Click-qPCR: an ultra-simple tool for interactive qPCR data analysis

Authors

Azusa Kubota[1,*], Atsushi Tajima[1]

[1]Department of Bioinformatics and Genomics, Graduate School of Advanced Preventive Medical Sciences, Kanazawa University, Kanazawa, Japan

*Corresponding author:

Azusa Kubota, Master of Med. Sci.

Department of Bioinformatics and Genomics, Graduate School of Advanced Preventive Medical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8640, Japan

Fax: +81-76-234-4249; Phone: +81-76-265-2715

E-mail: kubotaexperiments@gmail.com

Abstract

Real-time quantitative PCR (qPCR) is a pivotal technique for gene expression and DNA copy number variation analysis. However, the limited availability of easy-to-use software tools remains a significant challenge for experimental biologists without programming expertise. To address this issue, we developed Click-qPCR, a user-friendly, web-based Shiny application that requires no local installation. Click-qPCR streamlines ΔCt and ΔΔCt calculations using user-uploaded CSV data files. The application allows users to dynamically select genes and groups, and perform Welch's t-tests for group comparisons. Results are immediately visualized as interactive bar plots (mean ± SD with individual data points) and can be downloaded as publication-quality images, along with statistical summaries. Click-qPCR enables researchers to efficiently process, interpret, and visualize their qPCR data regardless of programming skills, thereby accelerating data processing and improving the accessibility of routine analysis. **Availability & Implementation:** The Shiny application of Click-qPCR is freely available at https://kubo-azu.shinyapps.io/Click-qPCR/. The source code and user guide for Click-qPCR are openly available on GitHub at https://github.com/kubo-azu/Click-qPCR.

Subjects: Software and Workflows, Bioinformatics, Data Visualization

Background

Real-time quantitative PCR (qPCR) is a cornerstone experiment in molecular biology, offering a highly sensitive and widely adopted method for profiling gene expression levels [1]. Its application currently spans diverse research areas, ranging from basic biological investigations to clinical diagnostics. In addition to assessing mRNA expression, relative qPCR is a robust method for detecting DNA copy number variations (CNVs), making it a common and effective technique. Although qPCR encompasses both absolute and relative quantification strategies, relative quantification is more commonly used in practice. This preference largely stems from the inherent complexities and specific requirements of absolute quantification, which often limit its utility to specialized applications, such as virus quantification. The ΔΔCt method, which is common for relative quantification, robustly compares mRNA transcript levels and CNVs. This comparison relies on a two-step normalization: first against a stable reference gene, and then against a control or calibrator sample [2]. This normalization process is crucial for accurately interpreting biological changes in gene expression and CNV analysis.

Despite its widespread adoption and critical importance for relative gene expression and CNV analysis, the ΔΔCt method often presents significant hurdles for researchers, particularly those in wet-lab environments, in terms of accessing and utilizing appropriate analysis software. Many existing software solutions are proprietary and commercially licensed, platform-specific, or demand a level of computational proficiency and programming knowledge that can be a barrier for many experimental

biologists. For instance, spreadsheet-based qPCR analysis is often error-prone and time-consuming for multiple genes, while advanced statistical software can be unnecessarily complex for routine tasks.

Recognizing these limitations, we developed Click-qPCR. Unlike many existing solutions, which may require local software installation, command-line operations, or a foundational understanding of programming environments, Click-qPCR is a fully web-based application that requires no setup beyond a standard internet browser. This tool is designed to provide a user-friendly, intuitive, and readily accessible web-based platform specifically designed for the rapid and interactive analysis of qPCR data. Its focus on operational simplicity and immediate visual feedback makes Click-qPCR ideal for researchers, students, and technicians with limited bioinformatics experience.

Implementation

The Click-qPCR application was developed using the R programming language [3] and the Shiny framework [4] to provide an interactive web interface. It relies on several publicly available R packages from the CRAN repository for its functionality. The application is platform-independent and can be run on any operating system with a standard R installation. Alternatively, users are required to have the following packages installed, which can be obtained using install.packages():

- shiny: Provides the core framework for the interactive web application [4]
- dplyr: Utilized for data manipulation, filtering, and summarization operations [5]
- tidyr: Assists in reshaping and tidying datasets for analysis [6]
- ggplot2: Handles graphical rendering of bar plots and error bars [7]

- DT: Integrates dynamic, interactive HTML tables for summary output display [8]
- RColorBrewer: Used for selecting and applying color palettes to the plots [9]
- fontawesome: Enables the use of 'Font Awesome' icons within the user interface [10]

The application's modular design supports both ΔCt-based relative quantification and ΔΔCt analysis workflows. All components are reactive and update automatically in response to user input, enhancing the fluidity and intuitiveness of the analytical experience.

Input and User Interface

The overview of Click-qPCR is shown in Figure 1. Users upload a tidy CSV file containing four columns: sample, group, gene, and Ct (Figure 1A). For convenience, a downloadable CSV template and a one-click example dataset loader are provided. Upon uploading the file or loading an example dataset (Figure 1B), the interface guides users to select a reference gene, one or more target genes, and two experimental groups for comparison (Figure 1C). These selections are made through dynamically generated drop-down menus, ensuring that only valid options based on the uploaded data are available. A preview of the data is shown in the sidebar.

In addition to ΔCt analysis, the user interface includes a dedicated section for ΔΔCt-based fold-change comparison. The overview of ΔΔCt analysis section is shown in Figure 2. Users are prompted to specify a single target gene and define a control and treatment group (Figure 2B). Note that the reference gene is automatically selected depending on the main panel (Figure 2A). These inputs are also validated through context-sensitive selectors.

Data Processing

For the ΔCt analysis, the app computes the ΔCt value for each sample by subtracting the Ct value of the reference gene from that of each target gene. The relative expression ($2^{-\Delta Ct}$) is then calculated. For group-wise comparison, Welch's *t*-tests are performed for each target gene to assess statistical significance. Significance levels are categorized as follows: * for $p < 0.05$, ** for $p < 0.01$, *** for $p < 0.001$, and *ns* (not significant) otherwise.

In the ΔΔCt mode, the app first calculates the ΔCt value for each sample as described above, then computes the ΔΔCt value by subtracting the mean ΔCt value of the control group from the ΔCt value of each sample (both control and treatment groups). The fold-change relative to the control group is subsequently derived as $2^{-\Delta\Delta Ct}$. Group-wise comparisons of these fold-change values are visualized (mean ± SD), and statistical significance between the control and treatment groups is determined using Welch's *t*-test on the $2^{-\Delta\Delta Ct}$ values, with annotations similar to those in the ΔCt analysis.

Output

The Click-qPCR application provides comprehensive outputs for both ΔCt and ΔΔCt analysis modes. These outputs are designed to be readily usable for reports and publications. In the ΔCt analysis mode, users obtain several key outputs. A bar plot (Figure 1E) is generated to visualize the mean ± SD of relative expression values ($2^{-\Delta Ct}$) for each selected gene across the compared groups. This plot is

further annotated with significance brackets derived from Welch's *t*-tests, which are performed between the two groups for each target gene. Along with this visualization, a detailed summary table (Figure 1F) presents statistics for each gene and group, including the mean relative expression, the standard deviation (SD) of the mean, the sample size (N), the *p*-value from the *t*-test, and the corresponding significance symbol. Both the statistical summary (as a CSV file) and the plot (as a PNG image) can be downloaded using the dedicated buttons in the user interface (Figure 1D).

For the ΔΔCt analysis mode, analogous outputs are generated to facilitate the interpretation of fold-change. A bar plot (Figure 2D) displays the mean ± SD of fold-change values ($2^{-\Delta\Delta Ct}$) for the selected target gene, comparing the treatment group to the control group; this plot is complete with significance brackets based on Welch's *t*-test performed on the $2^{-\Delta\Delta Ct}$ values. A corresponding summary table (Figure 2E) details statistics for each group, including the mean fold-change, the standard deviation (SD) of the mean, the sample size (N), and the standard error (SE) of the mean, when applicable. The table also includes the *p*-value and significance symbol for the comparison against the control group. Both this statistical summary (as a CSV file) and the fold-change plot (as a PNG image) can also be conveniently downloaded using the dedicated buttons in the user interface (Figure 2C).

In both analysis modes, all plots are generated using the ggplot2 package to ensure high-quality graphics. The application interface incorporates custom HTML/CSS enhancements for an improved user experience. Client-side JavaScript is used to manage timestamps for downloadable file naming, aiding in record-keeping and organization.

Reproducibility

An example dataset is embedded within the app and can be loaded with a single button click. Users may also download a template CSV file to format their own data. The app can be deployed locally or hosted on any standard Shiny server. Time-stamped file downloads support reproducible research practices, and all analysis steps are transparent to users.

Use Case

Click-qPCR is designed to streamline relative quantification analysis for researchers working with qPCR data, particularly those without a strong background in programming or statistics. The tool is intended for diverse user groups including:

- Experimental biologists conducting small- to medium-scale gene expression studies
- Technical staff responsible for routine data analysis in core facilities
- Undergraduate and graduate students learning about qPCR and statistical evaluation
- Collaborative research groups where ease of data sharing and reproducibility are essential

Limitations

While Click-qPCR provides an accessible and efficient platform for relative quantification analysis, several limitations should be noted:

- Assumption of normality: The app performs Welch's *t*-tests without explicitly testing for equal variances. Users should confirm that their data meets the assumption or consider non-parametric alternatives externally if needed.
- Lack of advanced normalization: Only single-reference gene normalization is supported. Analysis methods requiring multiple reference genes, such as geNorm [11], Normfinder [12], and BestKeeper [13], must be conducted outside this tool.
- Simplified statistical options: The app offers no correction for multiple comparisons (e.g., Bonferroni correction or FDR), which may be relevant when analyzing many target genes simultaneously.
- No support for technical replicates handling: The input format assumes that users have averaged technical replicates. Raw Ct values across technical replicates must be prepared in advance.
- Limited customization of plots: While the output figures are publication-quality, customization options (e.g., font sizes, color palettes, and axis scaling) are more limited than they would be with manual scripting in R.

These constraints reflect the app's design goals of simplicity and accessibility. Advanced users may choose to export intermediate results for further analysis using standard R workflows.

Concluding Remarks

Click-qPCR provides a user-friendly and accessible platform for the rapid and interactive analysis of qPCR data. It offers a crucial advantage over tools that necessitate programming skills or complex

local installations because it is immediately available through a web browser. The app focuses on ease of use and rapid visualization, making it suitable for researchers, students, and technical staff with limited bioinformatics experience. By simplifying common calculation and visualization workflows, it empowers users, regardless of their programming expertise, to efficiently process and interpret their experimental results. Click-qPCR will be a valuable addition to the molecular biology toolkit, facilitating quicker data turnaround and a more intuitive understanding of gene expression patterns and CNV analyses.

Availability of Supporting Source Code and Requirements

- Project name: Click-qPCR
- Project home page: https://kubo-azu.shinyapps.io/Click-qPCR/ (Shiny); https://github.com/kubo-azu/Click-qPCR (R source code)
- Operating System(s): Platform Independent
- Programming language: R
- License: MIT

Data Availability

The template CSV file for Click-qPCR can be found at https://github.com/kubo-azu/Click-qPCR/blob/main/example.csv.

Declarations

List of Abbreviations

PCR: polymerase chain reaction. qPCR: quantitative PCR. mRNA: messenger RNA. CNV: copy number variation. Ct: threshold cycle. SD: standard deviation. SE: standard error. FDR: false discovery rate.

Ethics approval

The authors declare that ethical approval was not required for this type of research.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Writing - original draft: AK. Writing - review and Editing: AT. Conceptualization: AK and AT. Software: AK. Funding Acquisition: AK. Supervision: AT.

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242 ## Figure legends

243 **Figure 1. Views of the main panel and ΔCt analysis section.**

244 **A.** Here is the guide section on how to prepare the data for upload. Users can try Click-qPCR by
245 clicking the "Use Example Data" button. Demo data is provided by this tool. Also, a template CSV
246 file is available to download by clicking the "Download Template CSV" button. **B.** Users can upload
247 a CSV file by clicking the "Browse…" button. **C.** Users select a reference gene and target genes for
248 data analysis. Two group labels are chosen for statistical testing. Analysis and visualization are started
249 by clicking the "Analyze" button. **D.** Users can download the analytical results as a CSV file and the
250 plot figure as a PNG file. **E.** Bar plots are shown in this section. **F.** The statistical summary is shown
251 in this section. This table contains the same data that users can download as a CSV file.

252

253 **Figure 2. Views of the ΔΔCt analysis panel and its result section.**

254 **A.** The reference gene is shown for the ΔΔCt analysis. This is automatically selected from the main
255 panel. **B.** Users select a target gene for the ΔΔCt analysis. Two group labels are chosen to calculate
256 the fold-change value. Analysis and visualization are started by clicking the "Run ΔΔCt Analysis"
257 button. **C.** Users can download the analytical results as a CSV file and the plot figure as a PNG file.
258 **D.** Bar plots are shown in this section. **E.** The statistical summary is shown in this section. This table
259 contains the same data that users can download as a CSV file.

Figure 1.

A

Prepare your data as a CSV file with the following four columns:
sample (sample identifier), **group** (e.g., treatment or condition), **gene** (gene name), and **Ct** (threshold cycle value).
Each row should represent the Ct value of one gene in one sample.
Hint: If you have several Ct values as the technical replicates, please use the mean value to analyze with this tool.

Use Example Data | Download Template CSV

B **Upload CSV File**

Browse... No file selected

Uploaded Data Preview:

sample	group	gene	Ct
Mouse_A	Treatment_X	Hoge	25.58
Mouse_A	Treatment_X	Fuga	27.23
Mouse_A	Treatment_X	Gapdh	20.27
Mouse_B	Treatment_Y	Hoge	26.21
Mouse_B	Treatment_Y	Fuga	27.65
Mouse_B	Treatment_Y	Gapdh	20.83
Mouse_C	Treatment_X	Hoge	25.79
Mouse_C	Treatment_X	Fuga	27.39
Mouse_C	Treatment_X	Gapdh	20.34

C **Select Reference Gene:**

Gapdh

Select Target Gene(s):

Hoge Fuga

Select Group 1 [usually, control]:

Control_X

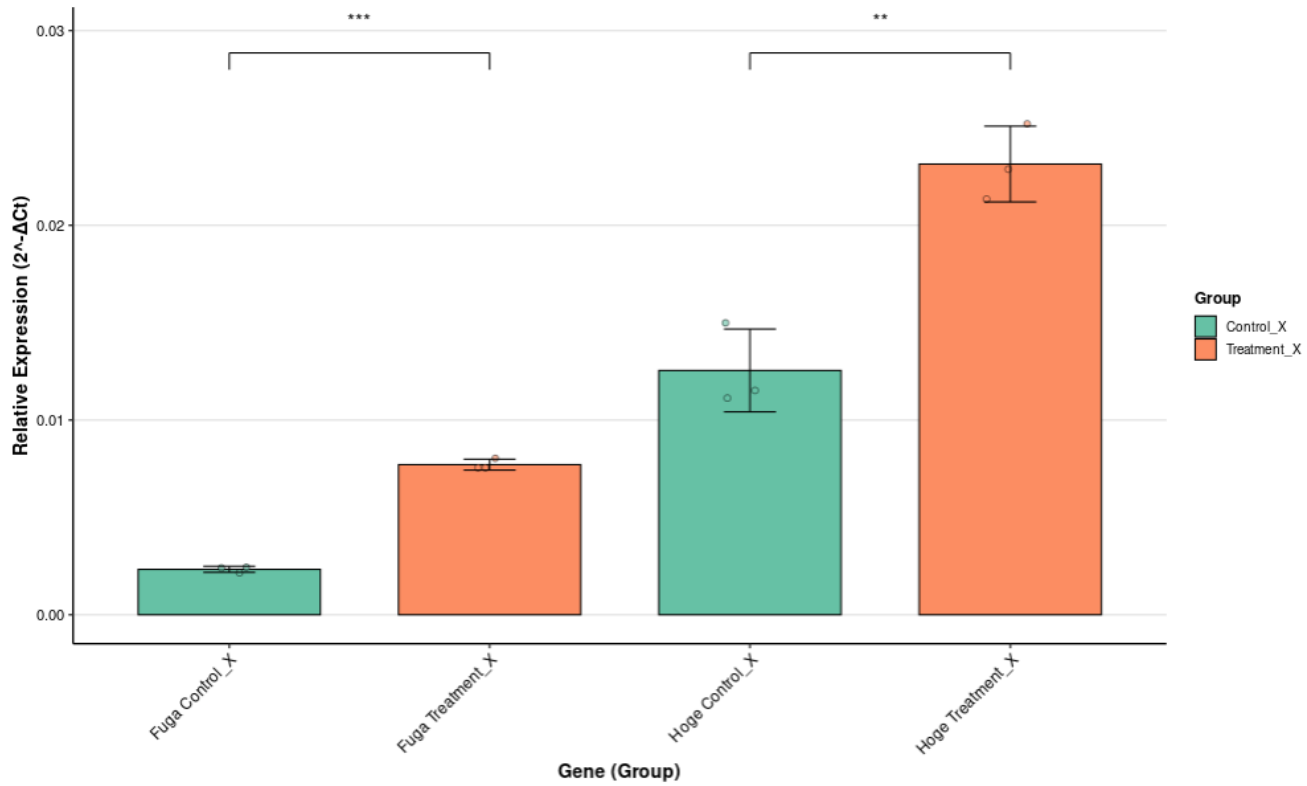
Select Group 2 [usually, treatment]:

Treatment_X

Analyze

D Download Stats (CSV) | Download Plot (PNG)

E **Analysis Plot:**



F **Statistical Summary Table:**

Show 10 entries Search:

gene	group	Mean	SD	N	label	p (Welch's t-test)	sig
Fuga	Control_X	0.002331627655028086	0.0001563284444648462	3	Fuga Control_X	0.0000649241525626705	***
Fuga	Treatment_X	0.007708298136733746	0.0002804546488488827	3	Fuga Treatment_X	0.0000649241525626705	***
Hoge	Control_X	0.01254387409254872	0.002126178640801588	3	Hoge Control_X	0.003198190329898205	**
Hoge	Treatment_X	0.02314275766220978	0.001945319076666127	3	Hoge Treatment_X	0.003198190329898205	**

Showing 1 to 4 of 4 entries Previous 1 Next

Figure 2.

A ΔΔCt Analysis Settings:

Reference Gene (from main analysis):

Gapdh

B **Target Gene:**

Hoge

Select Control Group to Calculate Fold-Change:

Control_X

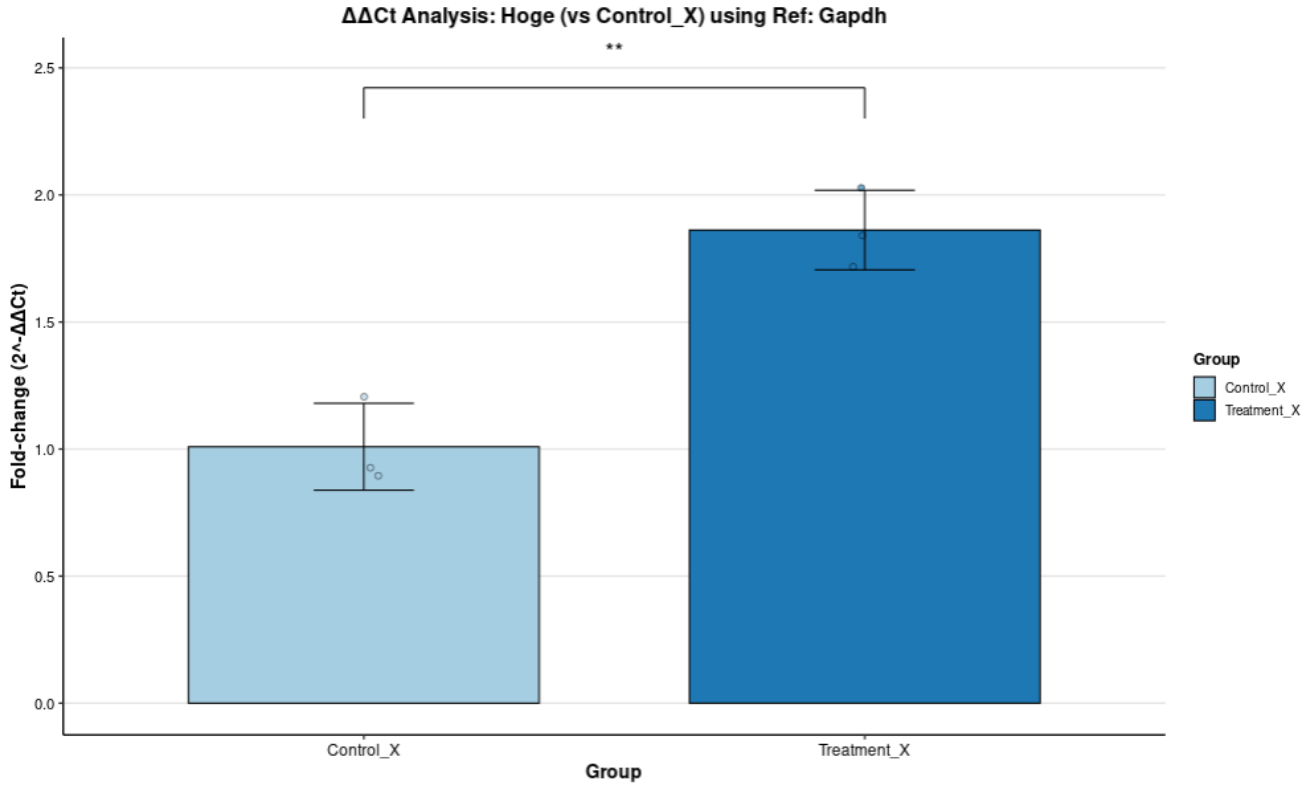
Select Target Group to Calculate Fold-Change:

Treatment_X

Run ΔΔCt Analysis

C Download ΔΔCt Stats (CSV) | Download ΔΔCt Plot (PNG)

D ΔΔCt Plot:



E ΔΔCt Table:

Show 10 entries

Search:

group	Mean_FoldChange	SD_FoldChange	N	SE_FoldChange	p-value (vs Control)	Significance
Control_X	1.009140320169701	0.1710486392390551	3	0.09875497790918762		
Treatment_X	1.861808377901772	0.1564986942132335	3	0.09035456323183531	0.0031981903298982	**

Showing 1 to 2 of 2 entries

Previous 1 Next