

ProntoPCR: Efficient qPCR Data Analysis Software

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Software

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Summary

Quantitative Polymerase Chain Reaction (qPCR) is an essential technique used to amplify and quantify the amount of DNA, gene or transcript in a sample by measuring the quantification cycle (Cq) threshold. qPCR is widely used in molecular biology, genetics, and diagnostics, and involves a standardised and routine analysis process. Inherently, this means qPCR analysis is repetitive and time-consuming, especially for large data sets. Therefore, an intuitive and versatile software application would be beneficial to automate and fast-track the analysis for a variety of disciplines. ProntoPCR is a software application that serves this purpose, aiming to efficiently and automatically perform routine qPCR calculations, such as averaging the housekeeper genes, calculating ΔCq , $\Delta\Delta Cq$, relative fold-change ($2^{-\Delta Cq}$), and fold-change ($2^{-\Delta\Delta Cq}$) of the target gene. It also provides the user with options to perform statistics and graphing of the data.

Statement of Need

Methods of quantifying relative gene expression through qPCR have remained largely unchanged since its implementation in 2001 ([Livak & Schmittgen, 2001](#)). These calculations rely on raw quantification cycle (Cq) results from a qPCR reaction, which can then be normalised in various manners (Table 1). These methods, however, are usually performed manually in programs such as Excel. Although this quantification is relatively simple, there are caveats to manual analysis such as being time consuming and prone to human or data entry errors. There is a growing need to automate these tasks, especially for handling large volumes of data. Presenting data in graphical formats is also a laborious task for many samples and targets. Therefore, we developed an application called ProntoPCR to address these issues by seamlessly semi-automating the calculations and analysis of qPCR data. A user can go from raw data to results, including statistics and graphs in just a few clicks.

Table 1: Description of terms and formulas used in qPCR calculations within ProntoPCR.

Term	ProntoPCR Alias	Formula/Explanation
Housekeeper Average	mean_hk	= Mean(Housekeeper Gene 1, ..., Housekeeper Gene n)
ΔCq (Delta Cq)	dcq_gene	= Cq (Gene of Interest) - Cq (Housekeeper Average)
$2^{-\Delta Cq}$ (Relative Fold Change)	fc_dcq_gene	= $2^{-\Delta Cq}$
ΔCq Control Average	dcq_ctrl_avg	The average ΔCq value for the control group for the selected gene. Used for $\Delta\Delta Cq$ calculations

Term	ProntoPCR Alias	Formula/Explanation
$\Delta\Delta Cq$ (Delta Delta Cq)	ddcq_gene	$= \Delta Cq$ (Gene of Interest) - ΔCq (Control Average)
$2^{-\Delta\Delta Cq}$ (Fold Change)	fc_ddcq_gene	$= 2^{-\Delta\Delta Cq}$

31 State of the Field

32 Several tools have been developed to support qPCR analysis across different research contexts
 33 and levels of technical expertise. Numerous established R packages support ΔCq and $\Delta\Delta Cq$
 34 workflows within a coding environment, including the CRAN/Bioconductor packages *tidyqpcr*,
 35 *qPCRtools*, *pqr* and *HTqPCR* (Ahmed & Kim, 2018; Dvinge & Bertone, 2009; Li et al., 2022;
 36 Wallace & Haynes, 2022). The *qpcR* package also focuses on amplification curve modelling
 37 and efficiency estimation (Ritz & Spiess, 2008). While these packages provide flexibility and
 38 PCR analysis support, they are primarily designed for scripted workflows and thus require
 39 familiarity with R programming.

40 Graphical applications with point-and-click interfaces have also been developed. For example,
 41 *shinyCurves* provides an interface for amplification curve visualisation and Cq extraction within
 42 viral detection contexts (Olaechea-Lázaro et al., 2021). The *SATqPCR* web application
 43 further extends this by implementing MIQE-guided analysis, incorporating efficiency correction
 44 and geNorm-based reference gene selection within a browser-based interface (Rancurel et al.,
 45 2019). *FaDA* is a broader laboratory data analysis platform that supports qPCR, amongst
 46 other assay types (Danger et al., 2021). General online relative gene expression calculators
 47 also exist. Although these applications improve accessibility, they focus on specific tasks
 48 rather than offering a single, integrated workflow for routine relative gene expression. *Qupid*
 49 is a Python-based application built using Streamlit that provides a local browser-rendered
 50 interface for $\Delta\Delta Ct$ analysis. It supports flexible data structures through the use of metadata
 51 “decorators,” which are text markers inserted within input files to designate assays, normalisers,
 52 and replicate groups (Kleinschmidt, 2023). In contrast, ProntoPCR is structured around
 53 commonly exported instrument formats and guided user selections, reducing the need for
 54 manual file modification while integrating normalisation, statistical testing, and graphical
 55 output within a single workflow.

56 Overview

57 ProntoPCR is an open-source, R shiny (Chang et al., 2021) software application available
 58 both online and locally as an R package (R Core Team, 2013), making it accessible to users
 59 with minimal programming experience. It is also designed to be compatible with the output
 60 generated by PCR machines, such as the Quantstudio 5 (Thermo Fisher Scientific) enabling
 61 the user to get instant results. Users only require a comma separated values (CSV) file of the
 62 raw data, including the quantification cycle (Cq) values, the target (gene) and sample name
 63 to get started. The app layout is split into multiple tabs including the About, Input Data,
 64 Calculations (Fig. 1A), Statistics (Fig. 1B), and Graphs (Fig. 1C) tabs.

65 Accessing and Using ProntoPCR

66 ProntoPCR has been designed to operate both online and locally. Whilst we aim to maintain
 67 online availability, the hosted platform may change or become unavailable. Therefore, it is
 68 recommended to rely on the local version, which functions with the same features as the online
 69 option. The local version also does not require internet access once installed. To run the
 70 application locally, the user needs to download R and RStudio. A step-by-step guide on how
 71 to install and use ProntoPCR is available: [ProntoPCR Handbook](#). The guide is aimed at users

72 with minimal programming expertise, making ProntoPCR accessible to all researchers. An
73 example dataset can be downloaded directly from the application. The online application is
74 available [here](#).

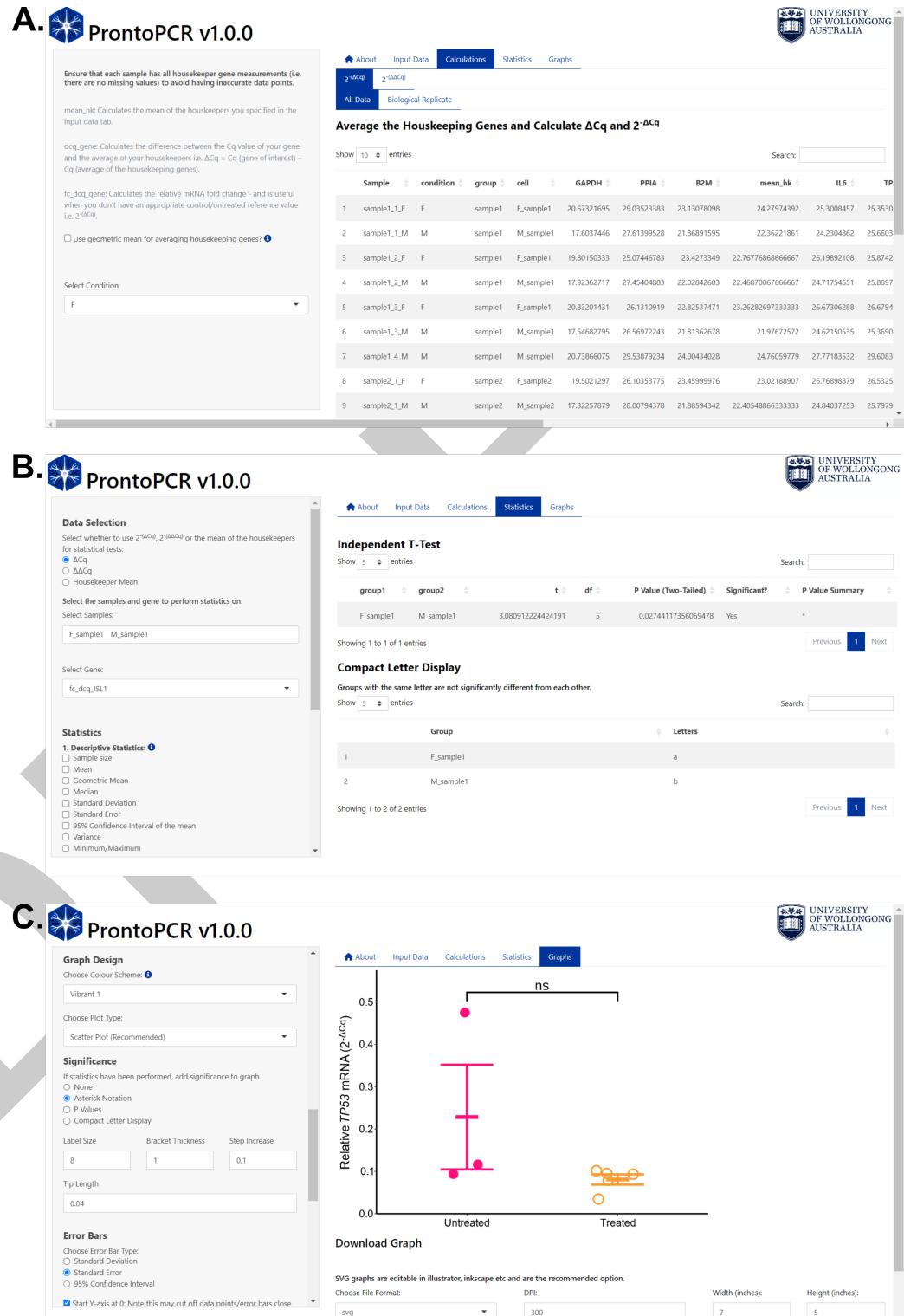


Figure 1: Overview of ProntoPCR graphical user interface including A) Calculations, B) Statistics, and C) Graphs tabs.

75 Requirements and Limitations

76 The main requirement for ProntoPCR to run is a correctly formatted .csv file, including the
77 sample names. If the inserted .csv file does not meet the requirements, a variety of error
78 messages can prompt the user to alter the .csv file until it is in the correct format (see [user](#)
79 [guide](#)). The application also does not include exhaustive options for statistics and graphing;
80 however, users can download the results of each calculation, which can be fed into their own
81 analysis pipelines.

82 Assumptions

83 ProntoPCR implements the Livak and Schmittgen $2^{-\Delta\Delta Cq}$ method for relative quantification
84 by default ([Livak & Schmittgen, 2001](#)). This approach assumes that amplification efficiencies
85 are between 90 - 110% and approximately equal between the target and reference (housekeeper)
86 genes. If amplification efficiencies differ substantially, efficiency-corrected models such as the
87 Pfaffl method ([Pfaffl, 2001](#)) should alternatively be used. Users are therefore encouraged to
88 experimentally validate primer efficiencies prior to using the ProntoPCR workflow.

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