How to analyze qPCR data

PREFACE

This document introduces statistical analysis of qPCR data in Qlucore Omics Explorer (QOE). It is assumed that the user is familiar with the basic functionality of QOE.

In addition to the statistical tests that are highlighted below other functionality such as plots and visualizations are also relevant for qPCR data analysis.

Contents

1. QPCR DATA 2

2. DATA PREPARATION 2

2.1. OPTION 1 2
2.2. OPTION 2 2

3. IMPORT DATA INTO QLUCORE OMICS EXPLORER 3

4. STATISTICAL TESTS 3

4.1. TWO GROUP TEST 3
4.2. MULTI GROUP TEST 4
4.3. TWO-WAY ANALYSIS 4

5. DISCLAIMER 5

1. QPCR DATA

Polymerase chain reaction, or PCR, uses repeated cycles of heating and cooling to make many copies of a specific region of DNA, like a gene. Real-time PCR (also called quantitative PCR or shorter qPCR) can be used to detect the amount of a gene that is present in a sample.

The CT (cycle threshold) value of a given gene represents the PCR amplification cycle at which the gene is detected above a certain threshold. It is a relative measure of the concentration of target in the PCR reaction. Higher CT values correspond to lower gene expression levels.

You often assume a 100% PCR amplification efficiency (two-fold increase with every cycle) across all samples, hence the use of a base two logarithm in the methods below. Note however that the real amplification efficiency value may not be 100%, i.e., not a two-fold increase.

2. DATA PREPARATION

The first step is to obtain CT values that are 'normalized'. To do so you apply a normalization method. There are several different options, two options are outlined here. The required steps for those are described below.

2.1. Option 1

You can use one or several housekeeping genes and computing normalization factors based on these.

The normalized CT values (the delta-CT values, ΔCT) are the difference between CT for each gene and CT for the housekeeping genes. It is common practice to use more than one housekeeping gene and to take the geometric mean of their CT-values before calculating the ΔCT.

ΔCT = CT (for a target gene) – CT (for the housekeeping genes)

Use for instance a spreadsheet for the normalization if the instrument manufacturer is not providing a dedicated tool for the task.

Note: The instrument manufacturer may denote “fold change” as the ratio between genes and housekeeping genes, i.e., the ΔCT values.

Again, a higher ΔCT value corresponds to lower expression, and many values will be negative (for genes that are more highly expressed than the housekeeping genes).

The ΔCT values should not be log-transformed, since they are already on the "log-scale" in the sense the abundance of the gene is proportional to 2^(- ΔCT). The ΔCT values can be used directly for PCA and linear models to look for differences between groups or association with a continuous phenotype.

2.2. Option 2

It is also possible to compute delta-delta-CT (ΔΔCT) values by comparing the delta-CT values for a sample with those for a calibration sample.

First you calculate the ΔCT value for each gene using the housekeeping genes.

ΔCT = CT (for a target gene) – CT (for the housekeeping genes)

Then you calculate the ΔΔCT value for each gene using the ΔCT for the same gene in the calibration sample.

ΔΔCT = ΔCT (for a target gene in sample) – ΔCT (for target gene in calibrator sample)

Then you can calculate the 2−ΔΔCT value using the method demonstrated by Schmittgen and Livak, 2008).

3. IMPORT DATA INTO QLUCORE OMICS EXPLORER

When data is normalized using one of the two options it shall be imported. Normally both data and annotations are imported. QOE provides import of data and annotations through Wizards. The starting point is to save your data from the normalization step in a *.txt file and then start QOE, select the file menu and the Import Wizard. The Wizard and steps are described in detail in the reference manual and in several videos that can be viewed at qlucore.com (Tip: Search for "Wizard" on https://qlucore.com/videos).

4. STATISTICAL TESTS

4.1. Two Group Test

A two-group comparison (t-test) can be used to test the significance of the difference between two conditions. The standard t-test assumes that the input values are normally distributed and the variance between conditions are comparable.

Below is an example with the data where a test has been made between "Normal" and "Infected" tissue. To the left a heatmap with clustering, highlighting the Tissue type, in the middle a Box plot for the "SGPL1" gene. The statistical test has been setup in the Statistics Window that can be seen to the right.

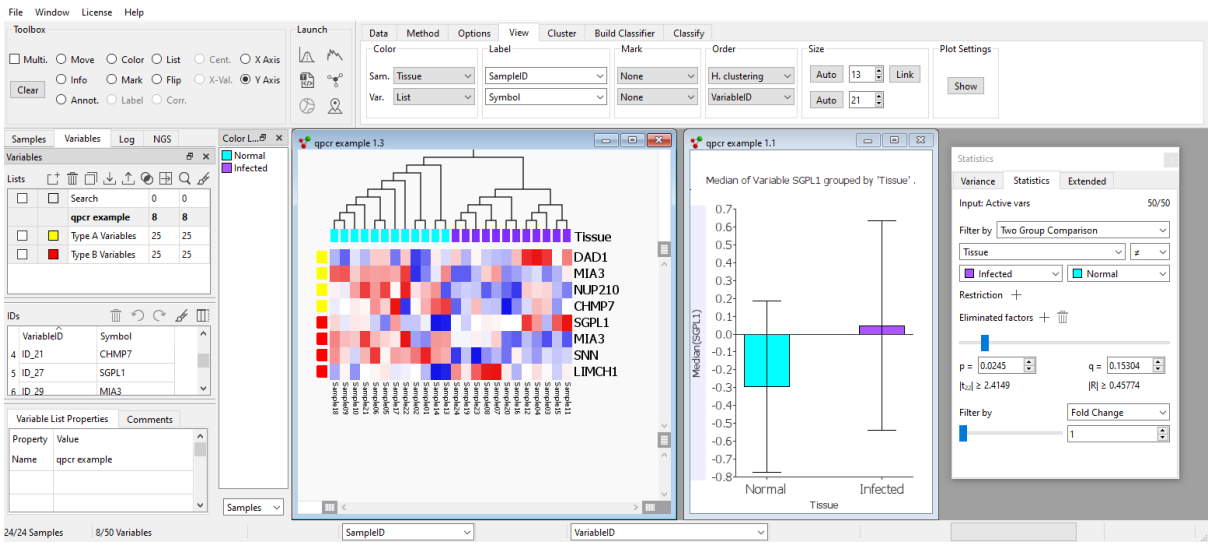

***Figure**: A t-test is performed using the Tissue annotation, a heatmap and a bar plot is shown*

As an alternative one can use the Mann Whitney U Test (Wilcoxon Rank Sum Test) when data is not normally distributed, and the number of samples are small, see the "Extended Statistics" in the inbuilt manual.

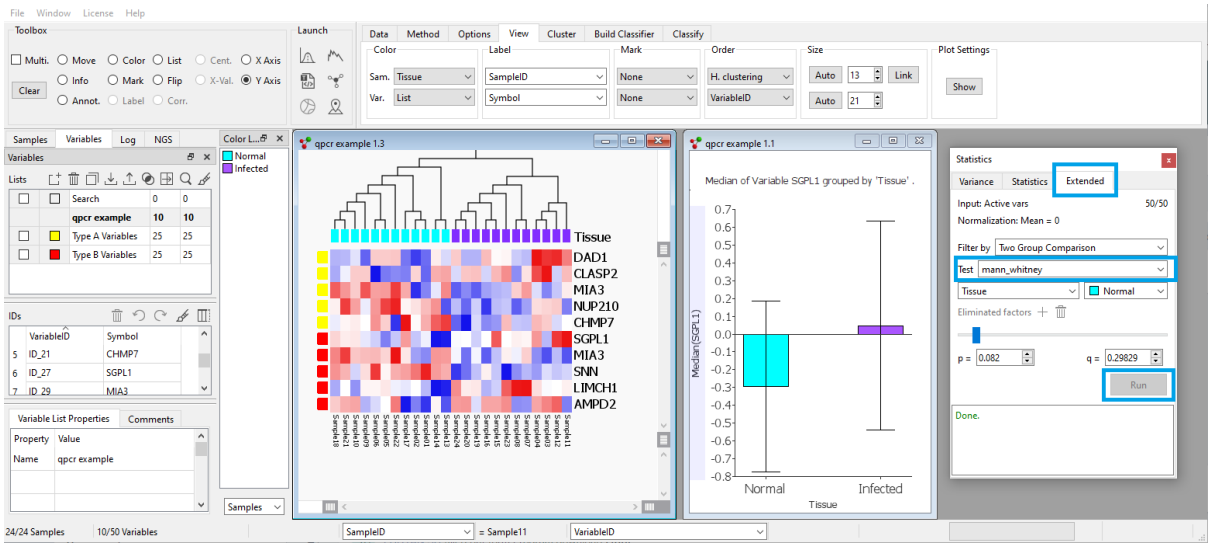

***Figure**: A Mann-Whitney test*

4.2. Multi Group Test

A multi group comparison (an ANOVA or F-test) can be used to test the significance of the difference between several conditions. ANOVA assumes that the input values are normally distributed and the variance between conditions are comparable. As an alternative one can use the Kruskal Wallis test when data is not normally distributed, see "Extended Statistics" in the inbuilt manual.

4.3. Two-way analysis

If you have two annotations, like Timepoint (in this case with two values) and Treatment (with three groups, here Placebo, Low Dose and High Dose) you can look for the interaction effect.

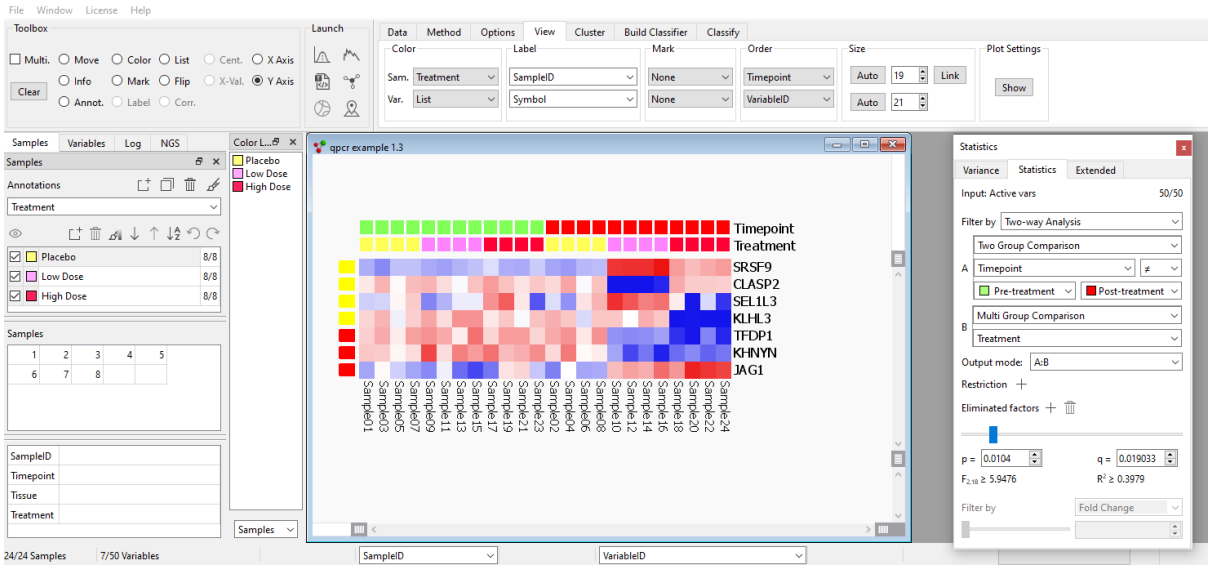

***Figure**: Two-way Analysis (interaction effect test)*

5. DISCLAIMER

The contents of this document are subject to revision without notice due to continuous progress in methodology, design, and manufacturing.
Qlucore shall have no liability for any error or damages of any kind resulting from the use of this document.
Qlucore Omics Explorer is only intended for research purposes.