

## RT-PCR Biostatistical Analysis

ACOBIOM is a Biotechnology company specialized in the discovery of new **Biomarkers** and in the development of innovative **Diagnostics**. ACOBIOM’s platform combines **molecular biology techniques** (NGS, high throughput sequencing, quantitative RT-PCR...) and specific tools & programs in **bioinformatics and biostatistics** to perform a large range of RNA/DNA analyses.

In case of Real-Time PCR (RT-PCR) analysis, there is a wide variety of applications. So, a single analysis pipeline doesn’t “work” for all cases, but a customized approach is much more appropriate.

Note that a crucial prerequisite for a successful RT-PCR study is that the generated data have the potential to answer the biological questions of interest. This is achieved first by defining a good experimental design (choice of the right primers and the probes, choice of the housekeeping genes and an appropriate number of replicates for the biological system under study), and second by planning an adequate execution of the PCR itself, ensuring that data acquisition does not become contaminated with unnecessary biases.

**Hence, Acobiom can design Your RT-PCR biostatistical analysis according to your specifications, for experimental design as well as for data analysis.**

Below, a generic roadmap for RT-PCR data analysis is described according to different stages. The major analysis steps are listed above the lines for pre-analysis, core analysis and advanced analysis. At last, we present visualization methods to confirm the integrity of the data and the process.

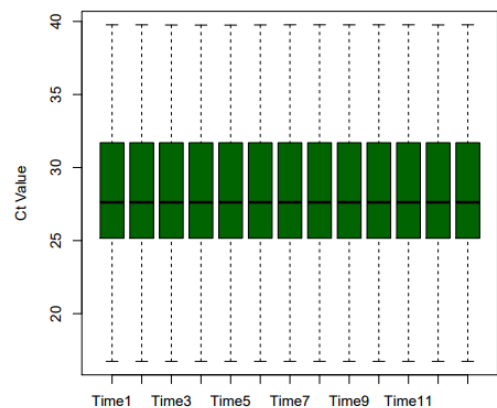
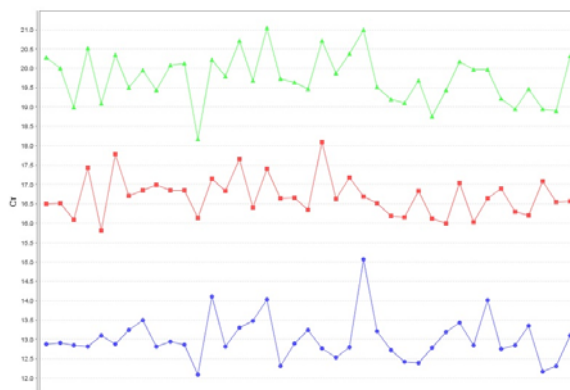
- **Input/Output (I/O)**

Input: Data Matrix (Cq raw reads), sample-level annotation metadata.

Output: Complete statistical tests answering any kind of problematic.

List of significant markers combined in a robust signature leading to a sample stratification/selection. (Premium Analysis)

- **Pre-processing:** Missing values, quality assessment/control, sample filtering (Numerical and graphical summaries of gene-level cq, gene filtering.)



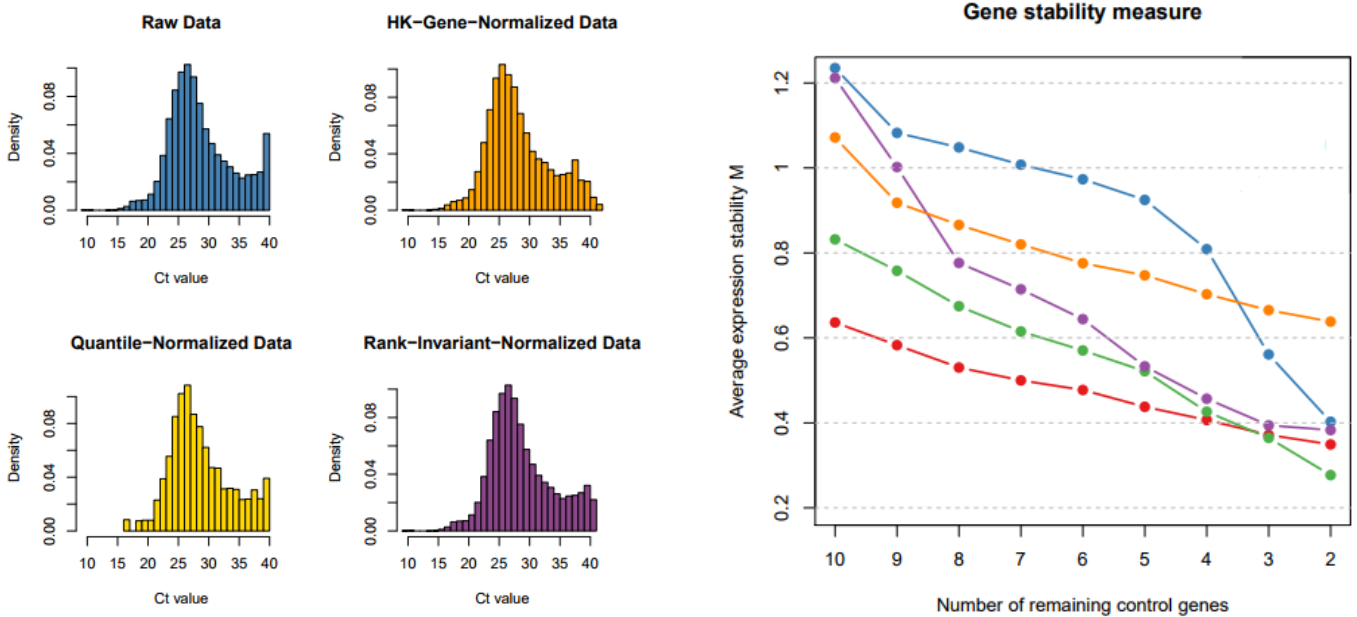
- **Normalization and expression quantitation** using adaptive methods regarding your data in order to determine the best normalization strategy.

- **Fitting into an adjusted model** after selection using the most adequate solution to your study. (Premium Analysis)
- **Optimization on the signature model:** using FDA-compliant Machine Learning and modeling methodologies. (Premium Analysis).

## 1. Pre-processing and Normalization

### • QA/QC

- Each Cq values are filtered in order to yield sufficient robust information across all predictive variables (conditions, pathological status, quantitative measure, etc.).



- Housekeeping genes are evaluated through an adapted M-values method (Vandesompele *et al*, 2002).
- Each run is evaluated through its replicates by controlling multiple parameters of distribution.

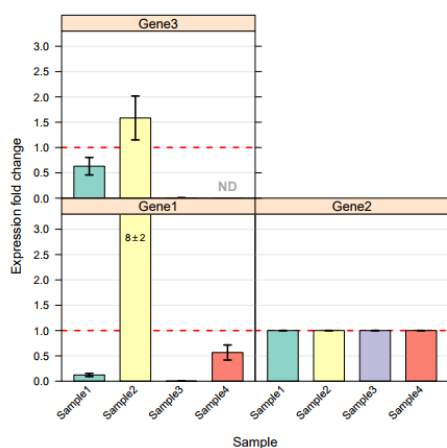
### • Normalization

Classic step of  $2\Delta\Delta Cq$  normalization (Livak *et al*, 2001).

$$\Delta Cq_{GOI} = [\text{Mean } Cq_{GOI}] - [\text{Mean } Cq_{HKG}]$$

### • Expression quantitation

All analyses are run on the  $\Delta Cq$  values only (after the first normalization of the  $2\Delta\Delta Cq$  method).



## 2. Model fitting

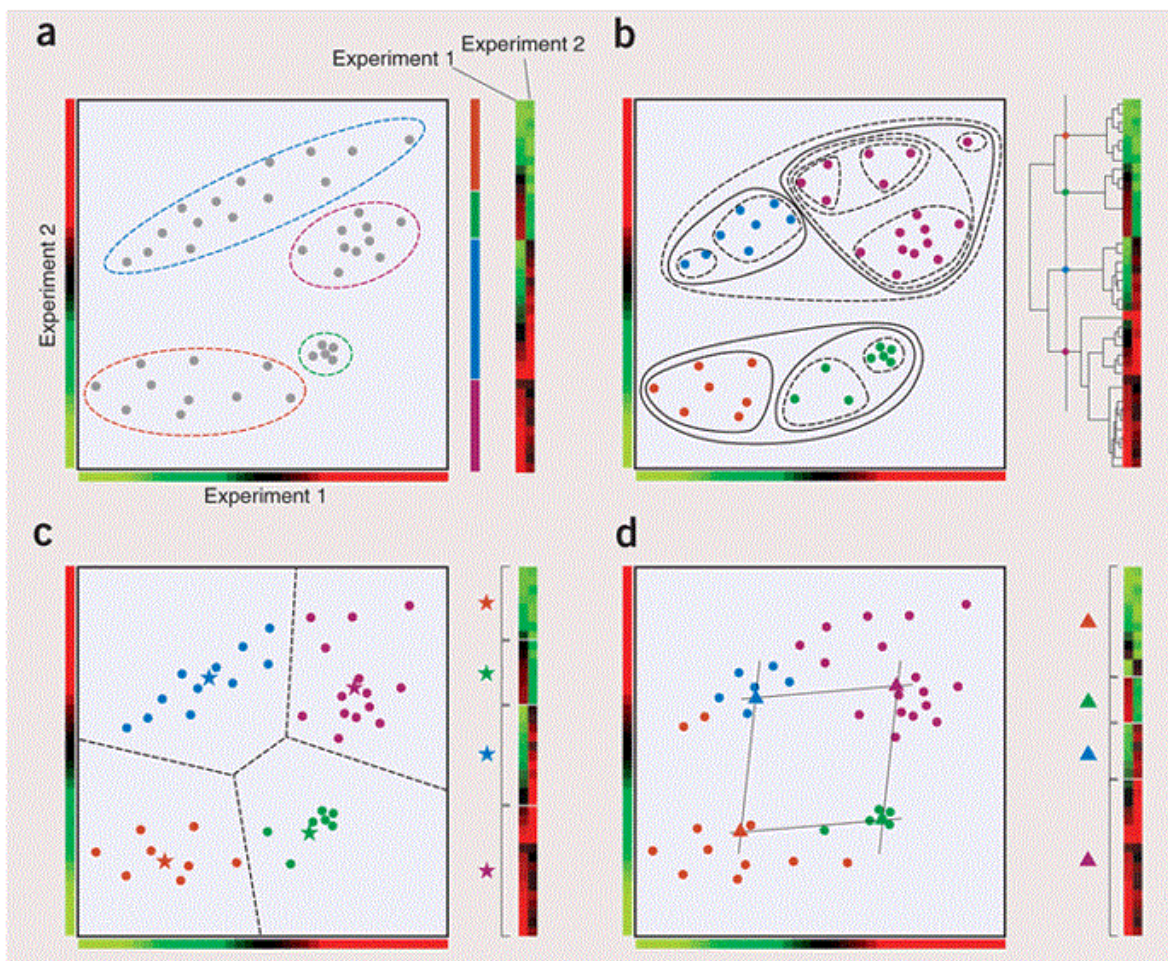
### Elastic-Net regression

- ACOBIOM's methodology is based on the « elastic-net » regression (Zou, Hui; Hastie, Trevor 2005).
- ACOBIOM's machine-learning algorithm repeatedly optimizes the model until convergence.
- The algorithm associates a range of genomic variables randomly comprised between 1 and  $n$  genes (any threshold of your liking can be used) in order to predict the output variable (survival, class selection, quantitative markers etc.).

Acobiom's team can compute any numbers of model (the optimized and sufficient number of computed models will be selected on line with your data) and all pertinent models related to the objective will be sorted.

For this selection, Acobiom associates for each gene a score based on its weight across all the models in order to discard the any kind of random effect association of variables a true and capable model.

Then, ACOBIOM computes a "masterkey" or a "new omics variable" to decipher all value of your qPCR experiment, from the selected genomic variables (the signature) in an « omics-score » or « index » associated to each sample.

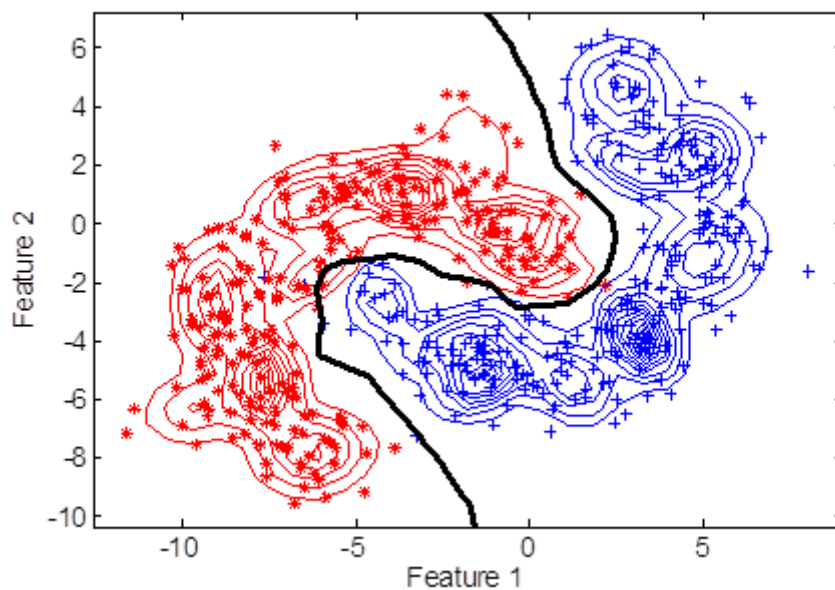


### 3. Optimization

#### Omics score

Once the signature and the « omics-score » have been computed, ACOBIOM can optimize the threshold of segregation between any numbers of conditions (« good responders » versus « bad responders » to a treatment for instance). ACOBIOM can define a buffer-zone (between the two populations following our example) to ensure the production of a safe and more accurate helping-decision tool (specificity and sensitivity wise).

These methodologies were built upon Health Authorities recommendation (FDA/EMA compliant) hence allowing our client a better understanding of regulatory demands regarding qPCR experiment.



The omics score can be used in addition to several variables, e.g. in personalized medicine the clinical variables, as the pain, localization of tumor, etc.

Data resulting from performance of your project will be confidential: you will be and stay the sole owner of the full data (raw and treated), that will be delivered on physical memory storage.

Acobiom's team is engaged in a constant scientific monitoring process for using the latest version of software and performing on the most recent omics analysis tools.

#### Contacts

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