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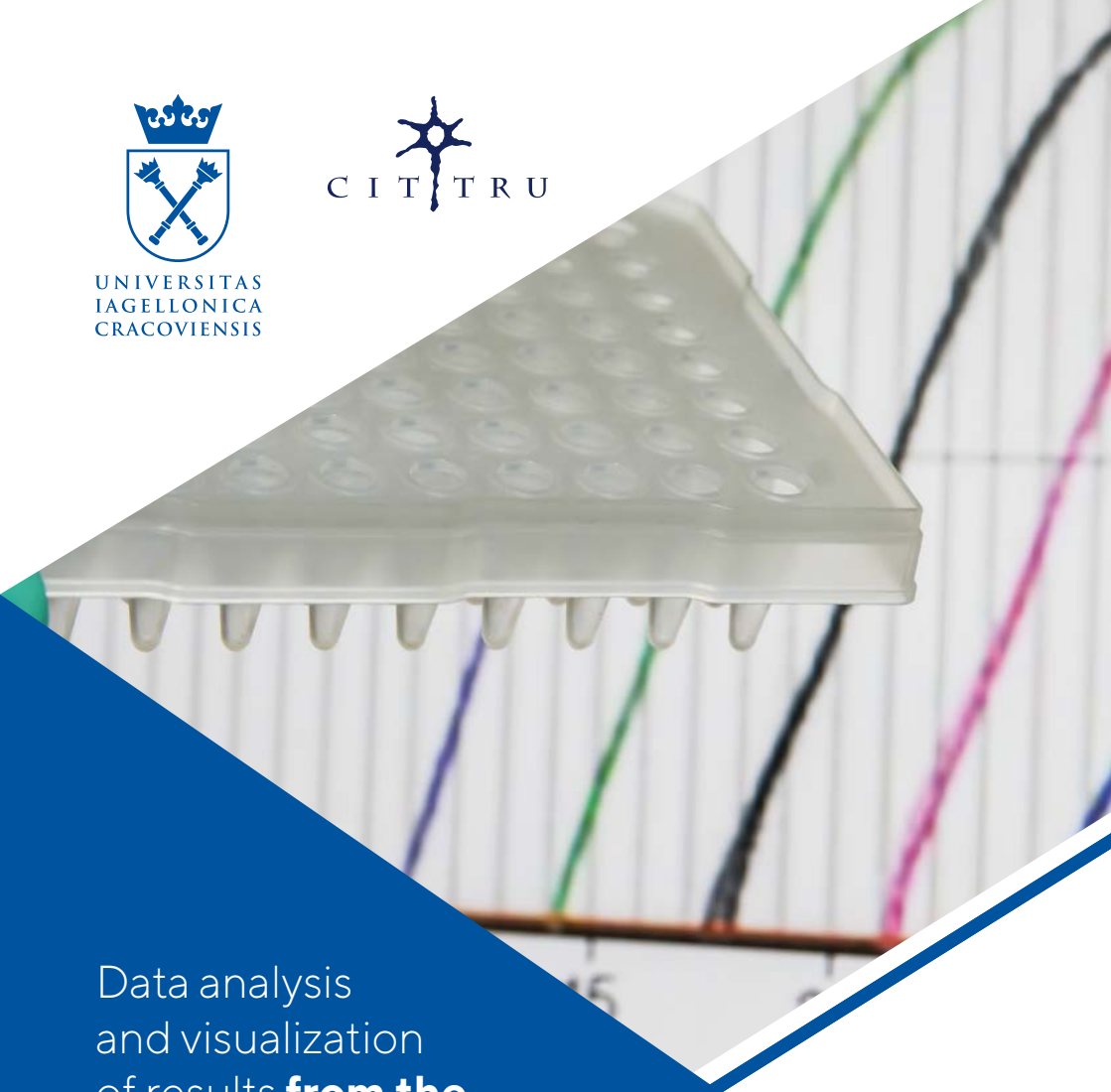

Data analysis and visualization of results **from the RT-qPCR method**

The computer program for data analysis and visualization of results from the RT-qPCR method

Fundusze Europejskie Inteligentny Rozwój

Rzeczpospolita Polska

Unia Europejska Europejski Fundusz Rozwoju Regionalnego

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1

qPCR method – challenges and limitations

Real-time quantitative polymerase chain reaction (RT-qPCR) is an analytical method widely used in research and diagnostics. In this method, the expression level of the target gene is calculated in relation to the reference gene/genes which are selected for its almost constant level of expression regardless of environmental factors to which the cell is subjected or its physiological **state**. Choosing the appropriate reference genes is crucial for the gene expression analysis and at the same time a limitation of the qPCR method. Their arbitrary selection only based on literature, may have consequences in an incorrect estimation of the studying genes expression, and thus in the faulty interpretation of the results. On the other hand, using available software such as geNorm, NormFinder or BestKeeper to select references gene, may lead to different results, which is related to the limitations of these algorithms.

2

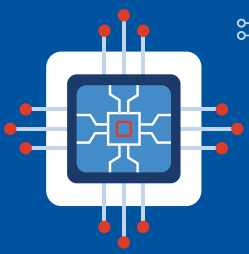
A new computational tool – the support of research

To solve this problem the research team from the Faculty of Biology of the Jagiellonian University developed an innovative method of **data analysis from the RT-qPCR method**. In this method, **the group of analyzed samples** (an experimental model) **is divided** into several sub-models consisting of a smaller number of samples (at least two samples) **and then in each of these models, the best reference gene/pair of genes is selected from the group** of potential reference genes using the NormFinder algorithm. The selected gene or pairs of genes being the best reference in a given model constitute the basis for further stages of the analysis, i.e. validation of the trend of target gene expression among particular samples.

Offer of cooperation in the implementation of the invention

The presented tool is the subject of the patent application. Further development of the invention is carried out by scientists from the Faculty of Biology of the Jagiellonian University.

Technology Transfer Center CITTRU UJ **is looking for entities interested in cooperation in the commercialization of the invention.**



The program allows for the estimation of the real level of expression of the genes tested and enables for correct biological interpretation of the results.

3

The coherence score

Within each model, statistical analyses are carried out based on appropriately selected tests, showing significant statistical differences in target gene expression between given samples across individual models. The next step is **to compare the consistency of the obtained results/values including statistics for a given target gene** within all tested models. The developed program sets the so-called coherence score describing the level of consistency of the obtained results. Achieving an unsatisfactory value of this coefficient – below the value acceptable by the researcher – leads to the next stage analysis, i.e. removal of the potential reference gene with the weakest stability value in each model, re-selection of the reference gene/pair of genes and re-validation of the results in all tested models.

4

Simultaneous and independent analysis of many target genes

The developed tool combines the functionalities of programs selecting the best reference genes, and spreadsheet programs, allowing for an **analysis of the expression level of the target gene, statistical analysis, and graphic interpretation of the results**. An important element of the solution is the implementation of a specific RT-qPCR data analysis scheme (removal of the potential reference genes with the weakest stability and subsequent analysis of raw data) and identification of the coherence score value defining the reliability/credibility of the analysis. The useful improvement of the entire process is also the automatic generation of box plots presenting the obtained results of the analysis, including the statistical significance marked on the graph between the examined samples of the experimental model (Fig. 1). The program enables the simultaneous, independent analysis of many target genes.

Fig. 1 A sample result of the analysis.

A

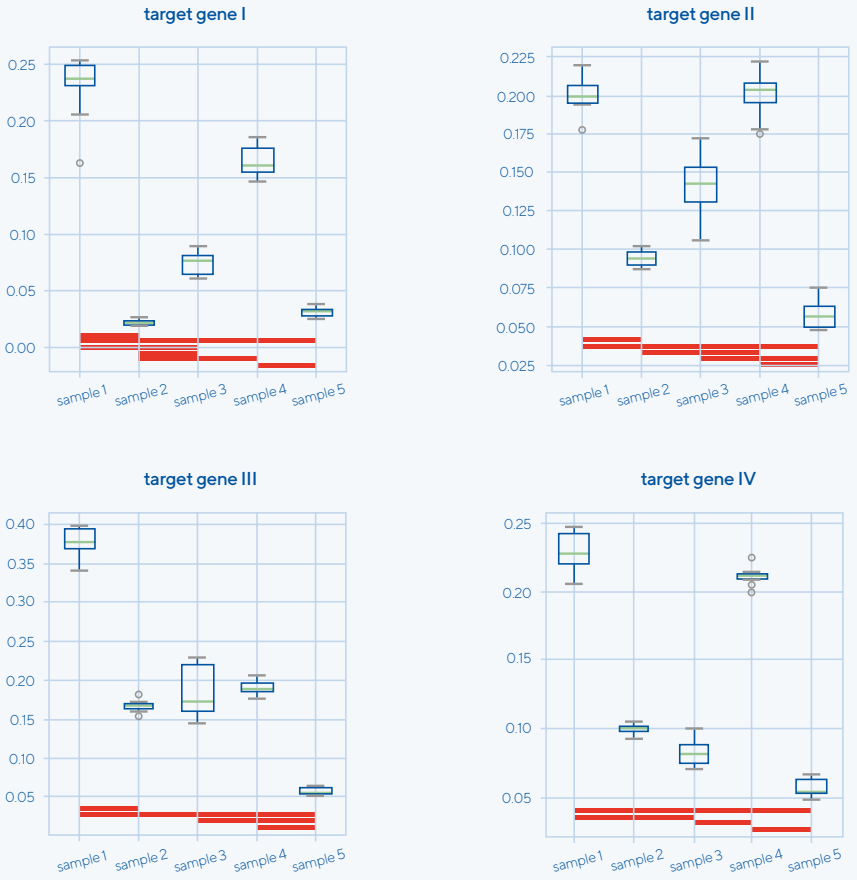
reference stability value
for the experimental model:

0.196718

B

range of stability values of references
for auxiliary models:

0.012949–0.227386



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