

**qPCR-DAMS: a Database Tool to Analyze, Manage, and Store Both Relative
and Absolute Quantitative Real-Time PCR data**

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For Install, How to use, and details of qPCR-DAMS, see Users Manual

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Abstract

Quantitative real-time PCR is an important high throughput method in biomedical sciences. However, existing software has limitations in handling both relative and absolute quantification. We designed qPCR-DAMS (**Q**uantitative **P**CR **D**ata **A**nalysis and **M**anagement **S**ystem), a database tool based on Access 2003, to deal with such shortcomings by the addition of integrated mathematical procedures. qPCR-DAMA allows a user 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 is a handy tool for real-time PCR users.

Availability: This software is free for academic use and downloadable at

<http://www.cvm.okstate.edu/research/Facilities/LungBiologyLab/>

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Quantitative real-time PCR (qPCR) is becoming increasingly important in biomedical research because of its accuracy, sensitivity, and high efficiency (3). 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. Most software, such as REST (5), Q-gene (4), and CARTA (1), were designed to process relative quantitative real-time PCR data. Absolute quantification has been widely used in microbiological detection and molecular diagnosis, as well as the determination of relative gene expression (2). Some software, such as ABI 7500 system software, provides packages for both absolute and relative quantification, but lacks data storage capacity and has limited data processing functions. For an absolute quantitative method, it cannot perform inter-plate calculations and gives normalized expression. Furthermore, it cannot handle the standard curve method for relative quantification. The qPCR-DAMS software provides 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 reference gene normalization (6) for both relative and absolute quantification. This 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.

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 (also see Supplementary Materials: Users manual, 6) Conceptions and Mathematical Structures for detail). 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, 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 inter-plate data and then 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).

Another drawback of most current software is the limited data storage and sorting capacity. Most tools only store Ct (threshold cycle) and the processed data. Q-gene provides a better coverage for the whole experiment, but Q-gene is

implemented on Microsoft Excel, which lacks database function (2). qPCR-DAMS is implemented on a MS Access 2003 relational database management system based on Visual Basic. As shown in the database structure (Fig. 2), 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. 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. Efficient data tracking is also critical for the accuracy and validation of real-time PCR. qPCR-DAMS has a “View Data” function on the main panel so that 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.

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 .

The qPCR-DAMS runs with a Windows XP or Windows 2000 environment. This software is free for academic use and downloadable at:

<http://www.cvm.okstate.edu/research/Facilities/LungBiologyLab/>

Supplementary Information can be found in the same website.

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References

1. **Bonanomi A, Kojic D, Giger B, Rickenbach Z, Jean-Richard-Dit-Bressel L, Berger C, Niggli FK and Nadal D.** Quantitative cytokine gene expression in human tonsils at excision and during histoculture assessed by standardized and calibrated real-time PCR and novel data processing. *J Immunol Methods* 283: 27-43, 2003.
2. **Bustin SA.** Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *J Mol Endocrinol* 25: 169-193, 2000.
3. **Ginzinger DG.** Gene quantification using real-time quantitative PCR: an emerging technology hits the mainstream. *Exp Hematol* 30: 503-512, 2002.
4. **Muller PY, Janovjak H, Miserez AR and Dobbie Z.** Processing of gene expression data generated by quantitative real-time RT-PCR. *Biotechniques* 32: 1372-1379, 2002.
5. **Pfaffl MW, Horgan GW and Dempfle L.** Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic Acids Res* 30: e36, 2002.

6. **Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A and Speleman F.** Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* 3: RESEARCH0034, 2002.

Figure legends

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

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.

Fig. 1A

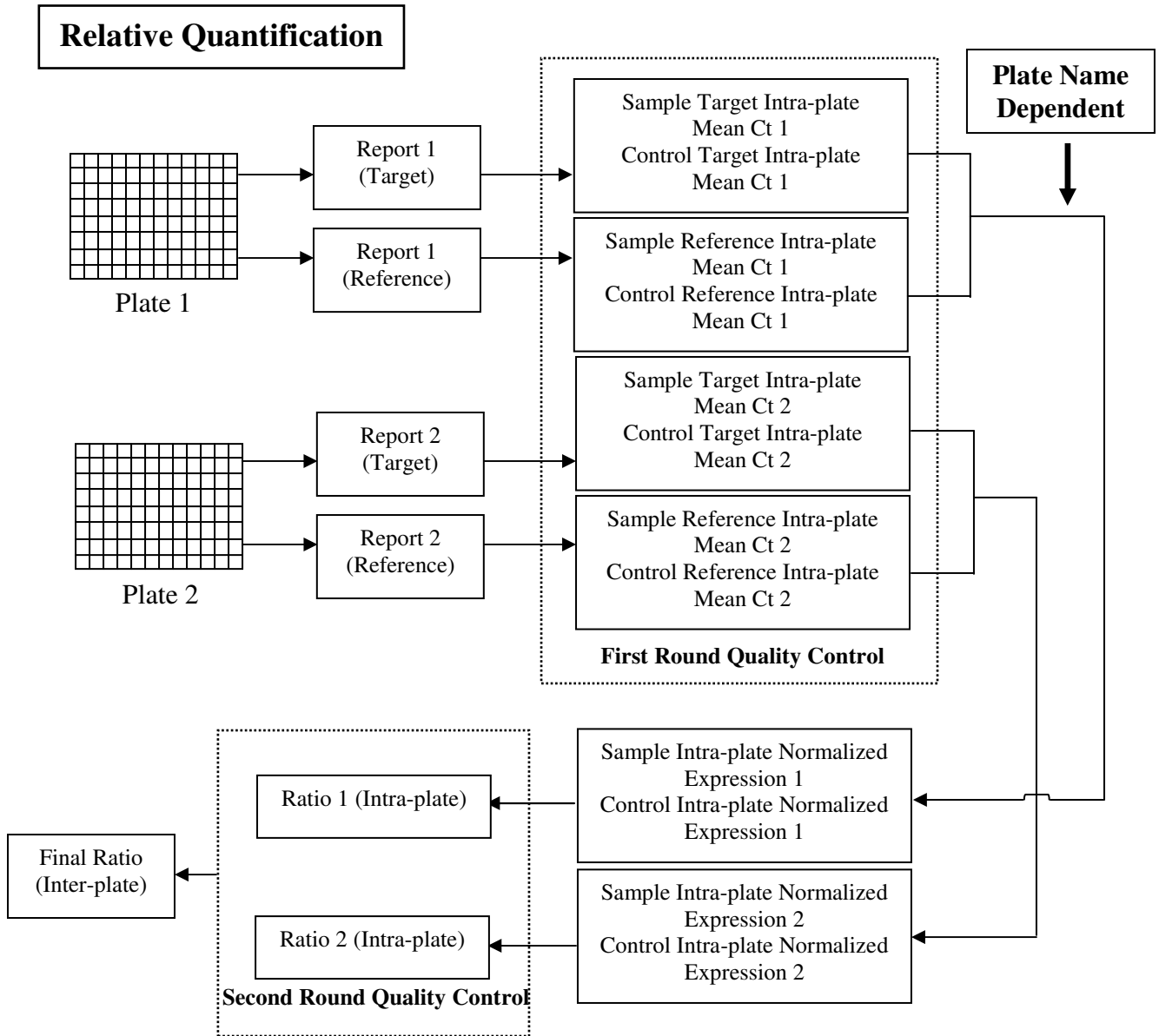


Fig. 1B

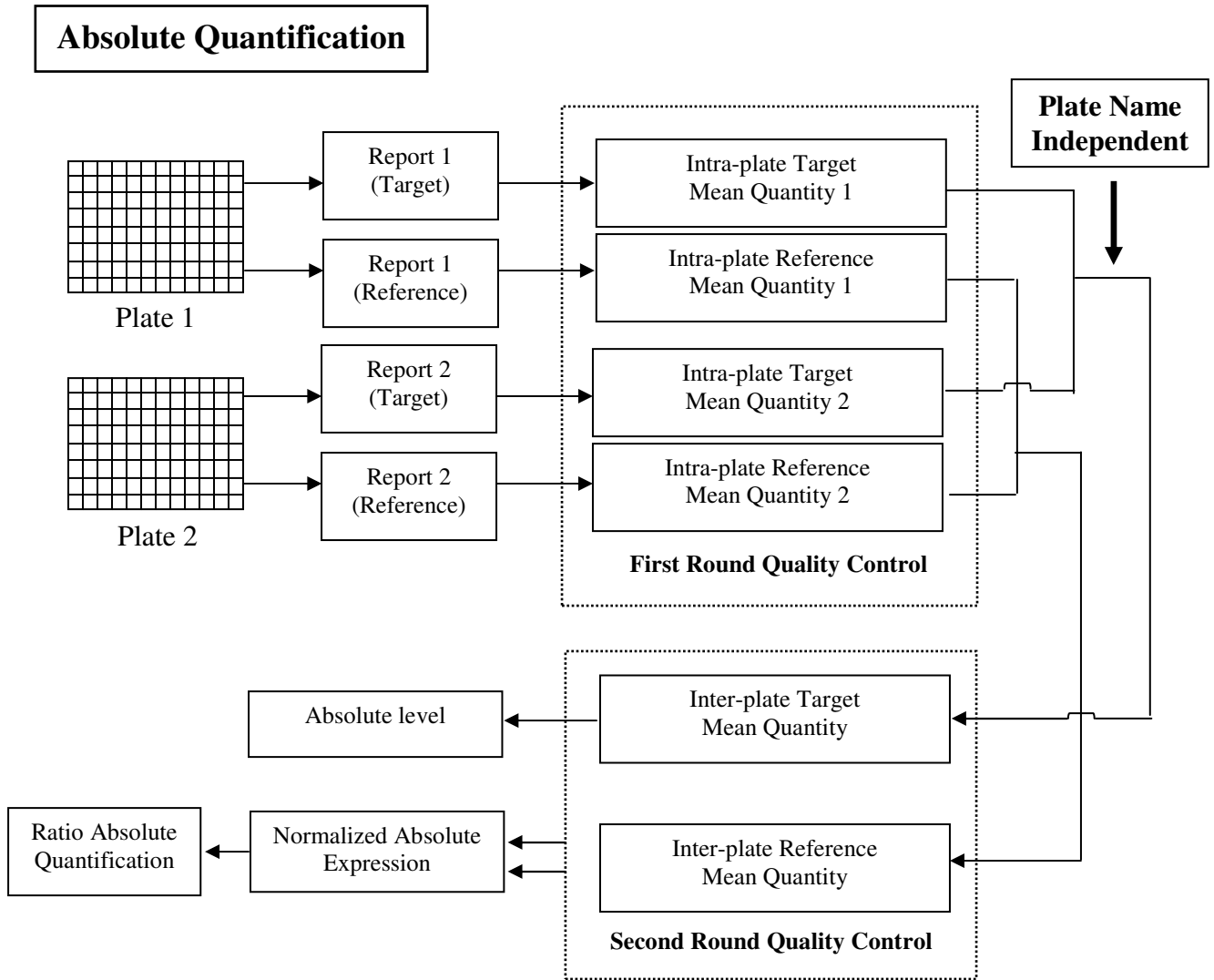


Fig. 2

