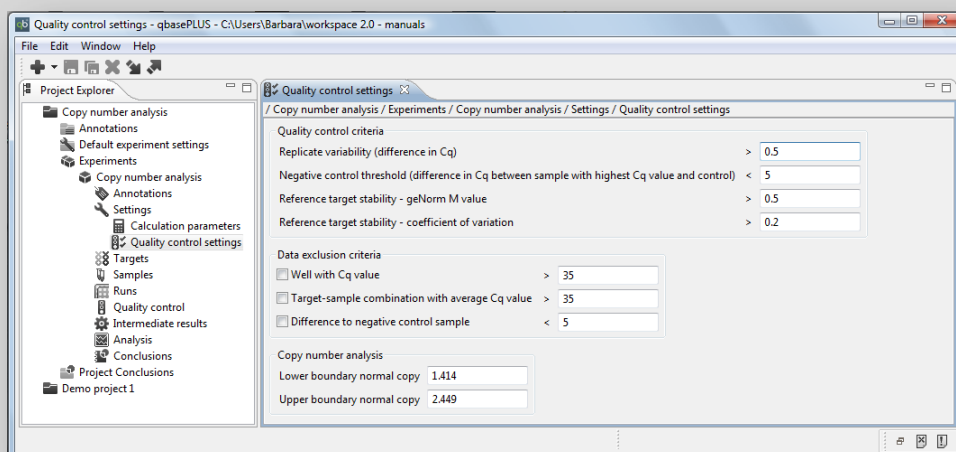
Quality control is an important feature in qbase<sup>PLUS</sup>. The program contains several types of quality control that can be accessed by double-clicking the Quality control icon  in the project explorer:

- quality control on technical replicates 
- quality control on positive and negative controls 
- quality control on the stability of reference targets 

A fourth type of quality control, that on the Normalization factors  is available in the Intermediate results section . More information can be found in a dedicated manual chapter on quality control.

Specific for qPCR-based copy number analysis is the definition of the thresholds for the Lower boundary for normal copy and the Upper boundary for normal copy in the Copy number analysis box. These thresholds are used for conditional bar coloring for easy detection of deletions and amplifications (see below) and are by default set at 1.414 (geometric mean of 1 and 2 copies) and 2.449 (geometric mean of 2 and 3). The default settings are recommended for a diploid organism (like human, mouse and rat) (Figure 6).

▼ Figure 6 – Quality control parameters



## Step 7 - Copy number analysis

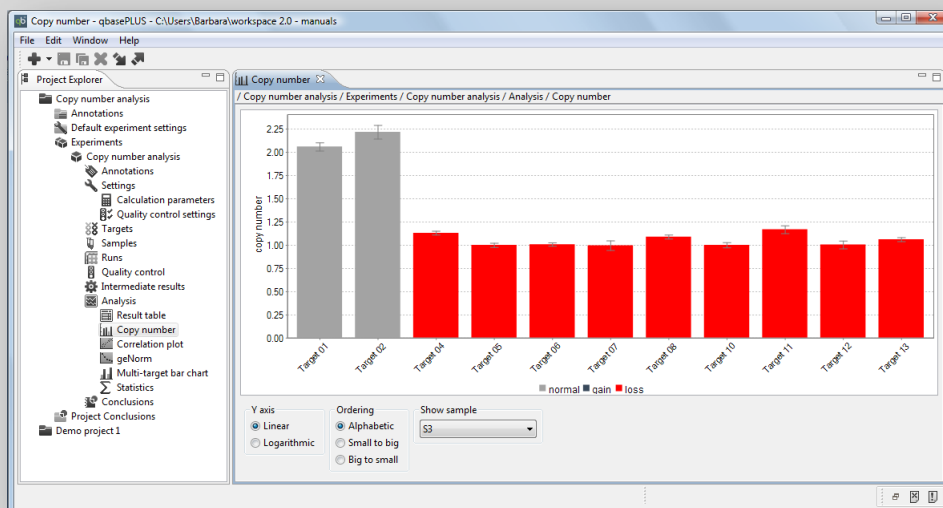
To visualize the copy numbers on a per sample basis, open the Copy number window (Project Explorer > Analysis > Copy number). The identified copy numbers are visualized on a per sample basis and conditional bar coloring is applied for easy detection of deletions and amplifications (Figure 7). The Y-axis indicates the copy number.

Use the Show sample drop down menu at the bottom to evaluate the results of the different samples.

## Step 8 - Export results

The results can be exported for reporting or further processing using different formats. Individual charts can be printed and exported as figures, or the entire result set can be exported in tabular format by clicking the upward pointing arrow (↑) in the qbasePLUS toolbar and selecting Export result table (CNRQ). You will be given the choice to export results only (calibrated normalized relative quantities) or to include the errors (standard error of the mean) as well. Scale can be linear or logarithm (base 10).

▼ Figure 7 – Copy number window



## References

D'haene B, Vandesompele J, Hellemans J.

Accurate and objective copy number profiling using real-time quantitative PCR. *Methods*. 2010 Apr;50(4):262-70

