THE DATABASE IMPLEMENTATION AND ALGORITHM DESIGN OF QPCR-DAMS: A DATABASE TOOL TO ANALYZE, MANAGE, AND STORE QUANTITATIVE REAL-TIME PCR DATA

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CHAPTER I

INTRODUCTION

Quantitative real-time PCR is an important high throughput method in biomedical sciences. However, existing software has problems on storage and processing real-time PCR data. One of the main shortcomings of existing software is that they cannot handle both relative and absolute quantification. Therefore, we designed qPCR-DAMS (**Q**uantitative **PCR** **D**ata **A**nalysis and **M**anagement **S**ystem), a database tool based on Access 2003, to deal with such problem through an embodied mathematical procedure. qPCR-DAMA allows a user to choose among four methods for data processing within a single software package: (I) Ratio relative quantification, (II) Absolute level, (III) Normalized absolute expression, and (IV) Ratio absolute quantification. qPCR-DAMS also provides a tool for multiple reference gene normalization. qPCR-DAMS has three quality control steps and a data display system to monitor data variation. In summary, qPCR-DAMS could be a handy tool for more real-time PCR users.

1.1 PCR and Quantitative real-time PCR

PCR (polymerase chain reaction) is a modern biological technique that occurs *in vitro* to allow the target DNA to be exponentially amplified at certain sequence and certain length by using DNA polymerase, a naturally occurring enzyme that catalyzes the formation and repair of DNA. Applications of PCR have been essential to the progression of certain areas in science. These include the mapping of the human genome project, single sperm analysis, molecular archaeology and ancient DNA, molecular ecology and behavior, disease diagnosis and drug discovery. Theoretically, the amount of amplicon

(amplified PCR product) has an exponential relationship with the initial input of the template: $A_f = (1+E)^N A_i$, which A_f is the final amount of PCR product, A_i is the initial input, E is the amplification efficiency (ideally 100%), and N is the number of PCR thermal cycles. However, PCR products can never be doubled because of the limitation of primers, activity of enzymes, and the reanealing of PCR products. Quantification of PCR products normally depends on illustration of the PCR products on an ethedium bromide (EB) stained agarose gel. Therefore, it is a semi-quantitative method.

Quantitative real-time PCR is a more accurate and high-throughput method in comparison to conventional PCR. By using fluorescent dyes that intercalate with double-strand DNA (such as SYBR Green I), or modified DNA oligonucleotides (called probes) that fluoresce when hybridized with a complementary DNA, Quantitative real-time PCR can quantify and amplify DNA simultaneously. Quantitative real-time PCR is widely used in biological sciences to quantify low abundance messenger RNA, enabling a researcher to quantify relative gene expression at a particular time, or in a particular cell or tissue type (Ginzinger 2002).

Like conventional PCR, quantitative real-time PCR also has 3 stages during the whole reaction: the baseline stage, the exponential stage, and the plateau stage. Only at the exponential stage, the final product and the initial input have the relationship of $A_f = (1+E)^N A_i$. Threshold cycle (Ct) is the central concept of quantitative real-time PCR. In real-time PCR, the fluorescence generated by the amplicons will increase with thermal cycles. Those fluorescent signals can be detected by means of a laser integrated in the sequence detector, for example, the TaqMan ABI Prism 7700 Sequence Detection System (Perkin Elmer, Foster City, CA). The PCR cycle number at which fluorescence

reaches a threshold value of 10 times the standard deviation of baseline emission is used for quantitative measurement. This cycle number is called the threshold cycle (Ct) and it is inversely proportional to the starting amount of target cDNA. Ct is usually set at the exponential stage of real-time PCR. The Ct is a relative value because it can be set manually by the researcher based on the researcher's experience.

As to the quantification method, quantification real-time PCR can be categoried into relative and absolute quantitative real-time PCR. Absolute quantitative method also named a relative standard curve method, is useful for investigators that have a limited number of cDNA samples and a large number of genes of interest. Relative quantification is also called the comparative CT method, which is useful for investigators who have a large number of cDNA samples and a limited number of genes of interest.

1.2 Relative quantitative real-time PCR

Relative quantification is the method used to reveal the relative mRNA levels of a gene in different samples or the mRNA level changes of this gene in a certain sample under different treatments. The mRNA levels of the interested gene (target gene) are normalized to an internal control gene (reference gene). The final result is normally reported as ratios (Pfaffl et al 2002). The relative expression ratio is calculated only from the real-time PCR efficiencies and the Ct of an unknown sample versus a control. Therefore, this model needs no calibration curve. Although high accuracy and reproducibility can be reached by this model, single reference gene normalization may inevitably generate biased quantitative results because the selected reference gene itself

may have differential expressions in many cases. However, multiple reference gene normalization can improve the accuracy of the quantification (Vandesompele 2002).

1.3 Absolute quantitative real-time PCR

Absolute quantification is the method used to determine the accurate mRNA quantity of an interested gene in a certain unit of sample by using a highly accurate and reproducible calibration curve (standard curve). Absolute quantification has been widely used in microbiological detection and molecular diagnosis, as well as the determination of relative gene expression. The final results can be expressed as absolute levels. Similarly to relative quantification, the results of absolute quantification can also be expressed as normalized expression and or ratios (Bustin 2000).

1.4 Current data processing software and their limitations

Unlike DNA microarray, real-time PCR, is the other widely used high throughput technology to examine differential gene expressions. Normally there is no software provided by the manufacturers to evaluate, process and store real-time PCR. Some software can perform minimal data process functions, for example, the software from the ABI system can calculate the absolute quantity from the standard curves from a single plate-run. However, it cannot process data from different plate-runs or process data with multiple reference genes. Absolute and relative quantification are both important for real-time PCR data processing; however, in the ABI software, those two methods are in two different packages and all the operations are different. Most researchers do not use the relative methods of the ABI software because they cannot be applied if their run contains

a standard curve. However, processing data of a single run by both absolute and relative quantification methods is quite common for researchers. To overcome those difficulties, some laboratories have developed software to administer relative quantitative real-time PCR data, such as REST, Q-gene and CARTA.

REST is a good tool to figure out the significance of differential expressed genes. The mathematical model of REST is also based on the PCR efficiencies and the mean crossing point deviation between the sample and control group. Before the generation of REST, all published equations and available models for the calculation of relative expression ratio allow only for the determination of a single transcription difference between one control and one sample, which is the biggest disadvantage that limits the application of those models. However, REST (relative expression software tool), compares two groups, with up to 16 data points in a sample and 16 in a control group, for reference and up to four target genes. It is a breakthrough in comparison with those old models. Further, the expression ratio results of the four investigated transcripts can be tested for significance by a randomization test. The biggest limitation of REST is its limitation for the number of genes and samples to process; the capacity to sort and display data also needs to improve (Pfaffl et al. 2002).

Q-Gene is a system based on MS Excel coded in Visual Basic for Applications. Q-Gene focuses on the mathematical evaluation and analysis of the data generated by quantitative real-time PCR, the calculation of the final results, the propagation of experimental variation of the measured values to the final results, and the statistical analysis. Since a good data sorting and reference function was designed in Q-Gene, Q-Gene manages and expedites the planning, performance, and evaluation of quantitative

real-time PCR experiments, as well as the mathematical and statistical analysis, storage, and graphical presentation of the data. Therefore, Q-Gene software application is a tool to cope with complex quantitative real-time PCR experiments at a high-throughput scale and considerably expedites and rationalizes the experimental setup, data analysis, and data management while ensuring highest reproducibility (Muller et al. 2002). Because this system monitors the error propagation during the data processing, it can process data from both simplex and multiplex relative quantitative real-time PCR (Simon 2003). Unfortunately, this system has the limitation of Excel and the database function is not strong enough

CARTA appears to be a database system that stores all the information about experiments, samples, and researchers. It also has a complicated data evaluation process. However, this software does not account the difference of amplification efficiency of reference gene and target genes and it uses the simplest data processing formula, which largely lowers the data accuracy (Pfaffl et al 2002;Bonanomi et al. 2003).

Except for those main data processing tools, some other software such as qBASE, DART-PCR (Stuart 2003), GENEX, qCalculator, and SoFAR (Wilhelm J 2003a and 2003b) were developed and can be downloaded from http://gene-quantification.info.

Except for those software mentioned above, very few software were designed to handle data from both relative and absolute quantitative real-time PCR. The ABI 7500 system software provides packages for both absolute and relative quantification. However, the packages for relative and absolute quantification are actually two distinct packages. Even though it can perform inter-plate calculation for relative quantification; it cannot perform inter-plate calculation and gives normalized expression for an absolute

quantitative method. Furthermore, it cannot handle the standard curve method for relative quantification. Therefore, those software packages have limited data processing functions.

1.5. Specific aims and significance

Quantitative real-time PCR (qPCR) is becoming increasingly important in biomedical research because of its accuracy, sensitivity, and high efficiency. With the wide application of this technique, efficiently managing and processing raw data is becoming more complex than acquiring the data. Although many laboratories and companies have developed software to manage real-time PCR data, there are limitations in existing software.

An apparent problem is that no software can efficiently manage both relative and absolute quantitative real-time PCR data because most software was designed to process relative quantitative real-time PCR data. Another problem of current software on real-time PCR data processing is the poor data storage functions. Furthermore, quality control is essential for real-time PCR data processing whereas most software do not have an efficient quality control system. Therefore, in this research, our purpose is to develop a database tool, qPCR-DAMS based on Access 2003. This tool will overcome the disadvantages of current systems and at the same time meet the requirement of more real-time PCR users. We have the following specific aims:

Specific Aim I

To provide a single software package to process, manage, and store both relative and absolute quantitative real-time PCR data.

Specific Aim II

To allow a user to choose among four methods: (I) Ratio relative quantification, (II) Absolute levels, (III) Normalized absolute expression, and (IV) Ratio absolute quantification, to process data; in the advanced option, to allow the user use multiple reference gene normalization for both relative and absolute quantification.

Specific Aim III

To monitor the coefficient of variation at each step during data processing and to further improve the accuracy by an easy data tracking and display system.

CHAPTER II

METHODS USED

2.1 System requirement

Operating system: Windows 2000 or above, Windows XP, Access 2000 or above

Memory: 256 MB of RAM

Hard disk: 10 MB of hard disk space

Monitor: 1024 x 768 or greater monitor resolution

Internet: not necessary for the current version but may be need for higher versions

2.2 Algorithms and data structure [1]

The qPCR-DAMS software should provide a single software package to process, manage, and store both relative and absolute quantitative real-time PCR data. A user is allowed to choose among four methods: (I) Ratio relative quantification, (II) Absolute levels, (III) Normalized absolute expression, and (IV) Ratio absolute quantification. In the advanced option, a user can also use multiple referenced gene normalization (Vandesompele 2002) for both relative and absolute quantification. To accomplish those functions, specific mathematical models and data structure have to be built.

Fig. 1 shows the mathematical model of how qPCR-DAMS works and how we resolve the internal conflict in data processing procedures for relative and absolute quantification methods. When there are multiple runs for the relative quantification, the normalized expression (NE) and relative expression (ratio) of a gene is first calculated from intra-plate data, and then the final ratio is calculated from inter-plate data. However,

[1] Used with permission, see appendix B

because data from different runs are comparable, for absolute quantification, first the mean quantities of the reference gene and the target gene are calculated from the inter-plate data and then the NE is calculated from the mean quantities. qPCR-DAMS assigns each stored plate report a plate name. For relative quantification, plate reports of the target gene and the reference gene generated from the same plate-run share the same plate name. When processing data, the integrated mathematical procedures are plate name dependent. Therefore, NE is calculated from reports with the same plate name (Fig. 1A). However, the absolute quantitative method is plate name independent. Before calculating NE, the inter-plate calculation is performed based on gene ID and sample names only (Fig. 1B).

Figure 2 shows the database structure. qPCR-DAMS is implemented on a MS Access 2003 relational database management system based on Visual Basic. qPCR-DAMS stores all the experiment related information (Gene, Sample, Plate, and Experiment) and a clear hierarchical relationship is established as gene>sample>plate>experiment.

Fig. 1A

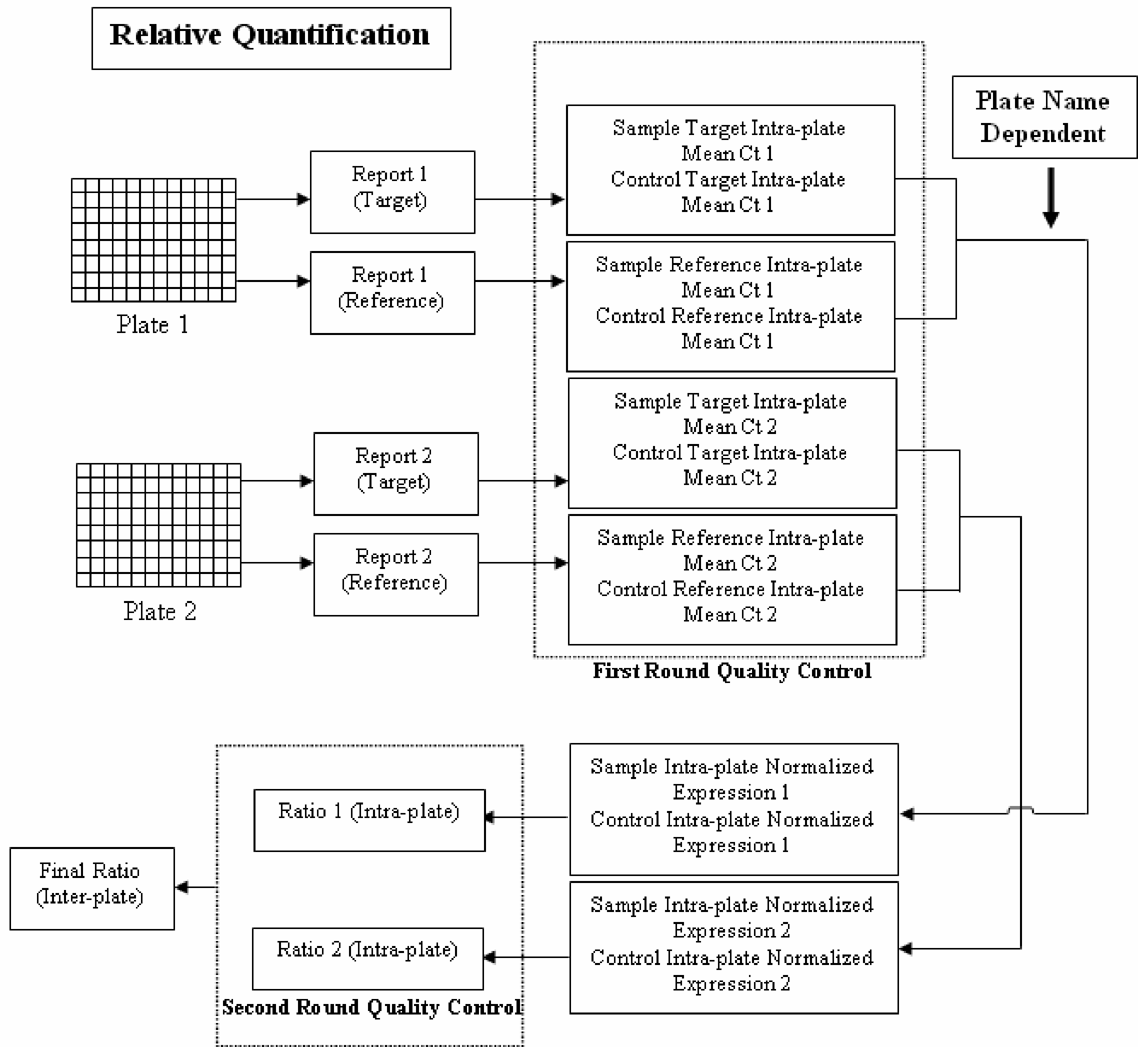

Fig. 1B

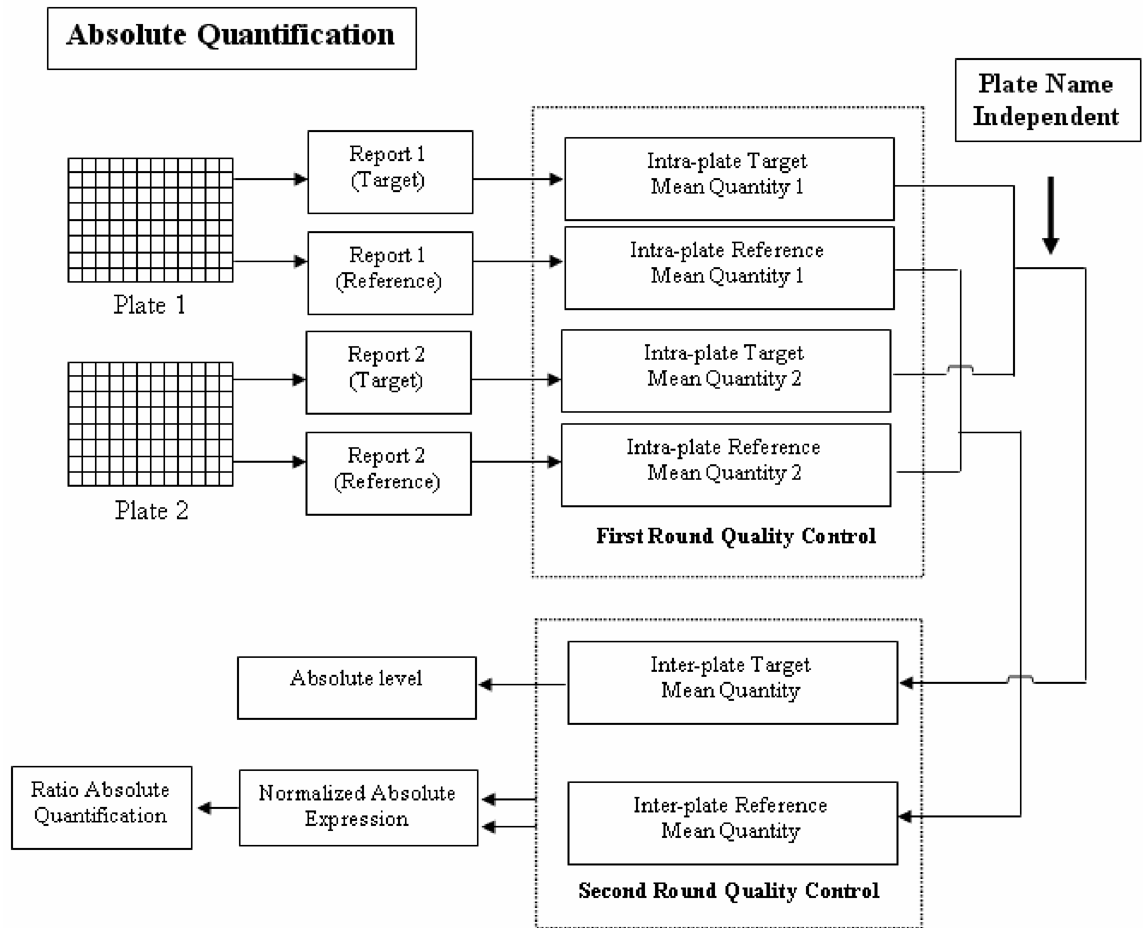

Figure 1. Mathematical strategies of relative and absolute quantification methods in qPCR-DAMS. (A) The relative quantification method calculates the intra-plate normalized expression, followed by the intra-plate ratio and the final ratio. The calculation is plate-name dependent. (B) The absolute quantification method calculates the intra- and inter-plate mean quantities first and then the normalized expression. The calculation is plate-name independent.

Figure 2.

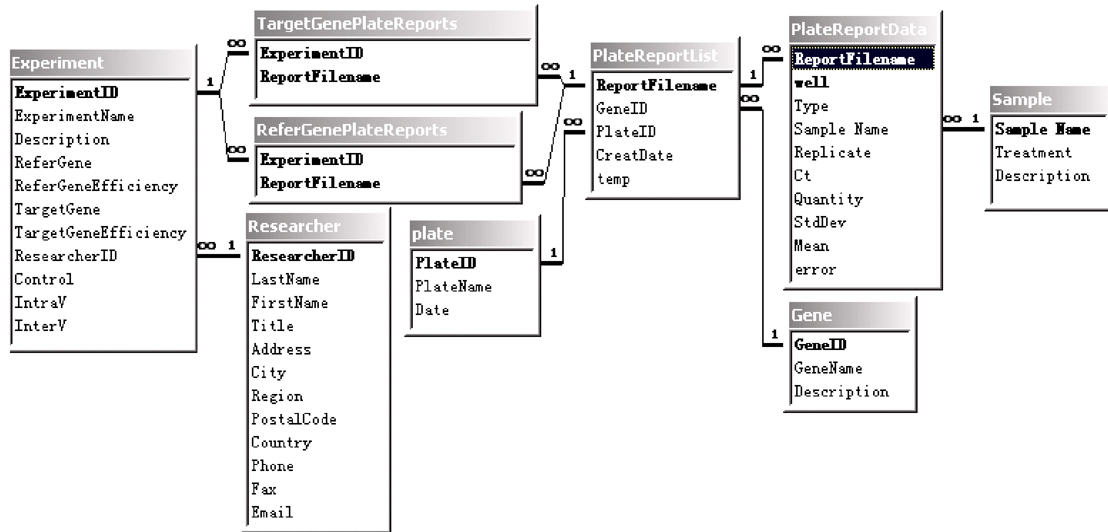

Figure 2. Database structure of qPCR-DAMS. qPCR-DAMS hosts gene, sample, and plate information. All the tables are linked so that efficient data processing and tracking functions can be implemented.

2.3 Principles in the development of qPCR-DAMS

2.3.1 General Principles

I. **Relative quantification**

Is the method used to reveal the relative mRNA levels of a gene in different samples or the mRNA level changes of this gene in a certain sample under different treatments. The mRNA levels of the interested gene (target gene) are normalized to an internal control gene (reference gene). The final result is normally reported as ratios.

II. **Absolute quantification**

Is the method used to determine the accurate mRNA quantity of an interested gene in a certain unit of sample by using a highly accurate and reproducible calibration curve (standard curve). The final result can be expressed as absolute levels, normalized absolute expression, or ratios.

III. **Control**

On the aspect of real-time PCR, control means the group of samples (e.g. untreated group or 0 time) whose expression will be used as the calibrator and the expression of other group of samples will be expressed as a value relative to it.

IV. **Normalized expression**

The expression levels of the target gene are expressed as a relative value to a reference gene in order to correct the difference of samples (eg. different starting materials and mRNA reverse transcription efficiency).

2.3.2 qPCR-DAMS related Principles

I. **Ratio relative quantification**

A sample, e.g. blank, untreated or 0 time, is selected as a control. The normalized expression of the control is defined as 1. The expression levels of other samples are ratio relative to the control. To get a more accurate quantification, multiple reference genes are recommended to be used in the normalization. The final ratio is the geometric means of the ratios derived from each reference gene.

II. **Absolute level**

The quantity (e.g. copy number) of the target gene is determined by a standard curve. The final result can be reported as copies/ng RNA, copies/cell, copies/ml blood, or copies/genome, etc.

III. **Normalized absolute expression**

The quantities of the target gene and the reference gene (normally a house-keeping gene) in a sample are both determined with standard curves. Because many house-keeping genes are steadily expressed in multiple samples, the final expression level of the target gene is then expressed as a normalized value in comparison with the reference gene (copies of the target gene per copy of the reference gene).

IV. **Ratio absolute quantification**

A sample, e.g. untreated or 0 time is chosen as a control. The normalized expression of all the sample groups is determined with the absolute standard quantification method, and then ratios relative to the control are calculated. Multiple reference gene normalization can also be used in this data processing module to get a more accurate quantification.

2.4 Functions of qPCR-DAMS

We have designed five main panels: **Gene**, **Plate**, **Experiment**, **View Data**, and **Process Data** on the database tool. Inside each panel, there are some more options to allow users to operate.

2.4.1 Gene

The main function is to host and manage gene information in the database.

1. **Add New Gene**

Allows users to input gene name, gene ID, and description.

2. **Edit Gene Information**

Allows users to edit the existing gene information including gene name, gene ID, and description in the database.

3. **View Gene List**

Allows the user to check the following items:

1. Gene list in the database
2. Report file name list associated with a gene
3. Plate report generated by the detector system in a report file name

2.4.2 Plate

It is the central part of the database system that brings genes, samples, and experiments all together. The main function is to host and manage plate information as well as data generated by the detector system in the database. There are two options but

only one option is needed for users to input plate reports into the database. The import application requires comdlg32.ocx in the %systemroot%\System32 folder. (See troubleshooting for how to extract comdlg32.ocx to the folder).

1) **Import Plate Report.**

   a) **Add New Plate Name**

   Allows users to enter the name of a new plate. Plate name here means the unique name we give to each real-time PCR plate. Therefore, when a target gene and several reference genes are run on the same plate, they should have the same plate name (**Note**: plate name is important for relative quantification because normalization is plate-name dependent.)

   b) **Import New Plate Report**

   Allows users to choose report from file, to select genes from the gene list, to select plate names from the plate name list, and to import plate reports generated by the detector system into the database. The "report file name" is the name we give to each individual plate report generated by the detector system. It is different from the plate name. If a target gene is run on a plate with three reference genes, there will be only one plate name but 4 plate file names.

   c) **Edit Sample Information**

   Allows users to edit sample information including treatment and description. (**Note**: Correct treatment input is important for data processing because some calculations are dependent on treatment).

   d) **View and Edit Plate Information**

Allows users to view and edit plate names, plate file names, and report files. The most important function is for data quality control. If a sample produces abnormal data, this sample can be removed manually by users through this option. Users can also delete plate report files that are not needed and therefore the database will not accumulate useless information. In addition, users can edit report file names, gene names, and plate names.

2) **Add Plate Report Manually.**

   a) **Add New Sample.**

      i) **Add sample by batch**

         (1) Paste New Sample

         Allows users to enter a batch of sample names at the same time and thus save significant time for users.

         (2) Add Sample to Database

         Allows users to input the sample name into the database. If users input a sample name that is already in the database, the database system ignores it.

         (3) Edit Sample Information

         Allows users to edit sample information including treatment and description.

      ii) **Add Sample One by One**

         (1) Add New Sample

         Allows users to enter sample name, treatment, and description one by one. This is useful if only a limited number of samples need to be input.

         (2) Edit Sample Information

         Allows users to edit sample information including treatment and description.

b) Add New Plate Name

Allows users to enter the name of a new plate.

c) Add New Plate Report Manually

Allows users to enter report file name, to select genes from the gene list, to select plate names from the plate name list, and to paste plate reports generated by the detector system into the database.

d) View and Edit Plate Information

Allows users to view and edit plate names, plate file names, and report files.

2.4.3 Experiment

Experiment panel provides a platform for users to organize all the information related to an experiment together.

1) **Researcher Information**

Allows users to enter name, title, address, city, region, Zip code, country, phone number, fax number, and email address of researchers.

2) **Add New Experiment**

Allows users to set up a new experiment (**Note**: only for single reference gene normalization! See “Advanced options” in “Process Data” for multiple references gene normalization). The following functions can be carried out by users:

- **a.** Enter the name of a new experiment
- **b.** Select researcher ID from the researcher list
- **c.** Select a target and a reference gene from the gene list
- **d.** Select a group of samples that will be used as control from the sample list

e. Enter the amplification efficiency of the target gene and the reference which will be used in the relative quantification. If no number is entered, the default value will be 2

f. Enter the threshold value of intra-plate and inter-plate variation that will be used in the data quality control

g. Select report files for the target gene and the reference gene that will be used in data processing

3) **Edit Experiment Setting**

Allows users to change the settings of an existing experiment in the database which include experiment name, description, target gene, reference gene, target gene amplification efficiency, reference gene amplification efficiency, control, threshold value of intra-plate and inter-plate variation, report files for data processing.

4) **View Experiment Setting**

Allows users to check the experiment list as well as their settings in the database. In addition, users can check the plate list involved in each experiment by plate name, the plate report file list under each plate name, as well as the detailed report file.

2.4.4 View data

This panel provides an easy way to track the archived data in the system. Furthermore, users can validate their processed results by directly sorting the data to the raw data exported from the detector system. This function may be extremely important for clinical laboratories that handle thousands of samples, or laboratories using real-time PCR for microarray data validation, who work with numerous genes because all the data

table in the database system are linked. No matter at which point the user starts viewing data, he can finally reach the raw data exported from the detector system. This panel is composed of four sub-panels: **View data by gene, View data by sample, View data by plate,** and **View data by experiment.**

2.4.5 Process Data

This panel allows users to choose the appropriate data processing module and to calculate the expression levels of the interested gene under certain quality controls for the selected experiments. Four basic data processing modules are provided by qPCR-DAMS to process data with single a reference gene normalization and an advanced option allows users to use a multiple reference gene normalization. The main functions covered by this panel are as follows:

1) **Basic data processing**

    a. **Ratio relative quantification**

    Allows users to perform relative quantification and express the results as ratios.

    b. **Absolute level**

    Allows users to perform absolute quantification without a reference gene.

    c. **Normalized absolute expression**

    Allows users to perform absolute quantification with normalization from a reference gene.

    d. **Ratio absolute quantification**

    Allows users perform absolute quantification and express the results as ratios.

2) **Advanced option**

Allows users to calculate more accurate relative expression levels by using multiple reference gene normalization. This function is based on the experiment's result generated from the basic data processing modules.

a. **Multiple references relative quantification**

Allows normalization with multiple reference genes based on relative quantification experiments.

b. **Multiple reference absolute quantification**

Allows normalization with multiple reference genes based on absolute quantification experiments.

2.4.6 Quality control

Helps users to improve the validity of the processed data by three error checking steps. The errors found by step 1 and 2 can be marked and excluded manually by users.

1) **Fix unrecognized Ct or quantity error**:

If Ct is undetermined or the quantity is 0, qPCR-DAMS updates the values of 40 to Ct or 0.01 to quantity to avoid the mathematical problem of calculating the ratio. This option will permanently change the data in the system, so please carefully check data before selecting **Yes**.

2) **Step 1 error check**:

A threshold value for the intra-plate variation can be set to find out the abnormally amplified samples from the replicates on the same plate.

3) Step 2 error check:

A threshold value for the inter-plate variation can be set to find out the abnormally amplified samples from the replicates on the different plates.

2.4.7 Data display:

qPCR-DAMS can output separate report for all the main calculation steps so as to meet the requirement of different users. This function also helps users to improve the data validity through step-by-step monitoring or re-checking of the processed data. Several factors such as bad sample, bad reaction, cross contamination, and pipetting error, may all cause misleading results. Many errors cannot be recognized by the built-in error check procedures, which are largely based on standard deviation calculations. This step helps users to find these “escaped” errors. This function should be even more helpful when using this function together with **View Data by Sample**. To know the meaning of each data-to-display, see **Concepts and Mathematical Structures**.

1) **Ratio relative quantification**

   Displays Ct, normalized expression, intra-plate sample ratio, inter-plate sample ratio and final ratio.

2) **Absolute level**

   Displays target intra-plate quantity, target inter-plate quantity, and absolute level.

3) **Normalized absolute expression**

   Displays target intra-plate quantity, reference intra-plate quantity, target inter-plate quantity, reference inter-plate quantity, normalized expression, and final normalized expression.

4) **Ratio absolute quantification**

Displays target intra-plate quantity, reference intra-plate quantity, target inter-plate quantity, reference inter-plate quantity, normalized expression, and final ratio.

5) **Multiple references relative quantification**

Displays inter-reference sample ratio and final ratio.

6) **Multiple reference normalization for absolute quantification**

Displays inter-reference sample ratio and final ratio.

2.5 Mathematical structures

The following mathematical structures give the main steps to calculate the expression levels of a gene of interest. In relative quantification, although both crossing point (CP) and threshold cycle (Ct) can be used in calculation in this software, only Ct is mentioned in the following text for the convenience of the description. Furthermore, in the following text, several letters will be used specifically to represent some meaning in each model. I represents the number of repeated wells on the same plate; J, the number of repeated plates; K, the number of reference genes chosen for normalization; L, the number of different groups of samples (treatment); N, the number of biological replicates of a sample.

2.5.1 Ratio relative quantification

I.a Single reference relative quantification

Step 1

Calculate intra-plate mean value of Ct of a sample (***Ct_{sample}***). This step is to calculate the mean value of Ct for the samples or controls from the replicated wells on the same plate-run.

Equation 1: Formula to calculate the *Ct_{sample}*

$$Ct_{sampleL} = \frac{Ct_{sampleL-well1} + Ct_{sampleL-well2} + \ldots\ldots + Ct_{sampleL-wellI}}{I}$$

$Ct_{sampleL}$ is the mean Ct of sample L from all the repeated wells; $Ct_{sampleL\text{-}wellI}$ is the Ct of sample L in well I.

Step 2

Calculate intra-plate normalized expression (***Intra-plate NE***). In this step, the normalized expression (NE) of each sample and control on the same plate-run is calculated. This step is plate name dependent, which is distinct from the absolute quantification method.

Equation 2: Formula to calculate the intra-plate NE

$$NE = \frac{(E_{ref})^{Ct_{ref}}}{(E_{target})^{Ct_{target}}}$$

E_{target}, target gene PCR amplification efficiency; E_{ref}, reference gene PCR amplification efficiency; Ct_{target}, intra-plate mean Ct of the target gene; Ct_{ref}, intra-plate mean Ct of the reference gene.

Step 3

Calculate the intra-plate sample ratio (***Intra-plate Ratio**$_{sample}$*). In this step, the relative expression of samples on the same plate-run is calculated as the ratio relative to the control. The *intra-plate Ratio*$_{sample}$ is calculated by 2 sub-steps.

1) Calculate $MNE_{control}$ by averaging all the biological replicates of the $NE_{control}$.
2) Calculate *intra-plate Ratio*$_{sample}$ by dividing NE_{sample} with $MNE_{control}$.

Equation 3: the combined formula to calculate the intra-plate Ratio$_{Sample}$

$$Intra-plateRatio_{sampleL} = \frac{NE_{sampleL}}{MNE_{control}} = \frac{NE_{sampleL}}{\left(\frac{NE_{control1}+NE_{control2}+......+NE_{controlN}}{N}\right)}$$

Intra-plate Ratio$_{sampleL}$ is the intra-plate ratio of sample L; $NE_{sampleL}$, NE of sample L; $NE_{controlN}$, NE for the Nth biological replicate of the control; $MNE_{control}$, mean value of $NE_{controlN}$ from all the biological replicates.

Step 4

Calculate the inter-plate sample ratio (***Inter-plate Ratio**$_{sample}$*). In this step, the mean values of the intra-plate ratio of individual samples from multiple runs are calculated.

Equation 4: formula to calculate the *inter-plate Ratio*$_{sample}$

$$Inter-plateRatio_{sampleL} = \frac{Ratio_{sampleL-plate1}+Ratio_{sampleL-plate2}+\ldots\ldots+Ratio_{sample-plateJ}}{J}$$

Inter-plate $Ratio_{sampleL}$ is the mean value of *intra-plate* $Ratio_{sampleL}$ from all the repeated plate-runs; $Ratio_{sample\text{-}plateJ}$ is the *intra-plate* $Ratio_{sampleL}$ on the *J*th repeated plate.

Step 5

Calculate the final ratio. In this step, the final ratio of a sample to control is obtained by averaging the inter-plate ratio from all the biological replications.

Equation 5: formula to calculate the final Ratio from single reference gene normalization

$$FinalRatio = \frac{Inter-plateRatio_{sample1} + Inter-plateRatio_{sample2} + \ldots\ldots + Inter-plateRatio_{sampleN}}{N}$$

I.b Multiple references relative quantification

Single reference gene normalization may generate biased quantitative results because the selected reference gene itself may have differential expression in many cases. However, multiple reference gene normalization can improve the accuracy of the quantification. This function is actually a further calculation on the results obtained from single reference gene normalization. Therefore, steps 1 to 4 are completely the same as described in the "Single reference relative quantification" and the additional steps are as follows:

Step 5

Calculate the inter-reference sample ratio (***Inter-ref*** $\boldsymbol{Ratio_{sample}}$): It is obtained from the geometric means of *inter-plate* $Ratio_{sample}$ derived from all different reference genes.

Equation 6: formula to calculate the *inter-ref Ratio*$_{sample}$

$$Inter-refRatio_{sampleL} = \sqrt[K]{Ratio_{sampleL-ref1} \times Ratio_{sampleL-ref2} \times ...... \times Ratio_{sampleL-refK}}$$

Inter-ref Ratio$_{sampleL}$ is the geometric mean of the Ratio of the sample L from all the reference genes; $Ratio_{sampleL\text{-}refK}$ is the inter-plate ratio of sample L calculated from the Kth reference gene.

Step 6

Calculate the final Ratio: In this step the final ratio of a sample to control is obtained by averaging the *inter-ref Ratio*$_{sample}$ from all the biological replicates.

Equation 7: formula to calculate the final Ratio from multiple reference gene normalization

$$FinalRatio = \frac{Inter-refRatio_{sample1} + Inter-refRatio_{sample2} + ... + Inter-refRatio_{sampleN}}{N}$$

2.5.2 Absolute levels

In absolute quantification, the theoretical relationship of the quantity and Ct can be expressed in the following formula which was derived from the standard curve:

$$Y = b + aX$$

Y, Log quantity of a standard or sample; X, Ct of the standard or sample; b, intercept point Y-value of the standard curve; and a, slope of the standard curve.

Because Log quantities but not quantities of unknowns have linear relationship with the Ct values, Log quantities are used in the calculations of absolute expression levels.

Step 1

Calculate Log Qty of individual wells (*Log Qty*$_{sample\text{-}well}$). In this step, the quantities (Qty) of the target (e.g. Copy number) in individual wells are transformed to Log value.

Step 2

Calculate intra-plate mean value of Log Qty (intra-plate Log Qty). In this step, the Log Qtys of samples from the replicated wells on the same plate-run are averaged.

Equation 8: Formula to calculate the intra-plate Log Qty

$$Intra-plateLogQty_{sampleL} = \frac{LogQty_{sampleL-well1} + LogQty_{sampleL-well2} + \ldots\ldots + LogQty_{sampleL-wellI}}{I}$$

Intra-plate LogQty$_{sampleL}$ is the mean Log quantity of sample L from I repeated wells on the same plate-run; *LogQty*$_{sampleL\text{-}wellI}$ is the individual Log quantity of sample L in the Ith well.

Step 3

Calculate final absolute level (***Inter-plate Log Qty*** or ***ME***). In this step, the Log Qtys of a sample from all the replicated plate-runs are averaged. This value can be considered as mean expression (ME) of that sample. This step is plate name independent, which is distinct from relative quantification method.

Equation 9: Formula to calculate the ME

$$ME_{sampleL} = \frac{LogQty_{sampleL-plate1} + LogQty_{sampleL-plate2} + \ldots\ldots + LogQty_{sampleL-plateJ}}{J}$$

$ME_{sampleL}$, mean expression of the sample L; $LogQ_{sampleL\text{-}plateJ}$, the intra-plate LogQty of sample L on the Jth plate.

2.5.3 Normalized absolute expression

In this method, in addition to the target gene, quantity of the reference gene is also determined by absolute quantification. Therefore steps 1 to 3 are exactly the same as described in "**Absolute levels**", however, two more steps are required. Those steps are plate name independent, which are distinct from the relative quantification method.

Step 4

Calculate the normalized mean expression (***NME***). In this step, the *ME* of the reference gene is subtracted from the target gene and the result is the normalized mean expression (*NME*).

Equation 10: Formula to calculate NME

$$NME = ME_{target} - ME_{ref}$$

ME_{target}, mean value of the log Qty of the target gene in a sample; ME_{ref}, mean value of the log Qty of the reference gene in a sample.

Step 5

Calculate final absolute level (**MNME**). In this step, the *NME* of samples from all the biological replicates is averaged. The result is the final normalized absolute expression of the target gene in that sample, which can be considered as mean normalized mean expression (*MNME*).

Equation 11: Formula to calculate MNME

$$MNME = \frac{NME_{sample1} + NME_{sample2} + \ldots\ldots + NME_{sampleN}}{N}$$

2.5.4 Ratio absolute quantification

IV.a Single reference gene normalization for ratio absolute quantification

All the other steps in the calculation are the same as those in "**Normalized absolute expression**". However, an additional step, which is constituted of 3 sub-steps, is required to obtain the relative expression (Ratio) of a sample to the control.

1) Calculate the Log Ratio of individual samples (Log $Ratio_{sample}$) by subtracting $MNME_{control}$ from NME_{sample}.
2) Calculate the Ratio of individual samples ($Ratio_{sample}$) by converting the Log $Ratio_{sample}$ to $Ratio_{sample}$.
3) Calculate the final Ratio, which is obtained by averaging all the biological replicates of $Ratio_{sample}$.

Equation 12: Combined formula to calculate the final Ratio from single reference gene normalization

$$Ratio = \frac{10^{LogRatio_{sample1}} + 10^{LogRatio_{sample2}} + \ldots\ldots + 10^{LogRatio_{sampleN}}}{N}$$

$$= \frac{10^{(NE_{sample1} - MNE_{control})} + 10^{(NE_{sample2} - MNE_{control})} + \ldots\ldots + 10^{(NE_{sampleN} - MNE_{control})}}{N}$$

$$= \frac{10^{[NE_{sample1} - \frac{(NE_{control1} + NE_{control2} + \ldots\ldots + NE_{controlN})}{N}]} + 10^{[NE_{sample1} - \frac{(NE_{control1} + NE_{control2} + \ldots\ldots + NE_{controlN})}{N}]} + \ldots\ldots + 10^{[NE_{sampleN} - \frac{(NE_{control1} + NE_{control2} + \ldots\ldots + NE_{controlN})}{N}]}}{N}$$

IV.b Multiple reference gene normalization for ratio absolute quantification

The theory behind this function is the same as the multiple reference relative quantification. After calculation of the $Ratio_{sample}$ from each individual reference gene, the geometric means of $Ratio_{sample}$ derived from all different reference genes is calculated, and then the final ratio of the sample is calculated from all the biological replicates. The formulae in the calculations are the same as Equations 6 and 7 and will not be repeated here.

2.5.5 Formulas used for all the models

I. Standard deviation (SD)

Equation 13: Formula to calculate standard deviation

$$SD = \sqrt{\frac{\sum (X - M)^2}{N - 1}}$$

SD, standard deviation; $\sum$, sum; X, individual value; M, mean of all the individual value; N, number of replications.

II. Coefficient of variation (CV)

Equation 14: Formula to calculate CV:

CV % = (SD/M) x 100%

CHAPTER III

RESULTS AND DISCUSSION

3.1 Data processing

The qPCR-DAMS software provides a single software package to process, manage, and store both relative and absolute quantitative real-time PCR data. Results of the sample experiment for (I) Ratio relative quantification, (II) Absolute levels, (III) Normalized absolute expression, (IV) Ratio absolute quantification, (V) Multiple Reference Gene Normalization Relative Quantification, (VI) Multiple Reference Gene Normalization Absolute Quantification are listed below. This qPCR-DAMS software may be especially useful in a core facility, where many researchers share the same detector system, but run real-time PCR and process data in different ways. The following results are obtained by processing the testing data by qPCR-DAMS. Testing data are listed in Appendix C.

3.1.1 Ratio Relative Quantification (Experiment 1):

Table 4: Ratio relative quantification result for experiment 1

Treatment	Final Ratio	SD Final Ratio
D0	1	0.209548
D1	1.743938	0.292553
D2	1.474616	0.059399

D3	2.773921	0.229582
D4	2.838308	0.738051
D5	1.934002	0.832779
D6	0.730862	0.002113

3.1.2 Absolute Levels (Experiment 1):

Table 5: Absolute levels result for experiment 1

Treatment	Mean Log Qty	StDev Log Qty
D0	3.776928	0.279465
D1	3.382855	0.170282
D2	3.213073	0.183467
D3	4.117435	0.228602
D4	4.149184	0.070496
D5	3.783094	0.148153
D6	3.818442	0.191278

3.1.3 Normalized Absolute Expression (Experiment 1):

Table 6: Normalized Absolute Expression result for Experiment 1

Treatment	AvgOfNE	StDevOfNE
D0	-2.58834	0.125609
D1	-2.32123	0.081542
D2	-2.42219	0.05125

D3	-2.17002	0.014316
D4	-2.20441	0.074602
D5	-2.26875	0.106302
D6	-2.68052	0.025788

3.1.4 Ratio Absolute Quantification (Experiment 1):

Table 7: Ratio Absolute Quantification result for Experiment 1

Treatment	AvgOfRatio	StDevOfRatio
D0	1	0.302815
D1	1.80651	0.333273
D2	1.41819	0.169115
D3	2.519575	0.083532
D4	2.357008	0.379941
D5	2.061153	0.49238
D6	0.77861	0.04683

3.1.5 Multiple References Relative Quantification:

If you choose experiment 1 and 2, the final result is as follows:

Table 8: Multiple References Relative Quantification result for experiment 1

Treatment	Final Ratio	StDev of Final Ratio
D0	0.998119	0.132892
D1	1.06107	0.145566

D2	0.689422	0.144023
D3	1.737226	0.031687
D4	1.577832	0.139648
D5	1.279272	0.337033
D6	0.925746	0.02834

If you choose experiment 1, 2, and 3, the final result is as follows:

Table 9: Multiple References Relative Quantification result for experiment 1

Treatment	Final Ratio	StDev of Final Ratio
D0	0.996138	0.097144
D1	0.915643	0.165894
D2	0.623733	0.095590
D3	1.539501	0.062812
D4	1.350501	0.085950
D5	1.117348	0.336757
D6	0.720779	0.028339

3.1.6 Multiple References Absolute Quantification

If you choose experiment 1 and 2, the final result is as follows:

Table 10: Multiple References Absolute Quantification result for experiment 1

Treatment	Final Ratio	StDev of Final Ratio

D0	0.999155	0.302663
D1	1.446325	0.322752
D2	0.897775	0.256326
D3	2.299829	0.070734
D4	1.97706	0.141585
D5	1.811127	0.295321
D6	0.902072	0.048961

If you choose experiment 1, 2, and 3, the final result will be as follows:

Table 11: Multiple References Absolute Quantification result for experiment 1

Treatment	Final Ratio	StDev of Final Ratio
D0	0.990916	0.193836
D1	1.108892	0.276209
D2	0.739287	0.18263
D3	1.751864	0.02755
D4	1.414202	0.103725
D5	1.405532	0.330699
D6	0.624024	0.025441

3.2 Data input

There are almost no limitations to the number of genes, samples and plates for qPCR-DAMS to handle. In addition to general information such as gene ID, sample name, treatment, sample description, researcher information and experiment description, qPCR-

DAMS also stores exported reports from the detector system. Therefore, the processed results are directly associated with the raw data, which can greatly improve the data validity in comparison to software that only host part of the raw data.

3.3 Data Display

Efficient data tracking is also critical for the accuracy and validation of real-time PCR. By using the "View Data" function on the main panel of qPCR-DAMS, a user can easily track the stored information in the database. A user can track data by genes, samples, plates or experiments. All the data tables are linked so that the user can trace back to the raw data from the detector systems starting from any point.

3.4 Quality control

Quality control is implemented through three error-checking steps during data processing. First, if Ct is undetermined or quantity is 0, the software assigns a value of 40 to Ct or 0.01 to the quantity to avoid the mathematical problem of calculating the ratio. Second, intra-plate variation is calculated as a coefficient of variation from replicated samples on the same plate. Third, inter-plate variation is calculated as a coefficient of variation from replicated samples on different plates. qPCR-DAMS allows a user to set a threshold value for intra-plate variation or inter-plate variation. If a sample has a variation above the threshold, the sample is marked and can be excluded manually by a user. There is one more advantage of qPCR-DAMS: the data display system. Most other software display final quantitative results directly. However, several factors such as bad sample, bad reaction, cross contamination, and pipeting error, may lead to misleading results.

qPCR-DAMS can output separate reports for all the calculation steps, which include intra-plate calculation, inter-plate calculation, NE, and MNE, etc .

CHAPTER IV

SUMMARY AND CONCLUSIONS

4.1 The database tool qPCR-DAMS has the following characteristics:

1. qPCR-DAMS contains 6 algorithms. These algorithms include 14 mathematic computations, which were completed by about 60 SQL queries and some VB scripts.
2. This single software package can process, manage, and store information and raw data of hundreds of experiments.
3. This software can process both relative and absolute quantitative real-time PCR data.
4. This software can efficiently control the data quality by the Quality Control, View Data, and Data Display systems.
5. This software provides a user friendly interface and allows easy data import output functions.

4.2 Significance of this study:

1. qPCR-DAMS is the first software to handle both relative and absolute quantificative real-time PCR data in a single software package.
2. qPCR-DAMS has the best data storage and data-tracking functions among all the real-time PCR software.
3. By using qPCR-DAMS, data processing, the most time-consuming step now becomes the easiest step of quantitative real-time PCR.

4. This is a good application of computer science methodologies to biological research and a contribution to bioinformatics; therefore, this work is also of relevance to computer science.

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APPENDIX A

BUG REPORT AND TROUBLESHOOTING

Troubleshooting:

If plate report cannot be imported, one possible reason is that the formats of the report have not been cleared. The other reason is because the Comdlg32.ocx is outdate or missing. Follow the listed steps to extract the Comdlg32.ocx.

1. Download the comdlg32.ocx (~60 Kb Zip)
2. Use WinZip or any other utility to unzip the file
3. Extract comdlg32.ocx to **%systemroot%\System32** folder (C:\WINNT\system32)
4. Click on Start > Run, and then type the following command:

   REGSVR32 %Systemroot%\System32\comdlg32.ocx

A dialog will show up like this:

RegSvr32

DllRegisterServer in C:\WINDOWS\System32\comdlg32.ocx succeeded.

OK

Restart Windows, and then launch your application.

APPENDIX B

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APPENDIX C

TEST DATA

Table 12. ACTB_03252005

well	Type	Sample Name	Replicate	Ct	Quantity	StdDev	Mean
D1	Standard			16.61	10000000		
D2	Standard			19.68	1000000		
D3	Standard			23.33	100000		
D4	Standard			26.54	10000		
D5	Standard			30.21	1000		
D6	Standard			33.45	100		
D7	Standard			36.25	10		
D8	NTC			40	0		
E1	Unknown	D0a		20.39	719513.9	0	0
E10	Unknown	D1b		21.86	261248.2	0	0
E11	Unknown	D1c		21.41	354641.7	0	0
E12	Unknown	D1c		21.38	362971.1	0	0
E13	Unknown	D2a		21.32	378938.5	0	0
E14	Unknown	D2a		21.01	467433.7	0	0
E15	Unknown	D2b		21.76	279190.3	0	0
E16	Unknown	D2b		22.17	210783.8	0	0
E17	Unknown	D2c		20.18	830465.7	0	0
E18	Unknown	D2c		20.2	819691.6	0	0
E19	Unknown	D3a		18.11	3480000	0	0
E2	Unknown	D0a		20.12	867956.4	0	0
E20	Unknown	D3a		18.4	2840000	0	0
E21	Unknown	D3b		19.95	976085.4	0	0
E22	Unknown	D3b		20.14	854610.1	0	0
E23	Unknown	D3c		18.78	2190000	0	0
E24	Unknown	D3c		19.33	1500000	0	0
E3	Unknown	D0b		18.04	3650000	0	0
E4	Unknown	D0b		18.22	3210000	0	0
E5	Unknown	D0c		17.67	4710000	0	0
E6	Unknown	D0c		18.43	2780000	0	0
E7	Unknown	D1a		20.02	930752		
E8	Unknown	D1a		20.1	880482.6	0	0
E9	Unknown	D1b		21.32	377468.3	0	0
F1	Unknown	D4a		18.05	3620000	0	0
F10	Unknown	D5b		19.25	1580000	0	0
F11	Unknown	D5c		20.29	769682.1	0	0
F12	Unknown	D5c		20.35	740423.8	0	0
F13	Unknown	D6a		17.99	3780000	0	0
F14	Unknown	D6a		18.06	3600000	0	0
F15	Unknown	D6b		19.08	1780000	0	0

F16	Unknown	D6b		19.19	1650000	0	0
F17	Unknown	D6c		18.17	3350000	0	0
F18	Unknown	D6c		17.97	3840000		
F2	Unknown	D4a		18.92	1980000	0	0
F3	Unknown	D4b		19.32	1510000	0	0
F4	Unknown	D4b		19.19	1650000	0	0
F5	Unknown	D4c		18.55	2560000	0	0
F6	Unknown	D4c		18.81	2140000	0	0
F7	Unknown	D5a		20.55	644167.4	0	0
F8	Unknown	D5a		20.44	695222	0	0
F9	Unknown	D5b		18.67	2360000	0	0

Table 13. ACTB_03302005

well	Type	Sample Name	Replicate	Ct	Quantity	StdDev	Mean
D1	Standard			16.8455	10000000		
D2	Standard			20.1732	1000000		
D3	Standard			23.3519	100000		
D4	Standard			26.7592	10000		
D5	Standard			30.1884	1000		
D6	Standard			33.6196	100		
D7	Standard			36.8698	10		
D8	NTC			40	0		
E1	UNKN	D0a		20.88	596962.4	0	0
E10	UNKN	D1b		21.47	397954	0	0
E11	UNKN	D1c		21.37	426268	0	0
E12	UNKN	D1c		21.51	387162.2	0	0
E13	UNKN	D2a		21.61	361445.7	0	0
E14	UNKN	D2a		21.22	472560.6	0	0
E15	UNKN	D2b		21.64	354069.1	0	0
E16	UNKN	D2b		22	276457.6	0	0
E17	UNKN	D2c		21.02	542197.1	0	0
E18	UNKN	D2c		20.75	652757.4	0	0
E19	UNKN	D3a		18.01	4291784	0	0
E2	UNKN	D0a		20.31	883265.3	0	0
E20	UNKN	D3a		18.63	2802649	0	0
E21	UNKN	D3b		19.71	1334106	0	0
E22	UNKN	D3b		19.88	1186986	0	0
E23	UNKN	D3c		18.82	2459541	0	0
E24	UNKN	D3c		18.97	2218601	0	0
E3	UNKN	D0b		17.96	4441838	0	0
E4	UNKN	D0b		18.05	4175398	0	0
E5	UNKN	D0c		17.52	6010383	0	0
E6	UNKN	D0c		18.01	4291784	0	0
E7	UNKN	D1a		19.87	1195172		

E8	UNKN	D1a		20.12	1006482	0	0
E9	UNKN	D1b		21.69	342107.9	0	0
F1	UNKN	D4a		18.45	3171746	0	0
F10	UNKN	D5b		19.03	2128969	0	0
F11	UNKN	D5c		20.35	859312.7	0	0
F12	UNKN	D5c		20.33	871206.7	0	0
F13	UNKN	D6a		17.87	4725281	0	0
F14	UNKN	D6a		17.95	4472473	0	0
F15	UNKN	D6b		18.93	2280443	0	0
F16	UNKN	D6b		19.04	2114386	0	0
F17	UNKN	D6c		18.02	4262387	0	0
F18	UNKN	D6c		17.99	4351188		
F2	UNKN	D4a		18.9	2327953	0	0
F3	UNKN	D4b		19.15	1960422	0	0
F4	UNKN	D4b		19.23	1855537	0	0
F5	UNKN	D4c		18.73	2616489	0	0
F6	UNKN	D4c		18.77	2545534	0	0
F7	UNKN	D5a		19.99	1100552	0	0
F8	UNKN	D5a		20.31	883265.3	0	0
F9	UNKN	D5b		18.61	2841441	0	0

Table 14. GAPD_03252005

well	Type	Sample Name	Replicate	Ct	Quantity	StdDev	Mean
J1	Standard			14.6	10000000		
J2	Standard			17.31	1000000		
J3	Standard			20.83	100000		
J4	Standard			24.58	10000		
J5	Standard			27.32	1000		
J6	Standard			29.48	100		
J7	Standard			33.56	10		
J8	NTC			40	0		
K1	Unknown	D0a		21.9	45259.14	0	0
K10	Unknown	D1b		22.19	35862.64	0	0
K11	Unknown	D1c		21.36	66420.26	0	0
K12	Unknown	D1c		21.89	44835.37	0	0
K13	Unknown	D2a		21.87	46268.28	0	0
K14	Unknown	D2a		21.93	44272.02	0	0
K15	Unknown	D2b		22.08	38813.36	0	0
K16	Unknown	D2b		22.19	35617.97	0	0
K17	Unknown	D2c		20.7	108538.7	0	0
K18	Unknown	D2c		20.8	100414.6	0	0
K19	Unknown	D3a		18.54	540624.1	0	0
K2	Unknown	D0a		21.68	53203.2	0	0
K20	Unknown	D3a		19.05	369743.1	0	0
K21	Unknown	D3b		20.43	132760.5	0	0

K22	Unknown	D3b		20.75	104590.4	0	0
K23	Unknown	D3c		19.24	322170.8	0	0
K24	Unknown	D3c		20.1	169576.2	0	0
K3	Unknown	D0b		20.23	153502	0	0
K4	Unknown	D0b		19.81	210331.8	0	0
K5	Unknown	D0c		20.29	147799.4	0	0
K6	Unknown	D0c		20.13	166314.9	0	0
K7	Unknown	D1a		20.4	135429.4	0	0
K8	Unknown	D1a		20.35	140919.5	0	0
K9	Unknown	D1b		21.87	45396.4	0	0
L1	Unknown	D4a		19.05	372106.4	0	0
L10	Unknown	D5b		20.29	147025.9	0	0
L11	Unknown	D5c		20.39	136874.1	0	0
L12	Unknown	D5c		20.3	145659.4	0	0
L13	Unknown	D6a		19.01	382694.5	0	0
L14	Unknown	D6a		18.5	558513.1	0	0
L15	Unknown	D6b		19.7	228514.6	0	0
L16	Unknown	D6b		19.64	239259.5	0	0
L17	Unknown	D6c		19.01	381746.1	0	0
L18	Unknown	D6c		18.53	546348.3	0	0
L2	Unknown	D4a		19.14	347257.6	0	0
L3	Unknown	D4b		19.65	236315.1	0	0
L4	Unknown	D4b		19.27	314524.6	0	0
L5	Unknown	D4c		18.89	418493.6	0	0
L6	Unknown	D4c		18.72	473376.3	0	0
L7	Unknown	D5a		21.22	73889.8	0	0
L8	Unknown	D5a		20.48	127886.4	0	0
L9	Unknown	D5b		20.14	164707.4	0	0

Table 15. GAPD_03302005

well	Type	Sample Name	Replicate	Ct	Quantity	StdDev	Mean
J1	Standard			15.6361	10000000		
J2	Standard			18.4338	1000000		
J3	Standard			21.5515	100000		
J4	Standard			24.7792	10000		
J5	Standard			28.0269	1000		
J6	Standard			31.3846	100		
J7	Standard			34.2323	10		
J8	NTC			40	0		
K1	UNKN	D0a		22.54865	54355.98	0	0
K10	UNKN	D1b		23.00643	38892.65	0	0
K11	UNKN	D1c		22.27453	66420.55	0	0
K12	UNKN	D1c		22.81198	44835.37	0	0
K13	UNKN	D2a		22.25369	67440.81	0	0
K14	UNKN	D2a		22.10982	74922.51	0	0

K15	UNKN	D2b		23.11879	35824.75	0	0
K16	UNKN	D2b		23.11258	35987.64	0	0
K17	UNKN	D2c		21.84848	90700.62	0	0
K18	UNKN	D2c		21.5815	110254.4	0	0
K19	UNKN	D3a		19.65015	452645.8	0	0
K2	UNKN	D0a		22.20538	69865.73	0	0
K20	UNKN	D3a		20.1093	323548.6	0	0
K21	UNKN	D3b		20.93755	176564.5	0	0
K22	UNKN	D3b		21.42305	123799.6	0	0
K23	UNKN	D3c		20.19889	303032.8	0	0
K24	UNKN	D3c		20.87616	184671.5	0	0
K3	UNKN	D0b		21.8631	89735.11	0	0
K4	UNKN	D0b		21.27535	137918.8	0	0
K5	UNKN	D0c		20.8359	190188.9	0	0
K6	UNKN	D0c		20.97869	171332.3	0	0
K7	UNKN	D1a		21.29795	135658.5	0	0
K8	UNKN	D1a		20.99812	168914.4	0	0
K9	UNKN	D1b		22.6906	48996.7	0	0
L1	UNKN	D4a		20.1646	310726.4	0	0
L10	UNKN	D5b		21.19763	145984.3	0	0
L11	UNKN	D5c		20.72129	206815.9	0	0
L12	UNKN	D5c		20.86152	186659.4	0	0
L13	UNKN	D6a		19.77651	412694.7	0	0
L14	UNKN	D6a		19.90562	375513.9	0	0
L15	UNKN	D6b		21.12885	153514.5	0	0
L16	UNKN	D6b		20.04446	339259.5	0	0
L17	UNKN	D6c		19.56989	480006.1	0	0
L18	UNKN	D6c		19.163	646348.3	0	0
L2	UNKN	D4a		19.94624	364522.4	0	0
L3	UNKN	D4b		20.36698	267983.1	0	0
L4	UNKN	D4b		20.12303	320316.3	0	0
L5	UNKN	D4c		19.75139	420346.2	0	0
L6	UNKN	D4c		19.48763	509766.2	0	0
L7	UNKN	D5a		22.24254	67992.8	0	0
L8	UNKN	D5a		21.28962	136487.3	0	0
L9	UNKN	D5b		21.02422	165721.2	0	0

Table 16. Rho 2_03252005

well	Type	Sample Name	Replicate	Ct	Quantity	StdDev	Mean
A1	Standard			14.28	10000000		
A2	Standard			16.21	1000000		
A3	Standard			20.11	100000		
A4	Standard			23.54	10000		
A5	Standard			26.85	1000		
A6	Standard			30.24	100		

A7	Standard		33.71	10		
A8	NTC		39.55	0		
B1	UNKN	D0a	25.85	1933.138	0	0
B10	UNKN	D1b	25.51	2423.52	0	0
B11	UNKN	D1c	25.9	1869.925	0	0
B12	UNKN	D1c	26.13	1604.744	0	0
B13	UNKN	D2a	26.65	1178.547	0	0
B14	UNKN	D2a	26.14	1594.109	0	0
B15	UNKN	D2b	26.82	1014.264	0	0
B16	UNKN	D2b	27.21	782.5796	0	0
B17	UNKN	D2c	25.37	2659.96	0	0
B18	UNKN	D2c	25.43	2555.928	0	0
B19	UNKN	D3a	22.18	22185.45	0	0
B2	UNKN	D0a	25.01	3379.358	0	0
B20	UNKN	D3a	22.6	16779.64	0	0
B21	UNKN	D3b	23.92	6975.885	0	0
B22	UNKN	D3b	24.11	6147.974	0	0
B23	UNKN	D3c	22.66	16123.38	0	0
B24	UNKN	D3c	23.49	9284.803	0	0
B3	UNKN	D0b	23.66	8292.412	0	0
B4	UNKN	D0b	23.47	9409.102	0	0
B5	UNKN	D0c	23.29	10605.42	0	0
B6	UNKN	D0c	24.21	5752.471	0	0
B7	UNKN	D1a	24.96	3493.598	0	0
B8	UNKN	D1a	25.02	3356.962	0	0
B9	UNKN	D1b	26.32	1414.29	0	0
C1	UNKN	D4a	23.08	12194.68	0	0
C10	UNKN	D5b	23.84	7357.01	0	0
C11	UNKN	D5c	24.71	4125.407	0	0
C12	UNKN	D5c	24.86	3733.795	0	0
C13	UNKN	D6a	24.27	6126.642	0	0
C14	UNKN	D6a	23.73	8905.29	0	0
C15	UNKN	D6b	25.22	3173.028	0	0
C16	UNKN	D6b	24.79	4273.786	0	0
C17	UNKN	D6c	23.69	9155.448	0	0
C18	UNKN	D6c	23.93	7753.369	0	0
C2	UNKN	D4a	23.23	11037.08	0	0
C3	UNKN	D4b	23.46	9471.875	0	0
C4	UNKN	D4b	23.24	10963.94	0	0
C5	UNKN	D4c	22.55	17346.88	0	0
C6	UNKN	D4c	23.08	12194.68	0	0
C7	UNKN	D5a	24.61	4409.043	0	0
C8	UNKN	D5a	24.17	5907.524	0	0
C9	UNKN	D5b	23.71	8021.251	0	0

Table 17. Rho2_03302005

well	Type	Sample Name	Replicate	Ct	Quantity	StdDev	Mean
A1	Standard			13.7631	10000000		
A2	Standard			17.0898	1000000		
A3	Standard			20.4165	100000		
A4	Standard			23.7432	10000		
A5	Standard			27.0699	1000		
A6	Standard			30.3966	100		
A7	Standard			33.7233	10		
A8	NTC			39.55	0		
B1	Unknown	D0a		25.22	3598.128	0	0
B10	Unknown	D1b		25.78	2441.977	0	0
B11	Unknown	D1c		25.96	2155.926	0	0
B12	Unknown	D1c		26.21	1813.361	0	0
B13	Unknown	D2a		25.72	2545.526	0	0
B14	Unknown	D2a		25.85	2326.482	0	0
B15	Unknown	D2b		26.64	1346.565	0	0
B16	Unknown	D2b		27.03	1028.002	0	0
B17	Unknown	D2c		26.01	2082.591	0	0
B18	Unknown	D2c		19.65	169984.2	0	0
B19	Unknown	D3a		22.34	26411.88	0	0
B2	Unknown	D0a		25.58	2804.537	0	0
B20	Unknown	D3a		22.6	22061.95	0	0
B21	Unknown	D3b		23.87	9159.761	0	0
B22	Unknown	D3b		24.01	8313.816	0	0
B23	Unknown	D3c		23.01	16611.11	0	0
B24	Unknown	D3c		23.2	14564.14	0	0
B3	Unknown	D0b		23.73	10091.78	0	0
B4	Unknown	D0b		24.01	8313.816	0	0
B5	Unknown	D0c		23.69	10375.09	0	0
B6	Unknown	D0c		24.12	7704.325	0	0
B7	Unknown	D1a		25.22	3598.128	0	0
B8	Unknown	D1a		24.78	4879.101	0	0
B9	Unknown	D1b		26.11	1943.319	0	0
C1	Unknown	D4a		23.09	15716.32	0	0
C10	Unknown	D5b		23.8	9614.485	0	0
C11	Unknown	D5c		24.82	4745.87	0	0
C12	Unknown	D5c		24.66	5301.656	0	0
C13	Unknown	D6a		23.98	8488.254	0	0
C14	Unknown	D6a		23.84	9351.948	0	0
C15	Unknown	D6b		24.95	4337.486	0	0
C16	Unknown	D6b		24.99	4219.045	0	0
C17	Unknown	D6c		23.75	9953.044	0	0
C18	Unknown	D6c		23.94	8726.544	0	0
C2	Unknown	D4a		23.15	15077	0	0
C3	Unknown	D4b		22.98	16959.64	0	0

C4	Unknown	D4b		23.32	13403.34	0	0
C5	Unknown	D4c		22.32	26780.05	0	0
C6	Unknown	D4c		23.21	14463.69	0	0
C7	Unknown	D5a		24.47	6046.795	0	0
C8	Unknown	D5a		24.25	7041.365	0	0
C9	Unknown	D5b		23.71	10232.46	0	0

Table 18. UBC_03252005

well	Type	Sample Name	Replicate	Ct	Quantity	StdDev	Mean
G1	Standard			15.41	10000000		
G2	Standard			18.96	1000000		
G3	Standard			22.6	100000		
G4	Standard			25.8	10000		
G5	Standard			29.19	1000		
G6	Standard			32.43	100		
G7	Standard			35.48	10		
G8	NTC			40	0		
H1	UNKN	D0a		22.09	119535.8	0	0
H10	UNKN	D1b		23.32	51127.17	0	0
H11	UNKN	D1c		22.33	101252.1	0	0
H12	UNKN	D1c		22.01	125984.2	0	0
H13	UNKN	D2a		22.18	112228.8	0	0
H14	UNKN	D2a		21.54	174504.2	0	0
H15	UNKN	D2b		22.42	95415.62	0	0
H16	UNKN	D2b		22.45	93083.3	0	0
H17	UNKN	D2c		20.58	336981.9	0	0
H18	UNKN	D2c		20.43	373138.8	0	0
H19	UNKN	D3a		19.89	540948.9	0	0
H2	UNKN	D0a		22.09	119499.3	0	0
H20	UNKN	D3a		20.01	499925.5	0	0
H21	UNKN	D3b		21.17	224844.3	0	0
H22	UNKN	D3b		21.63	163527.6	0	0
H23	UNKN	D3c		20.08	475554.5	0	0
H24	UNKN	D3c		20.74	302232.6	0	0
H3	UNKN	D0b		20.57	338589.7	0	0
H4	UNKN	D0b		19.32	799790.5	0	0
H5	UNKN	D0c		19.14	907121.3	0	0
H6	UNKN	D0c		20.28	414035	0	0
H7	UNKN	D1a		21.34	200336.5	0	0
H8	UNKN	D1a		21.34	199627.5	0	0
H9	UNKN	D1b		22.18	112613.6	0	0
I1	UNKN	D4a		19.99	505282.9	0	0
I10	UNKN	D5b		21.23	215702.4	0	0
I11	UNKN	D5c		21.45	184848.2	0	0
I12	UNKN	D5c		21.21	218828.4	0	0

I13	UNKN	D6a		20.36	391713.7	0	0
I14	UNKN	D6a		20.28	414147.4	0	0
I15	UNKN	D6b		21.15	227175.7	0	0
I16	UNKN	D6b		21.24	214451.8	0	0
I17	UNKN	D6c		20.16	449494.8	0	0
I18	UNKN	D6c		20.21	434151.2	0	0
I2	UNKN	D4a		20.73	304274.3	0	0
I3	UNKN	D4b		20.31	405757	0	0
I4	UNKN	D4b		20.38	387978.5	0	0
I5	UNKN	D4c		19.35	786688.8	0	0
I6	UNKN	D4c		20.18	444097	0	0
I7	UNKN	D5a		21.56	171869.7	0	0
I8	UNKN	D5a		21.32	202941.5	0	0
I9	UNKN	D5b		21.03	248262	0	0

Table 19. UBC_03302005

well	Type	Sample Name	Replicate	Ct	Quantity	StdDev	Mean
G1	Standard			15.37	10000000		
G2	Standard			18.67	1000000		
G3	Standard			22.32	100000		
G4	Standard			25.44	10000		
G5	Standard			28.74	1000		
G6	Standard			32.37	100		
G7	Standard			35.88	10		
G8	NTC			40	0		
H1	UNKN	D0a		22.23	93841.9	0	0
H10	UNKN	D1b		23.12	51409.95	0	0
H11	UNKN	D1c		22.1	102464	0	0
H12	UNKN	D1c		22.55	75583.74	0	0
H13	UNKN	D2a		22.08	103859.1	0	0
H14	UNKN	D2a		22.64	71121.31	0	0
H15	UNKN	D2b		22.32	88301.52	0	0
H16	UNKN	D2b		22.44	81419.8	0	0
H17	UNKN	D2c		20.32	341405.6	0	0
H18	UNKN	D2c		20.44	314798.4	0	0
H19	UNKN	D3a		19.75	501940.1	0	0
H2	UNKN	D0a		21.99	110375.6	0	0
H20	UNKN	D3a		20.21	367766.6	0	0
H21	UNKN	D3b		21.21	187034.2	0	0
H22	UNKN	D3b		21.5	153731.3	0	0
H23	UNKN	D3c		20.02	418182.7	0	0
H24	UNKN	D3c		20.84	240199.5	0	0
H3	UNKN	D0b		20.32	341405.6	0	0
H4	UNKN	D0b		19.56	570749.6	0	0
H5	UNKN	D0c		19.64	540696.5	0	0

H6	UNKN	D0c	20.21	367766.6	0	0
H7	UNKN	D1a	21.35	170141.4	0	0
H8	UNKN	D1a	21.67	137038.2	0	0
H9	UNKN	D1b	22.78	64697.66	0	0
I1	UNKN	D4a	19.99	426752	0	0
I10	UNKN	D5b	21.33	172457.8	0	0
I11	UNKN	D5c	21.27	179598.2	0	0
I12	UNKN	D5c	21.28	178387.9	0	0
I13	UNKN	D6a	20.36	332295.6	0	0
I14	UNKN	D6a	20.25	357953.2	0	0
I15	UNKN	D6b	21.11	200118	0	0
I16	UNKN	D6b	21.34	171295.7	0	0
I17	UNKN	D6c	20.17	377849.1	0	0
I18	UNKN	D6c	20.25	357953.2	0	0
I2	UNKN	D4a	20.72	260501.5	0	0
I3	UNKN	D4b	20.17	377849.1	0	0
I4	UNKN	D4b	20.55	292234.1	0	0
I5	UNKN	D4c	19.37	648992.1	0	0
I6	UNKN	D4c	20.05	409785.4	0	0
I7	UNKN	D5a	21.76	128947.5	0	0
I8	UNKN	D5a	21.31	174805.8	0	0
I9	UNKN	D5b	21.15	194778.1	0	0

APPENDIX D

USER MANUAL

1. Installing qPCR-DAMS

1. Go to web: **http://lungmicroarray.org/lbtl/info/qPCR-DAMS/index.htm**
2. Double click **qPCR-DAMS** and save to the chosen destination.
3. Double click "**qPCR-DAMS.zip**" from the destination.
4. Double "**qPCR-DAMS**", the whole package includes the software, test data, and users' manual.
5. Double click "**qPCR-DAMS.mdb**" to open the software.

If the software is run on Windows XP, a security warning will appear because of the system settings (for details please visit http://office.microsoft.com/en-us/assistance/HA011071331033.aspx). Ignore the warning and click **Open**. A panel containing the brief introduction of the software will appear. Check "Don't show this screen again" and click **OK**. A panel **Menu** will appear. If the software is run in Window 2000 environment, there will be no security warning and the introduction panel will appear directly.

2. Sample question

Here we present an example that is suitable for all the data processing modules of qPCR-DAMS. New users can try this example and the test data we provided. Test data can be found in the **Appendix C**.

Suppose we want to know the effect of a drug on the mRNA expression of GABA receptor rho2 subunit (rho2) in rat brain. Therefore, we applied the drug to rats and

collect whole brains from day 1 to day 6 (D1~D6) after the treatment. Rat brains collected on the day before drug application were used as controls (D0). Three independent experiments (a, b, and c) were carried out and the samples were named like D0a and D1c. Total RNA was isolated from those samples and 1 μg of the RNA was reverse transcribed into cDNA. The mRNA expression levels of rho2 were quantified with real-time PCR by both relative and absolute quantitative methods at the same time. Briefly, rho2 was run together with three house-keeping genes: β-actin (ACTB), Ubiquitin C (UBC), and Glyceraldehyde-3-phosphate dehydrogenase (GAPD) on the same 384-well-plate. The standard curves of each gene were also run on that plate. Duplicate wells were used for all the samples and the plate-run was repeated once (on 03/25/2005 and 03/30/2005, respectively). After exporting the experiment results (report file) from the detector system, the data were ready to process by qPCR-DAMS.

2.I Enter information

I.1 Enter New Gene

1. Select **Gene** on the **Menu** or press **G** on the keyboard. A **Gene Control Panel** appears.
2. Select **Add New Gene**. An **Add New Gene Information** panel will pop up. Enter gene information as follows:

Table 1: Add new gene information

Gene ID	***Gene Name***	***Description***
D38494	Rho2	GABA receptor rho2 subunit, enriched in retina

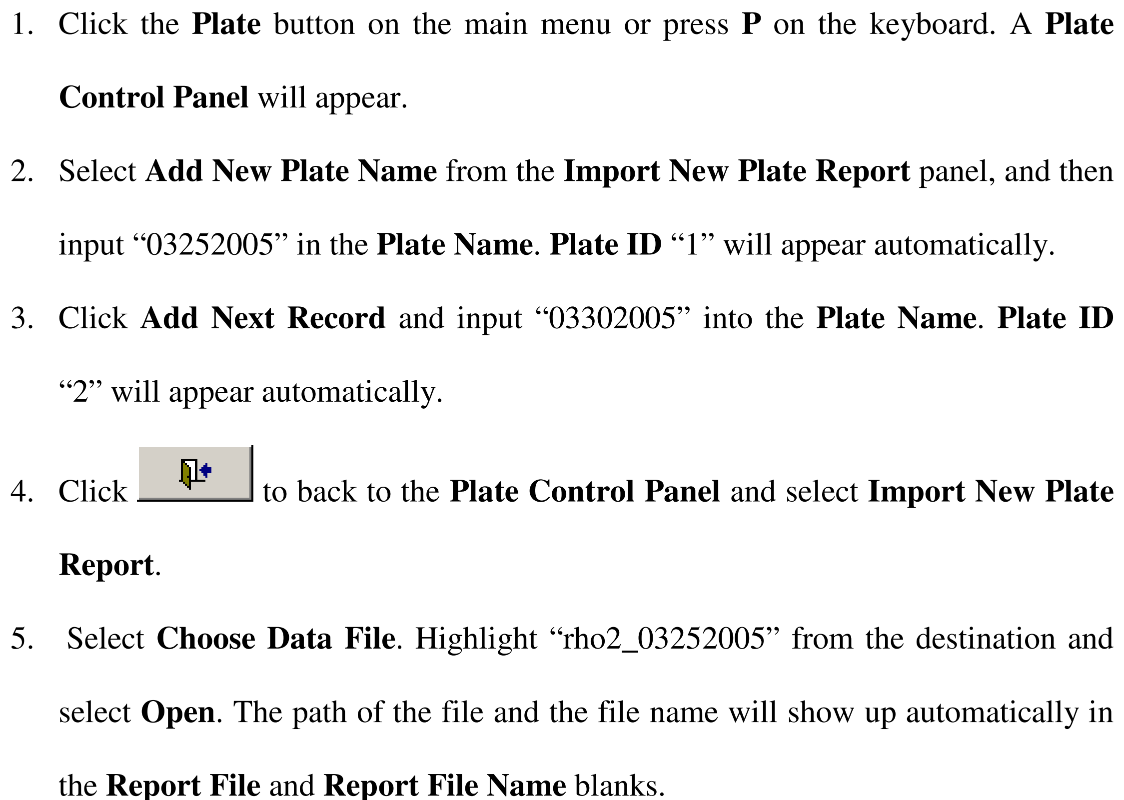
NM_031144	ACTB	beta-actin, a cytoskeletal structure protein
NM_017314	UBC	Ubiquitin C, protein degradation
X02231	GAPD	Glyceraldehyde-3-phosphate-dehydrogenase, oxidoreductase in glycolysis and gluconeogenesis

3. Close **Add New Gene Information** panel and Select from the Gene Control panel.

I.2 Enter New Plate

The users can either import plate report or add plate report manually. (Note: a comdlg32.ocx is required to run the import application. For details, see **Trouble Shooting**).

A. Import Plate Report

1. Click the **Plate** button on the main menu or press **P** on the keyboard. A **Plate Control Panel** will appear.
2. Select **Add New Plate Name** from the **Import New Plate Report** panel, and then input "03252005" in the **Plate Name**. **Plate ID** "1" will appear automatically.
3. Click **Add Next Record** and input "03302005" into the **Plate Name**. **Plate ID** "2" will appear automatically.
4. Click  to back to the **Plate Control Panel** and select **Import New Plate Report**.
5. Select **Choose Data File**. Highlight "rho2_03252005" from the destination and select **Open**. The path of the file and the file name will show up automatically in the **Report File** and **Report File Name** blanks.

6. Select “Rho2 ” from the **Gene ID** list.
7. Select “03252005” from the **Plate Name** list.
8. Select **Import Data** and input the data into the database (Note: the format of the report should be cleared before importing. To clear the format, open the plate report Excel file, click on the top left square and change the whole table black. Then select **Edit > Clear > Formats**.)
9. Select Choose Data File and enter more plate information according to the following table:

Table 2: plate information for sample question

Report File Name	***Gene Name***	***Plate Name***
ACTB_03252005	ACTB	03252005
UBC_03252005	UBC	03252005
GAPD_03252005	GAPD	03252005
Rho2_03302005	Rho2	03302005
ACTB_03302005	ACTB	03302005
UBC_03302005	UBC	03302005
GAPD_03302005	GAPD	03302005

[**Note**: If experiment report was generated by other system such as ABI 7500, arrange column of data to the same order as that system (Well, Type, Sample Name, Replicate, Ct, Quantity, Std Dev, Mean).

10. Click **Edit Sample Info** from the **Import Plate Report** panel. An **Edit Sample Info** panel will appear.
11. Sample information can be input according to the following table:

Table 3: Sample information for sample question

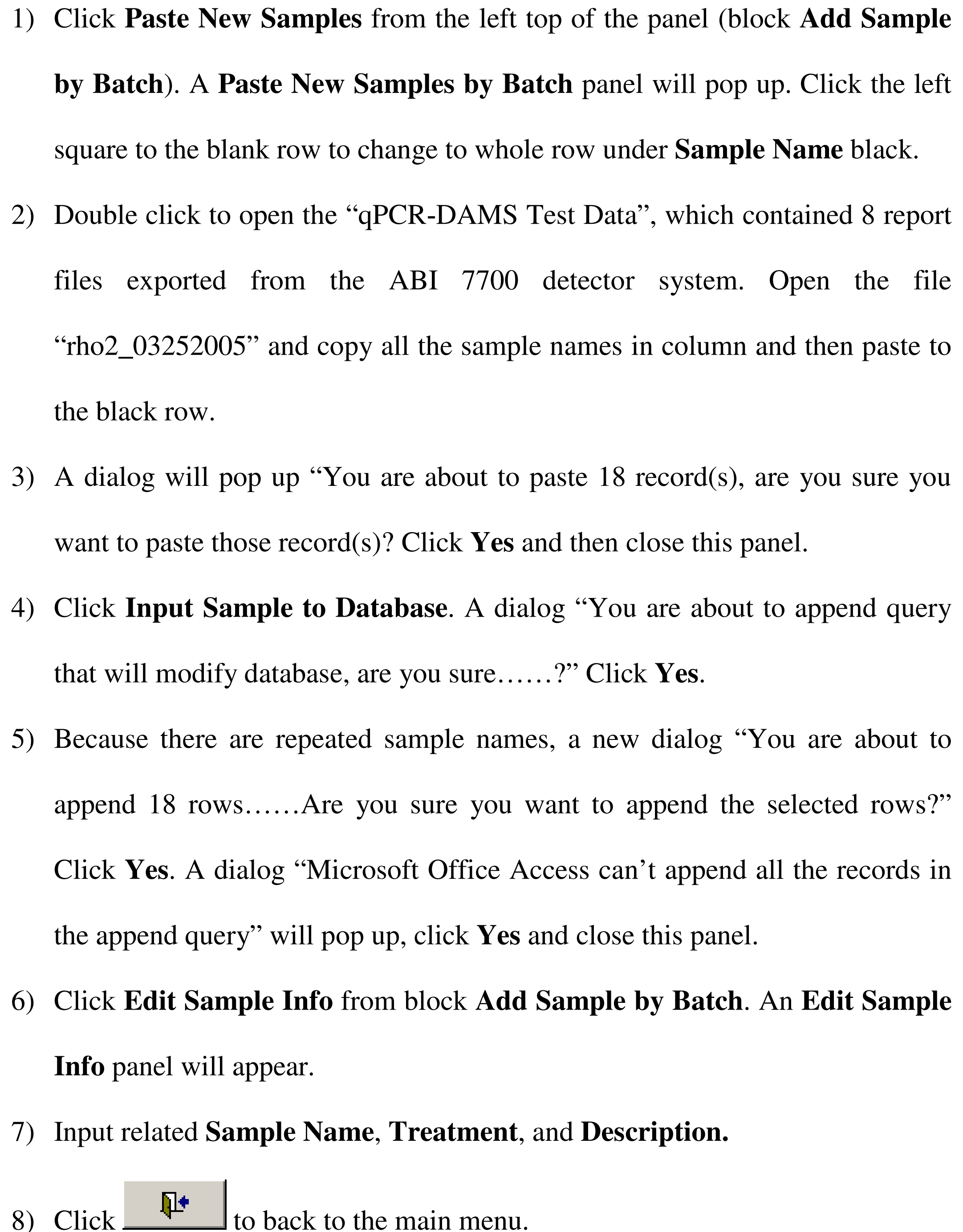
Sample Name	***Treatment***	***Description***
D0a, D0b, and D0c	D0	Brain tissue before drug treatment
D1a, D1b, and D1c	D1	Brain tissue after drug treatment for 1 day
D2a, D2b, and D2c	D2	Brain tissue after drug treatment for 2 days
D3a, D3b, and D3c	D3	Brain tissue after drug treatment for 3 days
D4a, D4b, and D4c	D4	Brain tissue after drug treatment for 4 days
D5a, D5b, and D5c	D5	Brain tissue after drug treatment for 5 days
D6a, D6b, and D6c	D6	Brain tissue after drug treatment for 6 days

12. Click to back to the main menu.
13. It is easy to make mistake when entering plate information. Go to **View and Edit Plate Information** from the **Plate Control Panel** to correct wrong information or delete unwanted plate information.
14. Close all other panels and back to the main Menu.

B. Add Plate Report Manually

1. Click the **Plate** button on the main menu or press **P** on the keyboard. A **Plate Control Panel** will appear.
2. Select **Add New Sample** from the **Add Plate Report Manually** panel and a **Sample Control Panel** will pop up.
3. You can enter new sample one by one when sample number is small or you can also enter sample by batch when sample number is large.

   (3.1) Enter sample by batch

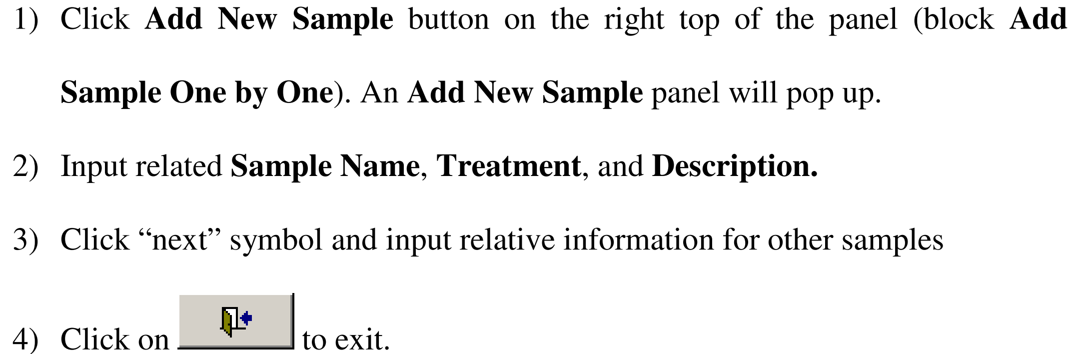
1) Click **Paste New Samples** from the left top of the panel (block **Add Sample by Batch**). A **Paste New Samples by Batch** panel will pop up. Click the left square to the blank row to change to whole row under **Sample Name** black.
2) Double click to open the "qPCR-DAMS Test Data", which contained 8 report files exported from the ABI 7700 detector system. Open the file "rho2_03252005" and copy all the sample names in column and then paste to the black row.
3) A dialog will pop up "You are about to paste 18 record(s), are you sure you want to paste those record(s)? Click **Yes** and then close this panel.
4) Click **Input Sample to Database**. A dialog "You are about to append query that will modify database, are you sure……?" Click **Yes**.
5) Because there are repeated sample names, a new dialog "You are about to append 18 rows……Are you sure you want to append the selected rows?" Click **Yes**. A dialog "Microsoft Office Access can't append all the records in the append query" will pop up, click **Yes** and close this panel.
6) Click **Edit Sample Info** from block **Add Sample by Batch**. An **Edit Sample Info** panel will appear.
7) Input related **Sample Name**, **Treatment**, and **Description.**
8) Click  to back to the main menu.

(3.2) Enter sample one by one (if you have not done add sample by batch, you can use this step)

1) Click **Add New Sample** button on the right top of the panel (block **Add Sample One by One**). An **Add New Sample** panel will pop up.

2) Input related **Sample Name**, **Treatment**, and **Description.**

3) Click "next" symbol and input relative information for other samples

4) Click on  to exit.

I.3 Enter New Experiment

1. Click **Experiment** button on the main menu or press **E** on the keyboard. An **Experiment Control Panel** will pop up.
2. Select **Researcher Information** and a researcher table will open. Input "He" and "Keyu" to the blank of **Last Name** and **First Name**, respectively. Other information is optional. Close this table.
3. Select **Add New Experiment**, and then input "Rho2 subunit expression in brain 1" under the **Experiment Name**. An **Experiment ID** "1" will be generated automatically.
4. Input "ACTB normalization" in the **Description**.
5. Select "ACTB" and "rho2" from the **Reference gene** and **Target gene** list, respectively.
6. Enter 1.99 and 2 for the **Refer E** (reference gene amplification efficiency) and **Target E** (target gene amplification efficiency), respectively. If amplification efficiency is not selected, the default number will be 2.

7. Select "Keyu" from the **Researcher ID** list and "D0" from the **Control** list. Input "6%" and "30%" for the **Intra-Plate Threshold** and **Inter-Plate Threshold** (Note: The users can enter their own threshold value).
8. Select "ACTB_03252005" and "ACTB_03302005" from list for the **Reference Gene Plate Report**; "rho2_03252005" and "rho2_03302005" for the **Target Gene Plate Report.**
9. Select "**Add Next Experiment**" and enter two more experiments according to the following settings:

Experiment 2

Experiment Name: Rho2 subunit expression in brain 2; **Description**: UBC normalization; **Target Gene**: rho2; **Reference Gene**: UBC; **Target E**: 2; **Reference E**: 1.98; **Control**: D0; **Researcher ID**: Keyu He; **Intra-plate threshold**: 6%; **Inter-plate threshold**: 30%; **Reference Gene Plate Report**: UBC_03252005, UBC_03302005; **Target Gene Plate Report**: Rho2_03252005, Rho2_03302005.

Experiment 3

Experiment Name: Rho2 subunit expression in brain 3; **Description**: GAPD normalization; **Target Gene**: rho2; **Reference Gene**: GAPD; **Target E**: 2; **Reference E**: 2.0; **Control**: D0; **Researcher ID**: Keyu He; **Intra-plate threshold**: 6%; **Inter-plate threshold**: 30%; **Reference Gene Plate Report**: GAPD_03252005, GAPD_03302005; **Target Gene Plate Report**: Rho2_03252005, Rho2_03302005.

10. Close all other panels and back to the main Menu.

2.2 Check Error and Process Data

A. Basic modules

I. Ratio Relative Quantification

1. Click **Process Data** and a **Choose Process Module** panel will appear. Select **Ratio Relative Quantification** from the new panel.
2. Under **Choose Experiment** select "Rho2 expression in brain 1" from the list.
3. Click "**Fix Unrecognized Ct Error**" and a dialog "You are about to run a query that will modify data in your table" will appear. Select **Yes** and a dialog "You are about to up-date 0 rows" will appear. Click **Yes**. If the dialog show "You are about to up-date N rows", it means that plate reports in this experiment include wells with "Undetermined" Ct. Click **Yes** and the "Undetermined" Ct will be changed to 40 by the system.
4. Click **Step 1 Error Check**. The pop-up table shows that "D2c" on report file Rho2_03302005 produced an intra-plate variation higher than 6%, the intra-plate threshold value. Close this table.
5. From the main **menu**, choose **View Data > View Data by Sample**. Click on the "+" in front of the "D2c" and the list of report data about "D2c" will show up. The report shows well "B18" is quite different from the other replicates. Close the **View Data** panel and select **Plate > Plate Control Panel > View and Edit Plate Information.** Select "03302005" > "Rho2_03302005". Check the well "B18" and return to the **Experiment Report** panel. Perform **Step 1 Error Check** again and

the pop-up table shows no sample has intra-plate variation higher than the threshold. Close this table.

6. Click **Step 2 Error Check**. The pop-up table shows D2a produces an inter-plate variation higher than 30%. Go **View Data> View Data by Sample>D2a**. No abnormal is found. Ignore this problem and close the **Check Error by Inter CV** table.
7. Select the result type (**Ct**, **Normalized Expression**, **Intra-plate Sample Ratio**, **Inter-plate Sample Ratio**, and **Final Ratio**) to process.
8. Select **Preview** to show results.
9. To export experiment result for further data processing, right click mouse on the report and select **Export** (We suggest choosing "Microsoft Excel 97-2003" as the file type). Click **Save** to save the exported report in the proper destination.
10. To print the processed data directly, click **Print** from the **Experiment Reports** panel.

II. Absolute levels

Procedures are basically the same as Ratio Relative Quantification. See Ratio Relative Quantification for details.

1. Choose **Process Data>Choose Process Module>Absolute levels**.
2. **Choose Experiment** "Rho2 expression in brain 1" from the list.
3. **Fix Unrecognized Quantity Error**. A dialog "You are about to run a query that will modify data in your table" will appear. Select **Yes** and a dialog "You are about to up-date 4 rows" will appear. From **View Data** (see detail later) it shows

the unrecognized quantity errors are from NTC but not from samples to quantify. Click **Yes** and the unrecognized quantity will be updated to 0.01 by the system.

4. **Step 1 Error Check** to find out and filter abnormal samples.
5. **Step 2 Error Check** to find out and filter more abnormal samples.
6. Select the result type (**Target Intra-plate Quantity**, **Target Inter-plate Quantity**, and **Absolute Level**) to process.
7. Select **Preview** to show results.
8. Right click on the experiment report to export.
9. Click Print from the **Experiment Reports** panel to print.

III. Normalized Absolute Expression

1. Select **Process Data>Choose Process Module>Normalized Absolute Expression**.
2. **Choose Experiment** from the experiment list.
3. **Fix Unrecognized Quantity Error** to update the unrecognized quantity.
4. **Step 1 Error Check** to find out and filter abnormal samples.
5. **Step 2 Error Check** to find out and filter more abnormal samples.
6. Select the result type (**Target Intra-plate Quantity**, **Reference Intra-plate Quantity, Target Inter-plate Quantity**, **Reference Inter-plate Quantity**, **Normalized Expression**, and **Final Normalized Expression**) to process.
7. Select **Preview** to show results.
8. Right click on the experiment report to export results.
9. Select **Print** to print results.

IV. Ratio Absolute Quantification

1. Choose **Process Data>Choose Process Module>Ratio Absolute Quantification**.
2. **Choose Experiment** from the experiment list.
3. **Fix Unrecognized Quantity Error** to update the unrecognized quantity.
4. **Step 1 Error Check** to find out and filter abnormal samples.
5. **Step 2 Error Check** to find out and filter more abnormal samples.
6. Select the result type (**Target Intra-plate Quantity**, **Reference Intra-plate Quantity, Target Inter-plate Quantity**, **Reference Inter-plate Quantity**, **Normalized Expression**, and **Final Ratio**) to process.
7. Select **Preview** to show results.
8. Right click on the experiment report to export results.
9. Select **Print** to print results.

B. Advanced modules

V. Multiple References Relative Quantification

1. Click **Process Data** and then select **Multiple References Relative Quantification** under the **Advanced Options**.
2. **Choose Experiment** from the experiment list and then click **Add**. If dialog "You are about to run an append……" pop up, click **Yes**. A dialog will remind you to add experiment "You are about to append N rows". Click **Yes**. This experiment

will display under the **Experiment Selected**. (**Note**: Please choose and add experiments one by one)

3. To delete a selected experiment, choose that experiment from **Experiment Selected** and click **Delete**. The same dialogs will appear. Click **Yes**.

Select the result type (**inter-ref sample ratio** and **final ratio**) and click **process**.

VI. Multiple References Absolute Quantification

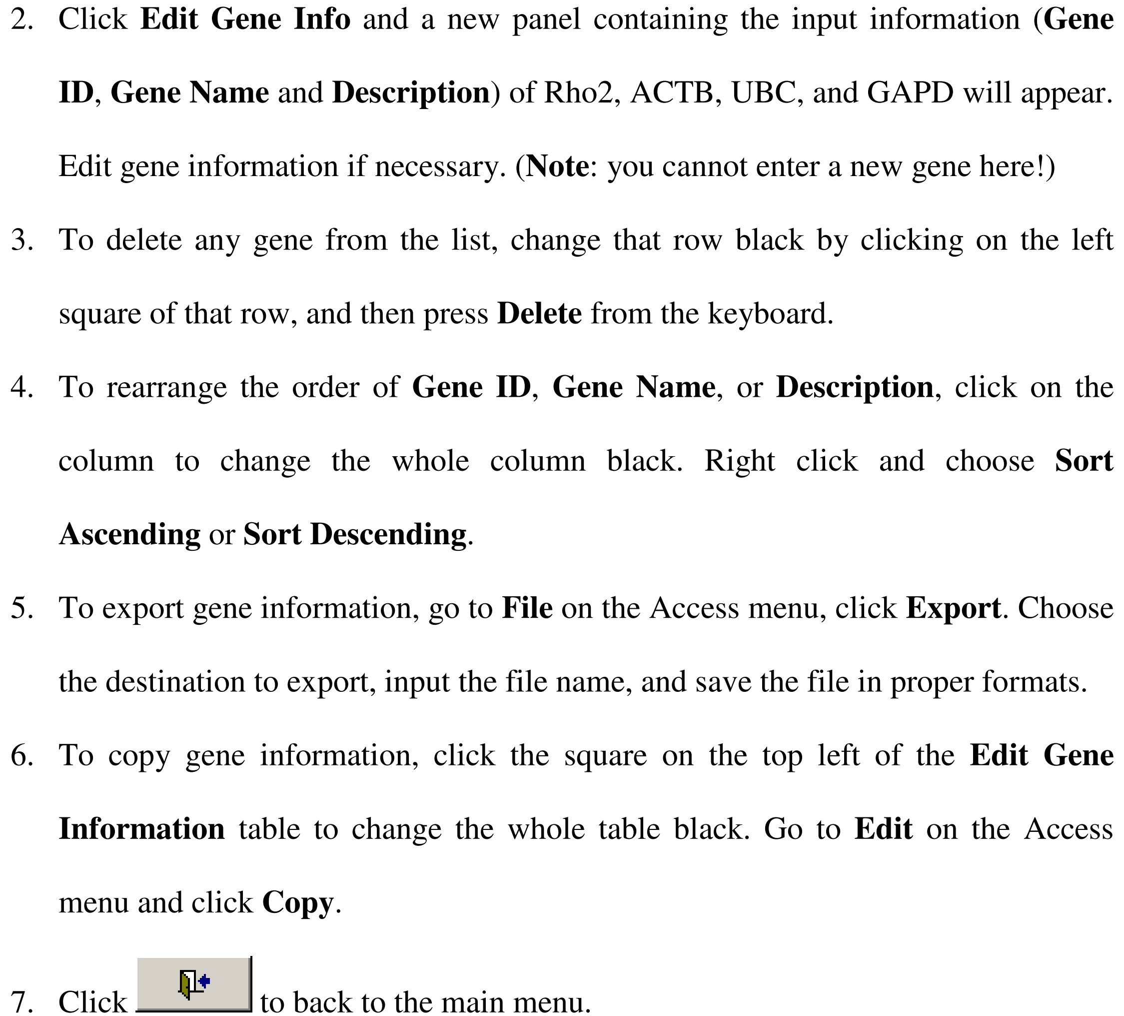
1. Click **Process Data** and then select **Multiple References Absolute Quantification** under the **Advanced Options**.
2. **Choose Experiment** from the experiment list and then click **Add**. If a dialog "You are about to run an append……" pop up, click **Yes**. A dialog will remind you the addition of experiment "You are about to append N rows". Click **Yes**. This experiment will show under the **Experiment Selected**. (**Note**: Please choose and add experiments one by one)
3. To delete a selected experiment, choose that experiment from **Experiment Selected** and click **Delete**. The same dialogs will appear. Click **Yes**.

Select the result type (**inter-ref sample ratio** and **final ratio**) and click **process**.

2.3. Edit Information

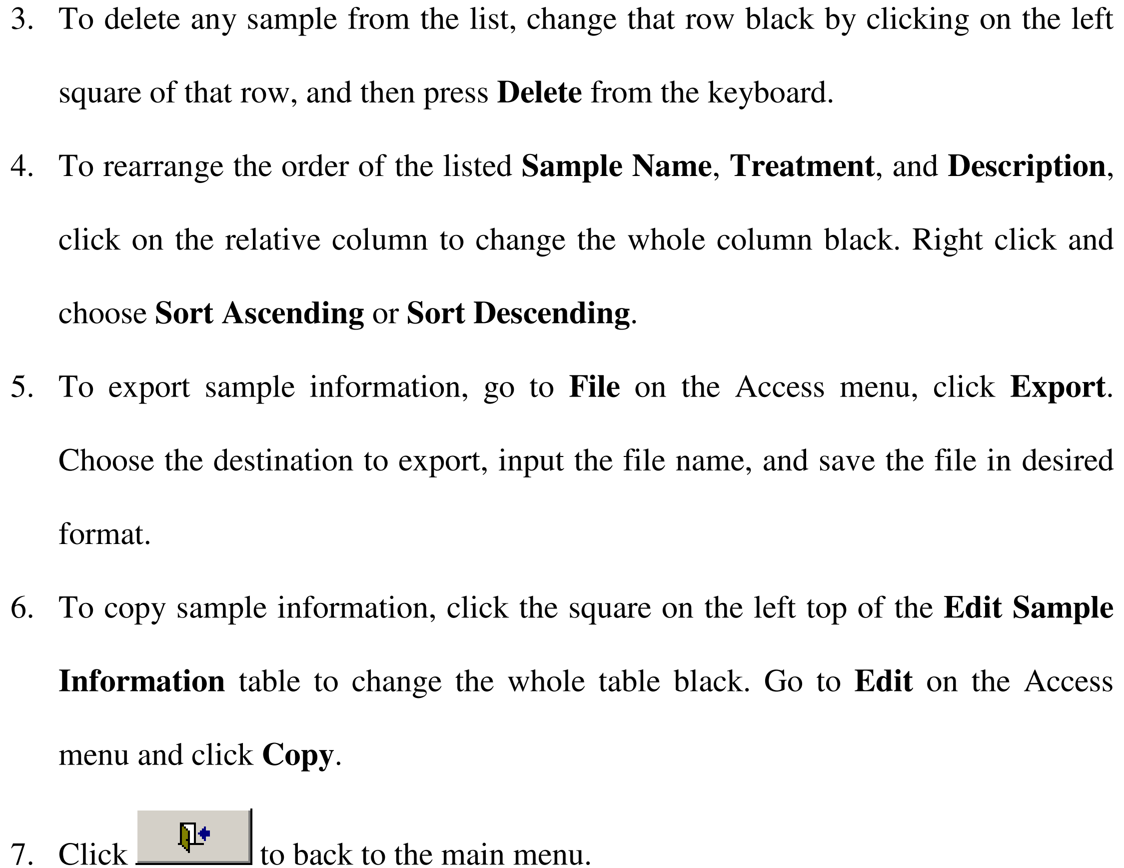
A. Edit Gene Information

1. Click **Gene** button on the main menu. The **Gene Control Panel** will appear.

2. Click **Edit Gene Info** and a new panel containing the input information (**Gene ID**, **Gene Name** and **Description**) of Rho2, ACTB, UBC, and GAPD will appear. Edit gene information if necessary. (**Note**: you cannot enter a new gene here!)
3. To delete any gene from the list, change that row black by clicking on the left square of that row, and then press **Delete** from the keyboard.
4. To rearrange the order of **Gene ID**, **Gene Name**, or **Description**, click on the column to change the whole column black. Right click and choose **Sort Ascending** or **Sort Descending**.
5. To export gene information, go to **File** on the Access menu, click **Export**. Choose the destination to export, input the file name, and save the file in proper formats.
6. To copy gene information, click the square on the top left of the **Edit Gene Information** table to change the whole table black. Go to **Edit** on the Access menu and click **Copy**.
7. Click  to back to the main menu.

B. Edit Sample Information

1. Click **Plate** button on the main menu. The **Plate Control Panel** will appear.
2. Select **Import Plate Report > Edit Sample Info.**, or **Add Plate Report Manually > Add New Sample > Edit Sample Info.** either from the block of **Add Sample by Batch** or **Add Sample One by One**. A panel containing the input information (**Sample Name**, **Treatment**, and **Description**) will appear. Edit sample information if necessary (Note: you cannot enter a new sample name here!).

3. To delete any sample from the list, change that row black by clicking on the left square of that row, and then press **Delete** from the keyboard.
4. To rearrange the order of the listed **Sample Name**, **Treatment**, and **Description**, click on the relative column to change the whole column black. Right click and choose **Sort Ascending** or **Sort Descending**.
5. To export sample information, go to **File** on the Access menu, click **Export**. Choose the destination to export, input the file name, and save the file in desired format.
6. To copy sample information, click the square on the left top of the **Edit Sample Information** table to change the whole table black. Go to **Edit** on the Access menu and click **Copy**.
7. Click  to back to the main menu.

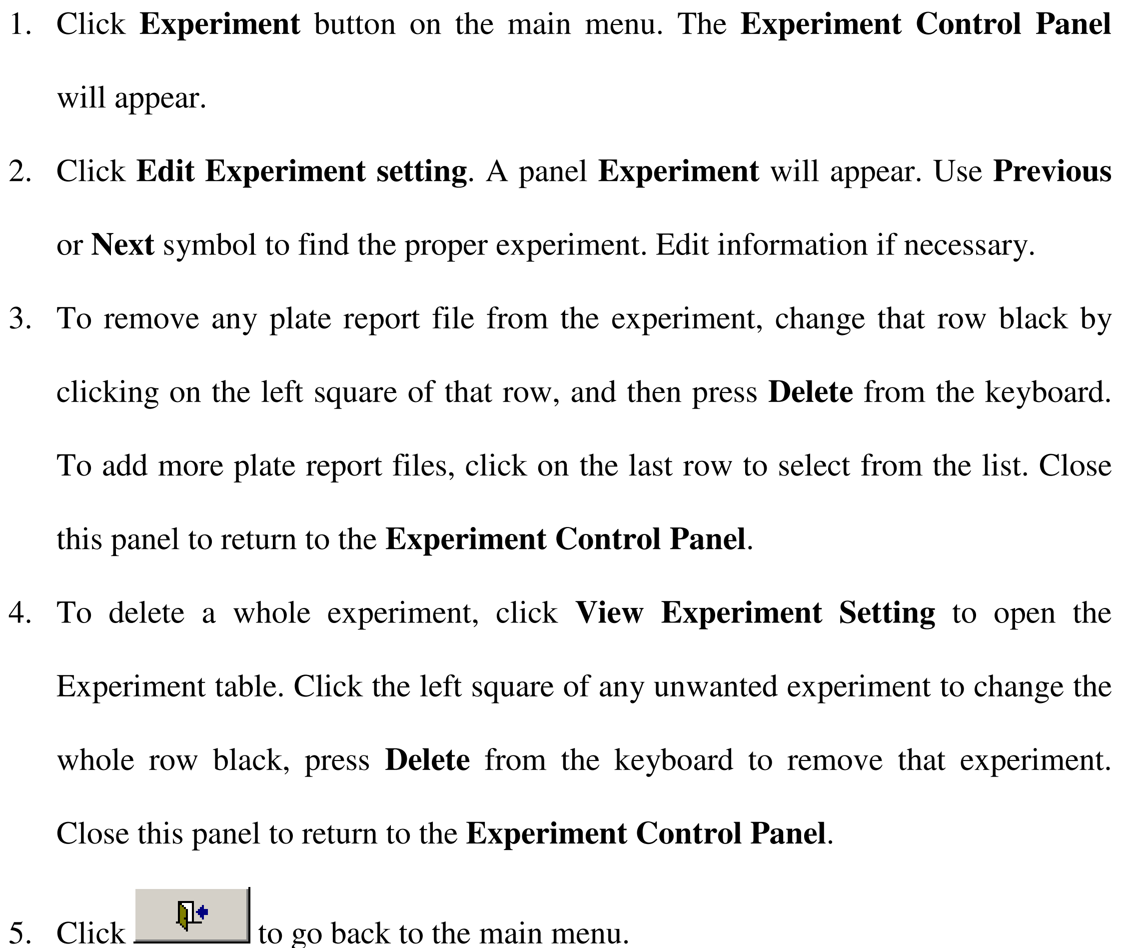
C. Edit Plate Information

1. Click **Plate** button on the main menu. The **Plate Control Panel** will appear.
2. Click **View and Edit Plate Information** and a new panel containing the input information (Plate ID, Plate Name, and Date) of “03252005” and “03302005” will appear.
3. To change a plate name, work on the plate table directly.
4. To change a report file name, eg. “Rho2_03252005” to “Rho2A” on plate “03252005”, click “**+**” before “03252005” to show the report file list in that plate, change “Rho2_03252005” to “Rho2A”.

5. To change the gene of a report file (if the user selects a gene for the report file), click "**+**" before the plate to show the report file list in that plate, select the right gene from the gene list.
6. To exclude abnormal wells from data processing, for example, well "B18" on plate report file "Rho2_03302005" is quite different from the other replicates, open plate list by clicking "**+**" before the plate "03302005", and then open the report file by clicking "**+**" before "Rho2_03302005". Mark well B18 with a "√" at the "error" column and the checked wells will not be used in further calculations. Use **Next** or **Previous** symbol to edit other plate reports. Close this panel to return to the **Plate Control Panel**. (Note: Don't change raw data such as **Well**, **Ct** and **Quantity**!!!)
7. To delete any unwanted reported file, go to **View and Edit Plate Information**, click the "**+**" on the left of a selected **Plate Name** to show the plate report file list, click on the left square of the selected report file to change the whole row black and then select **Delete** on the keyboard.
8. To rearrange the order of any plate information or plate report information, such as **Plate name** or **Sample Name**, click on the top of the corresponding column to change the whole column black. Right click and choose **Sort Ascending** or **Sort Descending**.
9. To export plate information or plate reports, open the proper panel, go to **File** on the Access menu, and click **Export**. Choose the destination to export, input the file name, and save the file in the desired format.

10. To copy plate information, click the square on the left top of the **Edit Plate Information** table to change the whole table black. Go to **Edit** on the Access menu and click **Copy**.
11. Close all the other panels and go back to the main **Menu**.

D. Edit Experiment Information

1. Click **Experiment** button on the main menu. The **Experiment Control Panel** will appear.
2. Click **Edit Experiment setting**. A panel **Experiment** will appear. Use **Previous** or **Next** symbol to find the proper experiment. Edit information if necessary.
3. To remove any plate report file from the experiment, change that row black by clicking on the left square of that row, and then press **Delete** from the keyboard. To add more plate report files, click on the last row to select from the list. Close this panel to return to the **Experiment Control Panel**.
4. To delete a whole experiment, click **View Experiment Setting** to open the Experiment table. Click the left square of any unwanted experiment to change the whole row black, press **Delete** from the keyboard to remove that experiment. Close this panel to return to the **Experiment Control Panel**.
5. Click  to go back to the main menu.

2.4. View Data

A. View Data by Gene

1. Click **View Data** button on the main menu or press **V** on the keyboard. A **Data Control Panel** will appear.
2. Click **View Data by Gene** button and the table **Gene** will appear.
3. Click "+" in the front of a gene ID, the "+" will change to a "-" and a list of plates containing this gene will append to the "-".
4. Click "+" in the front of a report file name, the "+" will change to a "-" and the details of that plate report will append to the "-".
5. Close the **Gene** table and go back to the **Data Control Panel**.

B. View Data by Sample

1. Click **View Data by Sample** button from the **Data Control Panel** or press **S** on the keyboard. A table **Sample** will appear.
2. Click "+" in the front of a sample name, the "+" will change to a "-" and a list of the details of this sample including a plate report containing the sample, wells containing the sample on that plate, and raw data (Ct, quantity, mean, etc) generated by the detector system about the sample will append to the "-".
3. Close the table **Sample** and go back to the **Data Control Panel**.

C. View Data by Plate

2. Click **View Data by Plate** button from the **Data Control Panel** or press **P** on the keyboard. A table **Plate** will appear.
3. Click "+" in the front of a plate ID, the "+" will change to a "-" and a list of plates with that plate ID will append to the "-".

4. Click “+” in the front of a report file name, the “+” will change to a “-” and the according report generated by the detector system will append to the “-”.
5. Close the table **Plate** and go back to the **Data Control Panel**.

D. View Data by Experiment

1. Click the **View Data by Experiment** button from the **Data Control Panel** or press **E** on the keyboard. A table **Experiment** will appear.
2. Click “+” in the front of an experiment ID, the “+” will change to a “-” and a list of plate reports related with that experiment append to the “-”.
3. Click “+” in the front of a report file name, the “+” will change to a “-” and the input report file generated by the detector system will append to the “-”.
4. Close all other panels and go back to the main **Menu**.

VITA

Keyu He

Candidate for the Degree of

Master of Science

Thesis: THE DATABASE IMPLEMENTATION AND ALGORITHM DESIGN OF QPCR-DAMS: A DATABASE TOOL TO ANALYZE, MANAGE, AND STORE QUANTITATIVE REAL-TIME PCR DATA

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Title of Study: THE DATABASE IMPLEMENTATION AND ALGORITHM DESIGN OF QPCR-DAMS: A DATABASE TOOL TO ANALYZE, MANAGE, AND STORE QUANTITATIVE REAL-TIME PCR DATA

Pages in Study: 73 Candidate for the Degree of Master of Science

Major Field: Computer Science

Scope and Method of Study:

Quantificative real-time PCR is an important technique for modern biomedical research. To obtain data by using quantificative real-time PCR is not difficult; on the contrary, to store, evaluate, process, and validate data is more difficult than obtaining of data. Some software has been developed for storing and processing PCR data. These software still have the data storage and data processing capacity problem. Further, none of these software can process relative quantification and absolute quantification data in a single software. Therefore, we developed a database tool that overcomes most of the disadvantages from previous software. Our purpose is to meet the requirements for real-time PCR users

Findings and Conclusions:

1. The qPCR_DAMS can store all the information related to experiments including gene name, plate name, plate-run data, researcher, and processed data, etc.
2. The qPCR_DAMS can process both relative and absolute quantification data with four basic models and two advanced modules (multiple reference gene normalization). These modules are: ratio relative quantification, absolute levels, normalized absolute expression, ratio absolute quantification, multiple reference relative quantification and multiple reference absolute quantification.
3. qPCR_DAMS can monitor data quality through three steps.
4. qPCR_DAMS can further improve the capacity for data validation by providing the data display function.

ADVISER'S APPROVAL: Dr. Johnson Thomas