

GenEx software—the complete solution for qPCR data analysis

Overview

Applied Biosystems™ GenEx™ software by Multid Analyses is a leading solution for the processing and analysis of qPCR data. The powerful functionalities of GenEx software coupled with its user-friendly interface make it a preferred choice for both novices and professionals to cover their data analysis needs. The software offers basic data preprocessing and management, advanced cutting-edge data analysis, selection and validation of reference genes, sample classification, grouping of genes, monitoring of time-dependent processes, and much more.

Features

Easy data import—broad instrument compatibility

GenEx software supports import of electronic data sheet (EDS) files from Applied Biosystems™ real-time PCR instruments.

Data preprocessing—missing data, normalization, inter-plate calibration, quality control

GenEx software contains comprehensive tools that help you perform all important data preprocessing steps, such as the handling of missing data, normalization, inter-plate calibration, gDNA background subtraction, and efficiency correction.

Absolute quantification—standard curves, reverse regression, LOD, LOQ

Construct standard curves that relate C_q values to concentrations for absolute quantification. With reverse regression, absolute quantities including confidence intervals are estimated for unknown samples. The limit of detection (LOD) and limit of quantification (LOQ) for the analysis are also determined according to current guidelines (Figure 1).

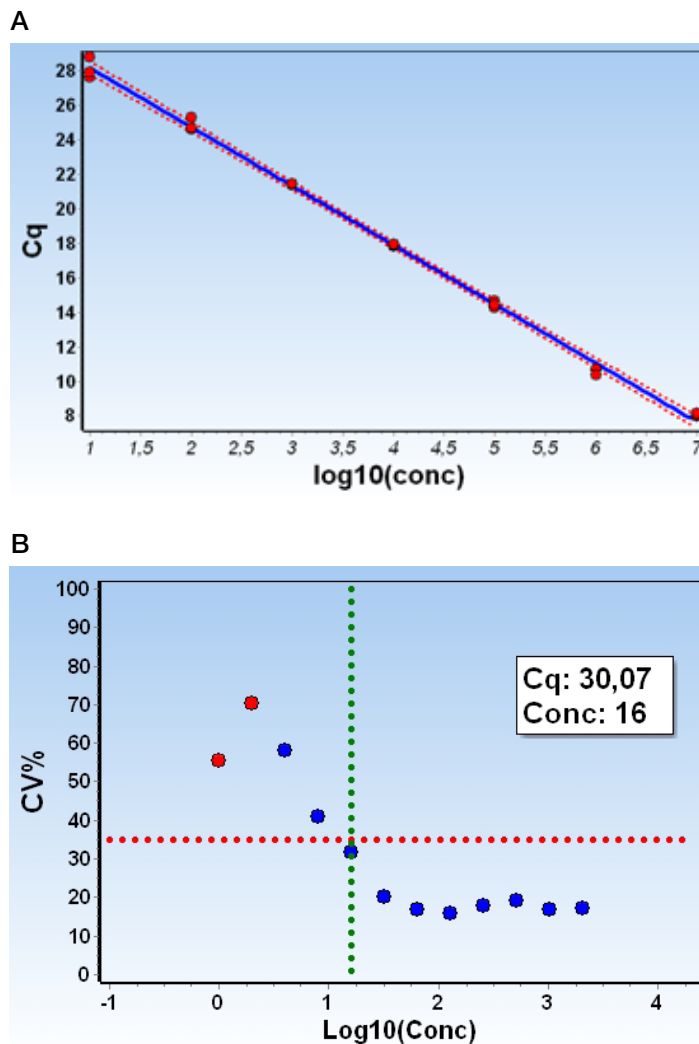


Figure 1. Absolute quantification. Examples of (A) standard curve with confidence interval and (B) limit of detection (LOD) with confidence interval. Screenshots provided by Multid Analyses AB, developers of GenEx software.

Relative quantification—reference gene identification, 1- or 2-way ANOVA, *t*-test

GenEx software contains powerful tools, including geNorm and NormFinder to identify the best reference genes and the optimum number of reference genes for normalization and relative quantification. Once normalized, the expression of genes of interest in two groups can be compared with parametric or nonparametric tests for unpaired as well as paired experimental design. When several genes are compared, GenEx software corrects for the multiple testing ambiguity. Several groups can be compared with 1-way ANOVA, and multifactorial studies are approached with 2-way ANOVA.

Expression profiling—PCA, hierarchical clustering, Kohonen self-organizing map

When many genes are analyzed, GenEx software offers a broad selection of profiling capabilities, including principal component analysis (PCA), hierarchical clustering analysis (HCA), volcano plot, and Kohonen self-organizing map (SOM), which exploit gene expression correlation to classify samples as well as genes based on common expression behavior (Figure 2).

Experimental design—optimizing a study based on requirement constraints

The key to successful qPCR analysis is, arguably, high-quality experimental design that balances statistical significance and experimental cost. By performing a fully nested pilot study, GenEx software estimates the variance contributions from the different experimental steps, advising you which steps may be improved to enhance data quality. It also indicates the number of subjects needed to achieve a desired resolution. Too few subjects may not prove or disprove your hypothesis, while too many subjects may improve resolution beyond what is practically relevant. GenEx experimental design tools predict optimized designs, taking into account budgetary restrictions based on pilot studies and estimated variance contributions.

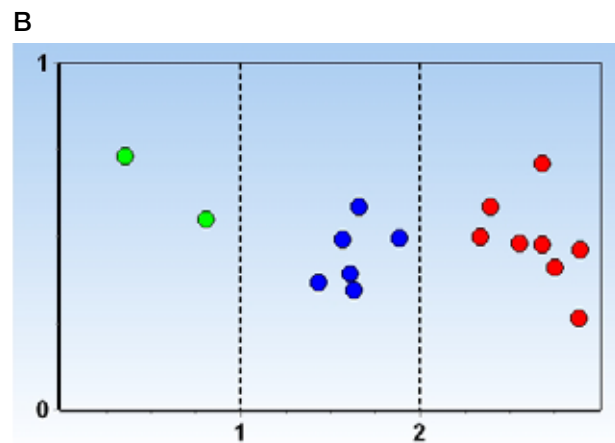
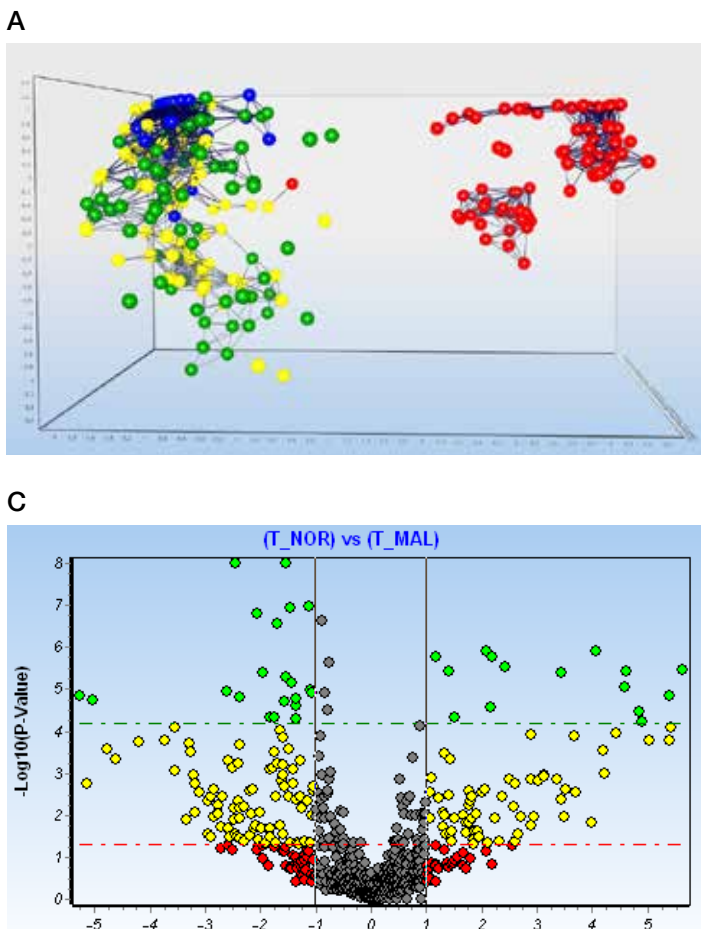


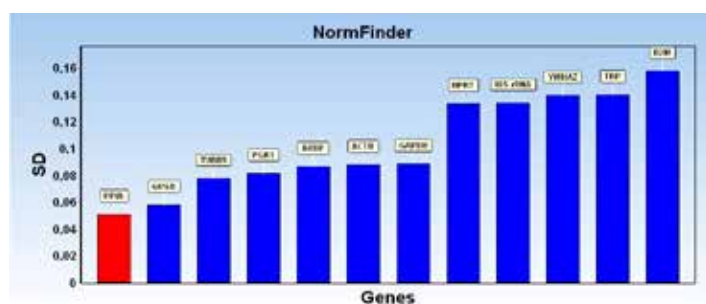
Figure 2. Expression profiling. (A) 3D PCA plot separating samples into groups, (B) clustering samples with Kohonen SOM, and (C) 2D volcano plot identifying the most significant genes. Screenshots provided by Multid Analyses AB, developers of GenEx software.

Finding the optimum reference genes—NormFinder

An appropriate approach for selecting reference genes is a special form of analysis of variance, which in qPCR literature is best known through the tool called NormFinder. NormFinder is applied to a panel of candidate reference genes that is analyzed in a set of representative samples.

In essence, NormFinder calculates the global average expression of all of the genes in all of the samples, to which the individual genes are compared. Based on this comparison, the SD for each candidate reference gene is estimated (Figure 3). Furthermore, if the samples are from different treatment groups, NormFinder separates the variation into intra-group and inter-group contributions.

A



B

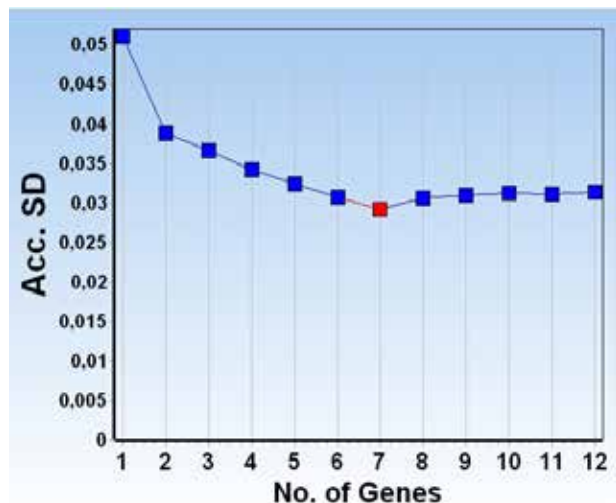


Figure 3. (A) NormFinder identifies the best reference gene, and (B) GenEx software calculates the optimum number of reference genes.

User reviews

Flexibility

Dr. Rafal Igielski, Department of Plant Biotechnology, Faculty of Biology, University of Szczecin, Poland

“I’m a plant biologist, and during the last years I’ve analyzed expression of 40 genes encoding enzymes related to gibberellins and abscisic acid metabolism. Working with a huge amount of qPCR data, which comes from multipoint analyses comprising tens of genes under various treatments, always was problematic. GenEx software solved all issues in an easy way, starting from preprocessing including normalization with reference genes, normalization with reference sample, relative quantities, and transformation to $\log_2$. What I love is that all the data are ready to copy and use to create custom charts. For me the most important benefits of having GenEx software are the possibility to select reference genes and of course full statistics, among them a simple *t*-test or 2-way ANOVA being just a few clicks away.”

Functionality

Prof. Mikael Kubista, founder and CEO of TATAA Biocenter, Sweden, and head of the Department of Gene Expression at Institute of Biotechnology, Czech Academy of Sciences

“Our clients request detailed precision and perfect control of the results we deliver, and GenEx software is the only software that provides all the statistical parameters needed to fully describe qPCR experiments, ranging from absolute quantification in diagnostics and relative quantification for classification to expression profiling and advanced single-cell work. The workflow of Applied Biosystems™ QuantStudio™ qPCR instruments and GenEx software is fully integrated, eliminating the risk of human error, making sure all experimental parameters describing the qPCR study are transferred and correctly considered in the statistical analyses of the data, and everything is done with just a few mouse clicks.”

Compatibility

Dr. Anders Stålberg, Sahlgrenska Cancer Center, University of Gothenburg, Sweden

“Our research lab uses GenEx software on a daily basis to perform a wide range of applications. The software is convenient for simple as well as complicated analyses such as standard curves, gene expression profiling, and single-cell analysis. We not only use GenEx software for all qPCR data but also for other types of data, including protein profiling, NGS, and array data. GenEx software enables all people in the lab to analyze and share data in a practical manner.”



Simplicity

Dr. Michael W. Pfaffl, Professor of Molecular Physiology, Technical University of Munich, Germany

“We are using the GenEx professional software package to analyze our real-time RT-qPCR results now for about 15 years. The transfer of the crude qPCR data from the PCR cyclers to the GenEx package is very convenient. The GenEx data analysis workflow is easy, intuitive, and MIQE compliant, which is very important for our work group and to publish the results in well-recognized journals. We are always happy with the results we retrieve. In particular, the advanced data analysis gadgets and statistical modules in GenEx software, for example HCA, PCA, or the dynamic PCA, make the identification of mRNA or microRNA biomarker signatures relatively simple. Our group can recommend GenEx professional software to any research or diagnostic real-time RT-qPCR laboratory worldwide.”



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