Short Note

qDATA Version 1.1 – Fold-Change Correction by Implementation of Jensen's Inequality

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Posted Date: 4 February 2026

doi: 10.20944/preprints202602.0172.v2

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qDATA Version 1.1 – Fold-Change Correction by Implementation of Jensen's Inequality

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Abstract

qDATA is an open-source R-based bioinformatics application designed to enable fast and reliable qRT-PCR data analysis within a robust and user-friendly graphical interface. The original software implementation is presented in detail elsewhere and the present paper describes qDATA v1.1 that contains new implementations for enhancing data analysis accuracy.

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1. Introduction

qDATA algorithm (https://github.com/DL-UB/qDATA) [1] relies on Livak's method for calculating the fold-change for a qRT-PCR raw cycle threshold (Ct) values dataset [2]. Although it can accommodate any number of biological replicates (BR) and technical replicates (TR), the software was designed bearing in mind the use of three BRs and three TRs. Although not a common practice, qDATA calculates all possible combinations of ΔCt values between the three TRs within a BR (27 ΔCt values), due to the specificities of a given biological sample. Furthermore, when calculating the ΔΔCt values, since each of the three BRs is independent, all possible combinations of ΔΔCt between experimental groups should be computed (729 ΔΔCt values).

The original algorithm uses each individual ΔΔCt value within the exponential function $2^{-\Delta\Delta Ct}$, then calculates the mean of these values, $\mu_2^{-\Delta\Delta Ct}$, in order to obtain the fold-change result. However, this method introduces upward bias in the final result, an issue that is solved by the new version of the software.

2. qDATA Version 1.1

The solution implemented in qDATA v1.1 is based on considering the Jensen's inequality [3] which states that for any strictly convex function f, the inequality

$$E[f(X)] \geq f(E[X]) \quad (1)$$

stands for an expected (E) statistic. Applied on analysis of qRT-PCR data using the Livak's method [2], E is the descriptive statistic of ΔΔCt dataset and $f(X)$ is the $2^{-\Delta\Delta Ct}$ function. The $2^{-\Delta\Delta Ct}$ function is always convex as it respects the inequality

$$f(\lambda x_1 + (1-\lambda) \cdot x_2) \leq \lambda f(x_1) + (1-\lambda) \cdot f(x_2) \quad (2)$$

for any x_1 and x_2 in the dataset and $\lambda \in [0,1]$ [3]. Thus, a descriptive statistic parameter (such as the mean) applied on the $2^{-\Delta\Delta Ct}$ dataset will be greater or equal than the value obtained when an average of the ΔΔCt dataset is used within the Livak's formula. The inequality can be visually represented by comparing a random normal distribution with the corresponding exponential function, providing a clear visual cue regarding the convex nature of the function and the possible differences between the $E[f(X)]$ and $f(E[X])$ (Figure 1).

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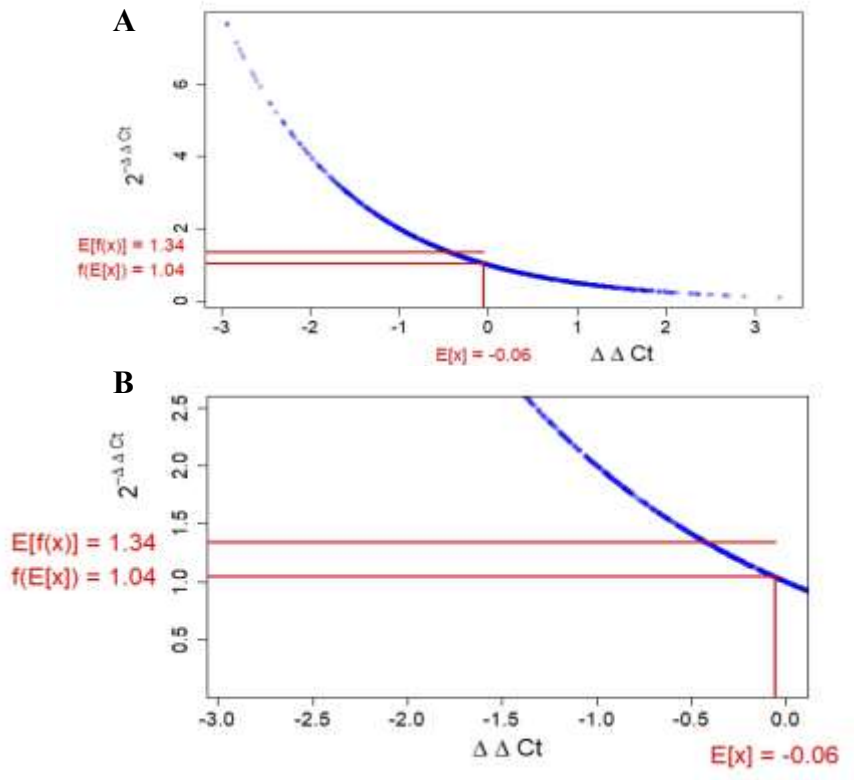

Figure 1. Visualization of the Jensen's inequality applied on $2^{-\Delta\Delta Ct}$ function of random ΔΔCt values. The figure presents the convex nature of $f(X)$ (A) and an enlarger section showing the inequality (B). Graph notations refer to mean dataset (X) value ($E[X]$) and $2^{-\Delta\Delta Ct}$ function ($f(X)$). The graph was generated using R.

Thus, considering that in the context of qRT-PCR the Ct and ΔΔCt values are on a $\log_2$ scale, judging the differences between the $E[f(X)]$ and $f(E[X])$ are actually equivalent to evaluating differences between the arithmetic and geometric mean FC. From both biologically and statistically standpoints, reporting the geometric mean is a more natural choice.

3. Conclusions

qDATA version 1.1 implements the bias correction revealed by Jensen's inequality in order to generate more accurate and meaningful fold-change results from a qRT-PCR raw dataset. The new software version is available on GitHub as release 1.1.

Funding: This research received no external funding.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: I acknowledge Denisa-Nicoleta Constantin for identifying unexpected results, which led to troubleshooting and creating the new software version, and Dr. Attila Cristian Ratiu for the technical review and proofread.

Conflicts of Interest: The author declare no conflict of interest.

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