

Short Note

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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.

Keywords: qDATA; data analysis; qRT-PCR; fold change

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 $\Delta\Delta Ct$ values, since each of the three BRs is independent, all possible combinations of $\Delta\Delta Ct$ between experimental groups should be computed (729 $\Delta\Delta Ct$ values).

The original algorithm uses each individual $\Delta\Delta Ct$ 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

$$E[f(X)] \geq f(E[X]) \tag{1}$$

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 $\Delta\Delta Ct$ 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

$$f(\lambda x_1 + (1-\lambda) \cdot x_2) \leq \lambda f(x_1) + (1-\lambda) \cdot f(x_2) \tag{2}$$

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 $\Delta\Delta Ct$ 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).

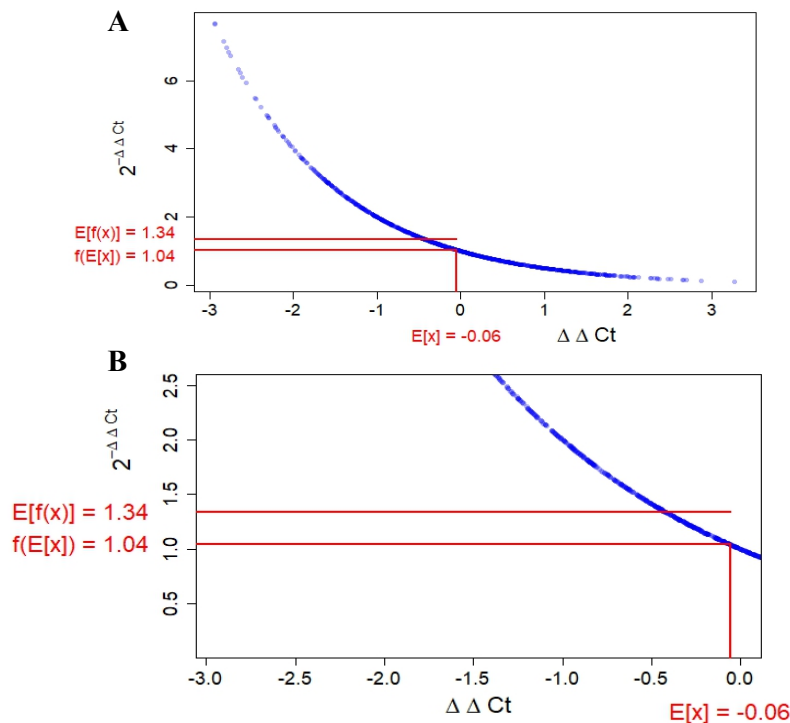


Figure 1. Visualization of the Jensen's inequality applied on $2^{-\Delta\Delta Ct}$ function of random $\Delta\Delta Ct$ values. The figure presents the convex nature of $f(X)$ (A) and an enlarged section showing the inequality (B). Graph notations refer to mean dataset (X) value ($E[X]$) and $2^{-\Delta\Delta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

Thus, considering that in the context of qRT-PCR the Ct and $\Delta\Delta Ct$ 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.

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