DataAssist™ – Data Analysis Software for TaqMan® Real-Time PCR Data

Matt Xia, Jon Sherlock, Patricia Hegerich, Xiaoqing You, Kathy Lee, Criss Walworth, and Eugene Spier

***Abstract*—A data analysis software, DataAssist™ software, has been developed for quick analysis and interactive visualization of TaqMan® real-time PCR data. It uses the comparative C_T method (also known as the $2^{-\Delta\Delta Ct}$ method) [1] to calculate relative quantities (RQ) of gene expression for sample comparison. The software uses a refined Grubbs' outlier test to remove outlier among technical replicates, provides a metric to measure control gene stability based on the geNorm algorithm [2] to assist with endogenous control selection, and allows using single or multiple control genes for data normalization. The software provides a function-rich graphic user interface (GUI), many content-rich tables and scalable graphics charts for easy, interactive, and rapid high-throughput data analysis and visualization.**

***Index Terms*—Comparative C_T method, Gene Expression, RT-PCR, TaqMan®.**

I. INTRODUCTION

Real-time RT-PCR is widely used to quantify gene expression levels by measuring the threshold cycle (C_T), an arbitrarily placed threshold which ensures the PCR is in the exponential phase of amplification. The C_T is reversely related to the amount of target molecules in the reaction. The classic comparative C_T method can be used to calculate the expression level of the gene of interest relative to a calibrator or reference sample using the C_T data [1].

Applied Biosystems provides a large collection of TaqMan® gene expression assays that are widely used for quantitative gene expression study. We have developed a data analysis tool, DataAssist™ Software, to quickly analyze and visualize the experiment data (C_T) generated by Applied Biosystems real-time PCR instruments, especially with TaqMan® Gene Expression Assays, TaqMan® Array Plates, or TaqMan® Array 384-Well Micro Fluidic Cards.

DataAssist™ Software is a simple, yet powerful data analysis tool for sample comparison. It uses the comparative C_T method to calculate relative quantity of gene expression. First it filters outliers among technical replicates using a refined Grubbs' test, and then normalizes the C_T data using single or multiple endogenous control genes:

$$\Delta C_T = C_T \text{ gene of interest} - \text{Normalization Factor} \qquad (1)$$

Normalization Factor is the arithmetic mean or geometric mean of C_T values of the selected control genes. If multiple genes are selected as controls, a gene stability measure is also calculated based on the geNorm algorithm to assist with selecting most stable control genes for data normalization [2]. The normalized ΔC_T data are used to calculate the relative gene expression fold change using a selected calibrator (reference sample):

$$\Delta\Delta C_T = \Delta C_T \text{ sample A} - \Delta C_T \text{ calibrator} \qquad (2)$$

$$\text{Fold Change} = 2^{-\Delta\Delta Ct} \qquad (3)$$

The fold change can also be calculated between sample groups of biological replicates, by grouping samples to biological replicates, the mean $2^{-\Delta Ct}$ of the biological replicates is used to determine the expression fold change [1]:

$$\text{Fold Change} = 2^{-\Delta Ct} \text{ group A} \,/\, 2^{-\Delta Ct} \text{ reference} \qquad (4)$$

Statistical analysis is performed to provide standard deviations for gene expression comparison between samples, and p-value from t-test for comparison between biological groups.

II. METHODS AND RESULTS

DataAssist™ software was developed using Java as a standalone desktop application for Windows XP and Vista® operating systems. Java Swing was used to implement the Graphic User Interface (GUI), and the open-source Java chart library JFreeChart [3] was used to implement most charts for data visualization. The software installer for Windows was created using open source tool Nullsoft Scriptable Install System (NSIS) [4]. DataAssist™ Software is freely available at http://www.appliedbiosystems.com/DataAssist.

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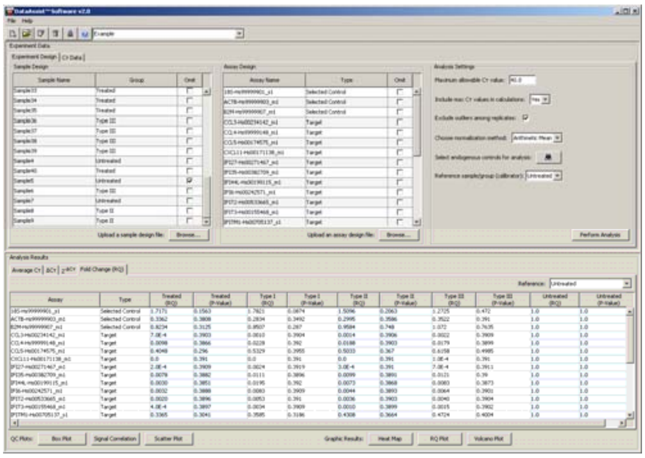

DataAssist™ Software v2.0
File Help
Example
Experiment Data
Experiment Design | CT Data
Analysis Results
Average CT | ΔCT | 2^-ΔCT | Fold Change (RQ)
Upload a sample design file: Browse...
Upload an assay design file: Browse...
Perform Analysis
QC Plots: Box Plot | Signal Correlation | Scatter Plot
Graphic Results: Heat Map | RQ Plot | Volcano Plot

Sample Design

Sample Name	Group	Omit
Sample33	Treated	☐
Sample34	Treated	☐
Sample35	Treated	☐
Sample36	Type III	☐
Sample37	Type III	☐
Sample38	Type III	☐
Sample39	Type III	☐
Sample4	Untreated	☐
Sample40	Treated	☐
Sample5	Untreated	☑
Sample6	Type III	☐
Sample7	Untreated	☐
Sample8	Type II	☐
Sample9	Type II	☐

Assay Design

Assay Name	Type	Omit
18S-Hs99999901_s1	Selected Control	☐
ACTB-Hs99999903_m1	Selected Control	☐
B2M-Hs99999907_m1	Selected Control	☐
CCL3-Hs00234142_m1	Target	☐
CCL4-Hs99999148_m1	Target	☐
CCL5-Hs00174575_m1	Target	☐
CXCL11-Hs00171138_m1	Target	☐
IFI27-Hs00271467_m1	Target	☐
IFI35-Hs00382709_m1	Target	☐
IFI44L-Hs00199115_m1	Target	☐
IFI6-Hs00242571_m1	Target	☐
IFIT2-Hs00533665_m1	Target	☐
IFIT3-Hs00155468_m1	Target	☐
IFITM1-Hs00705137_s1	Target	☐

Analysis Settings

Maximum allowable CT value: 40.0

Include max CT values in calculations: Yes

Exclude outliers among replicates: ☑

Choose normalization method: Arithmetic Mean

Select endogenous controls for analysis:

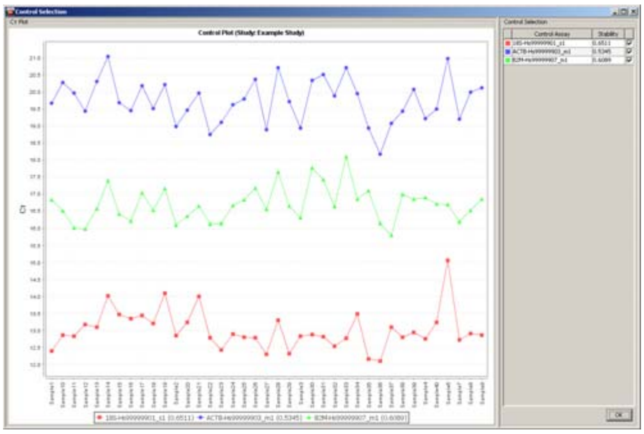
Reference sample/group (calibrator): Untreated

Reference: Untreated

Assay	Type	Treated (RQ)	Treated (P-Value)	Type I (RQ)	Type I (P-Value)	Type II (RQ)	Type II (P-Value)	Type III (RQ)	Type III (P-Value)	Untreated (RQ)	Untreated (P-Value)
18S-Hs99999901_s1	Selected Control	1.7171	0.1563	1.7821	0.0874	1.5096	0.2063	1.2725	0.472	1.0	1.0
ACTB-Hs99999903_m1	Selected Control	0.3362	0.3808	0.2834	0.3492	0.2995	0.3586	0.3522	0.391	1.0	1.0
B2M-Hs99999907_m1	Selected Control	0.8234	0.3125	0.8507	0.287	0.9584	0.748	1.072	0.7635	1.0	1.0
CCL3-Hs00234142_m1	Target	7.0E-4	0.3903	0.0010	0.3904	0.0014	0.3906	0.0022	0.3909	1.0	1.0
CCL4-Hs99999148_m1	Target	0.0098	0.3866	0.0228	0.392	0.0188	0.3903	0.0179	0.3899	1.0	1.0
CCL5-Hs00174575_m1	Target	0.4048	0.296	0.5329	0.3955	0.5033	0.367	0.6158	0.4985	1.0	1.0
CXCL11-Hs00171138_m1	Target	0.0	0.391	0.0	0.391	0.0	0.391	1.0E-4	0.391	1.0	1.0
IFI27-Hs00271467_m1	Target	2.0E-4	0.3909	0.0024	0.3919	3.0E-4	0.391	7.0E-4	0.3911	1.0	1.0
IFI35-Hs00382709_m1	Target	0.0078	0.3882	0.0111	0.3896	0.0099	0.3891	0.0121	0.39	1.0	1.0
IFI44L-Hs00199115_m1	Target	0.0030	0.3851	0.0195	0.392	0.0073	0.3868	0.0083	0.3873	1.0	1.0
IFI6-Hs00242571_m1	Target	0.0032	0.3888	0.0083	0.3909	0.0044	0.3893	0.0064	0.3901	1.0	1.0
IFIT2-Hs00533665_m1	Target	0.0020	0.3896	0.0053	0.391	0.0036	0.3903	0.0040	0.3904	1.0	1.0
IFIT3-Hs00155468_m1	Target	4.0E-4	0.3897	0.0034	0.3909	0.0010	0.3899	0.0015	0.3902	1.0	1.0
IFITM1-Hs00705137_s1	Target	0.3365	0.3041	0.3585	0.3186	0.4308	0.3664	0.4724	0.4004	1.0	1.0

Fig. 1 DataAssist™ software main window. It allows quick and interactive experiment setup including data analysis settings and sample grouping to biological replicates.

DataAssist™ software provides a function-rich GUI for easy data importation, experiment setup, and interactive, high-throughput data analysis (Fig. 1). The calculation in DataAssist™ software is very rapid and the results are provided in content-rich tables and scalable graphics charts that can be easily exported (Fig. 2 - 9).


Control Selection
CT Plot
Control Plot (Study: Example Study)
CT
18S-Hs99999901_s1 (0.6511)
ACTB-Hs99999903_m1 (0.5345)
B2M-Hs99999907_m1 (0.6089)
OK

Control Selection

Control Assay	Stability	
18S-Hs99999901_s1	0.6511	☑
ACTB-Hs99999903_m1	0.5345	☑
B2M-Hs99999907_m1	0.6089	☑

Fig. 2 Control Selection Plot and Table. The plot displays C_T values of control genes for all samples, which gives a quick overview of the expression profile of each control gene. The gene stability measure [2] is shown in the adjacent table to assist with selecting the most stable control genes for data normalization.

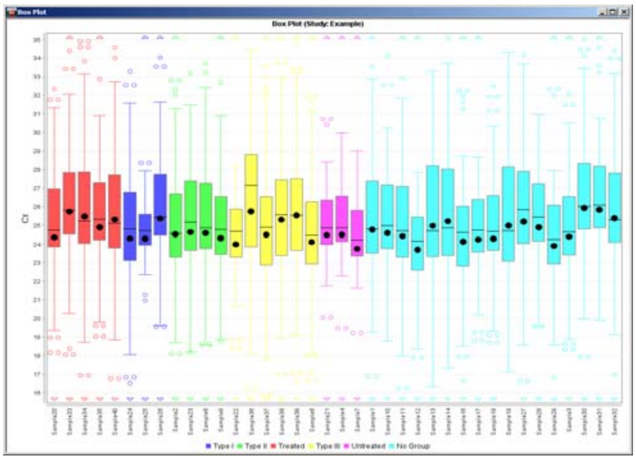


Fig. 3 Box-and-whisker Plot. It displays the overall range of C_T distribution for each sample from all genes in the experiment. The bar is colored based on the sample biological group if samples are grouped to biological replicates.

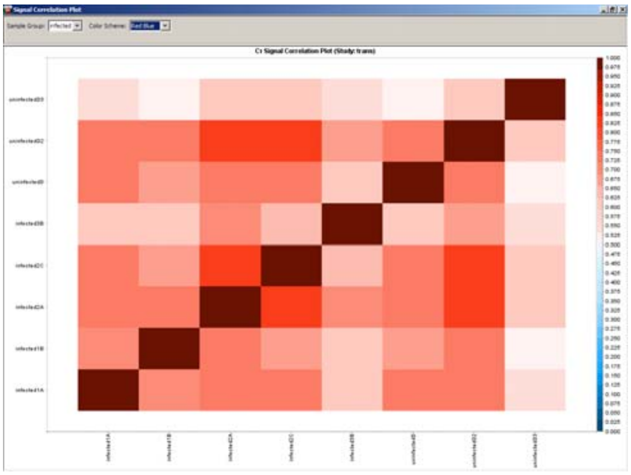


Fig. 4 C_T Signal Correlation Plot. The plot displays C_T signal correlation between samples in a selected biological group. Pearson’s product moment correlation coefficient (r) is calculated for each pair of samples and displayed as a color box either in red-blue or red-green color map.

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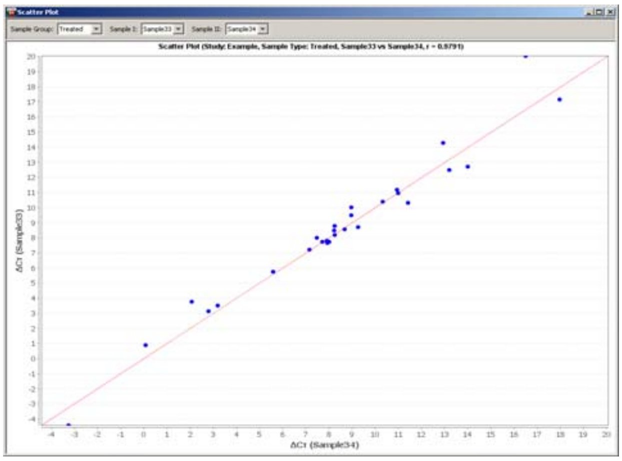
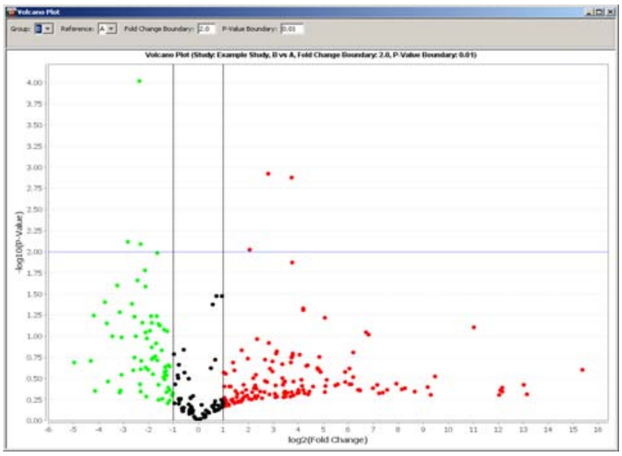

Scatter Plot

Sample Group: Treated Sample I: Sample33 Sample II: Sample34

Scatter Plot (Study: Example, Sample Type: Treated, Sample33 vs Sample34, r = 0.9791)

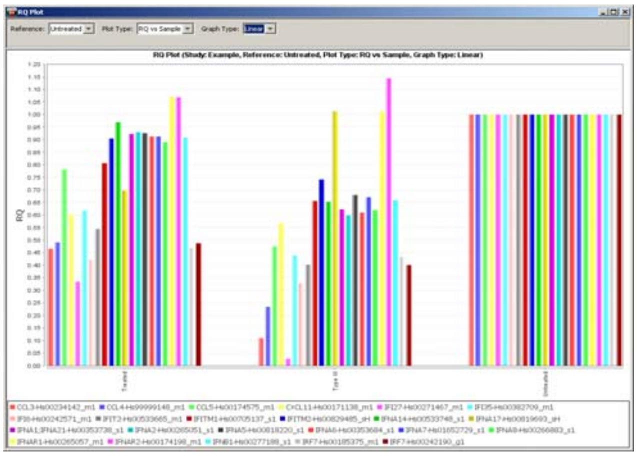
Fig. 5 Scatter Plot. It shows ΔC_T correlation between any two selected samples in a chosen biological group. Pearson's product moment correlation coefficient (r) is also calculated and included in the plot.


Volcano Plot

Group: B Reference: A Fold Change Boundary: 2.0 P-Value Boundary: 0.01

Volcano Plot (Study: Example Study, B vs A, Fold Change Boundary: 2.0, P Value Boundary: 0.01)

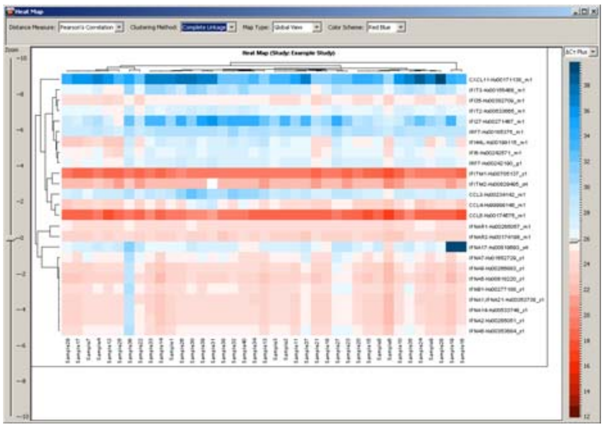
Fig. 6 Volcano Plot. It displays the fold change versus the p-value from t-test for comparison between sample groups, which gives a quick overview of the statistical significance of fold changes for all genes in the experiment. The fold change and p-value boundary can be adjusted to rearrange the genes in the plot.


RQ Plot

Reference: Untreated Plot Type: RQ vs Sample Graph Type: Linear

RQ Plot (Study: Example, Reference: Untreated, Plot Type: RQ vs Sample, Graph Type: Linear)

Fig. 7 RQ Plot. It shows the RQ (fold change) versus Target (gene) or RQ versus Sample, as Linear, Log_{10}, or Log_2 scale. The standard deviation is also displayed as error bar for each sample on log_2 scale when no biological group is specified.


Heat Map

Distance Measure: Pearson's Correlation Clustering Method: Complete Linkage Map Type: Global View Color Scheme: Red Blue

Heat Map (Study: Example Study)

Fig. 8 Dendrogram and Heat Map. It displays the dendrograms of both sample cluster and gene cluster along with gene expression heat map. Genes and samples are clustered using hierarchical clustering [5] with average linkage, complete linkage or single linkage method. The normalized gene expression data (ΔC_T) are used to calculate the distances between samples and genes using either Pearson's correlation coefficient or Euclidean distance. The heat map can be configured as either red-blue or red-green map, with red color box representing up-regulated gene expression level, and the middle expression level can be set using the adjustable color scale on the right side.

REFERENCES

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