qPCR Guru

User Guide

Complete reference for the qPCR Guru analysis platform

BETA Release • 2026

ΔΔCq & Pfaffl relative quantification

MIQE 2.0 compliance checking

geNorm & NormFinder reference gene validation

PCA, volcano plots, heatmaps, and more

Publication-ready figure export

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1. Getting Started

1.1 Supported File Formats

qPCR Guru accepts data exported from virtually any qPCR instrument. Simply export your results to one of the following formats and upload:

Format	Extension	Notes
Microsoft Excel	.xlsx, .xls	Most common; exported from Bio-Rad CFX, Applied Biosystems QuantStudio, Roche LightCycler, etc.
Comma-Separated Values	.csv	Universal text format; works with all instruments
Tab-Separated Values	.tsv	Alternative text format
RDML	.rdml	Standardized qPCR data exchange format (ZIP-wrapped XML)

1.2 Uploading Your Data

From the upload screen, you can drag and drop your file onto the upload area, or click **Browse Files** to select from your computer. qPCR Guru will automatically detect the file format, identify the data sheet, and parse sample names, target names, and Cq values.

Tip: If your file contains multiple sheets, qPCR Guru will look for sheets named 'Results', 'Quantification', 'End Point Results', or similar standard names. If no matching sheet is found, it will try each sheet until it finds valid qPCR data.

1.3 Understanding the Interface

After uploading, the interface has two main regions:

- **Left panel:** Analysis controls including reference gene selection, calibrator sample, chart options, statistical tests, and export tools.
- **Main area:** Tabbed views for QC Dashboard, Amplification Curves, Melt Curves, and the Analysis tab (charts, tables, visualizations).

Navigation tabs across the top let you switch between views. Each tab becomes active once the relevant data is available.

2. QC Dashboard

The QC Dashboard provides an at-a-glance quality assessment of every sample-target combination in your dataset. Each row shows the sample, target, number of valid replicates, mean Cq, standard deviation, coefficient of variation (CV%), and a pass/fail flag.

2.1 QC Metrics Explained

Metric	Description	Pass Criteria
Mean Cq	Average quantification cycle across replicates	Must be a valid number
SD	Standard deviation of Cq values within replicates	≤ 0.5 Cq (default threshold)
CV%	Coefficient of variation: (SD / Mean) × 100	Lower is better; flags high variability
N Valid	Number of replicates with valid Cq values	≥ 2 replicates recommended

2.2 NTC Validation

No-Template Controls (NTCs) should show no amplification or very late amplification. qPCR Guru flags NTC wells that amplify before cycle 35 (default), indicating potential contamination. The QC Dashboard highlights any NTC issues with a warning.

2.3 Replicate Consistency

Replicates with a standard deviation exceeding the threshold (default: 0.5 Cq) are flagged. You can optionally exclude QC-failing groups from downstream analysis using the **Exclude QC fails** toggle in the analysis settings.

2.4 Outlier Detection

When 3 or more replicates are present, qPCR Guru identifies statistical outliers using the Grubbs test. Outlier wells are flagged but not automatically removed, giving you full control over your data.

3. MIQE 2.0 Compliance

3.1 What Is MIQE?

The Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines provide standards for qPCR experimental design and reporting. qPCR Guru evaluates your data against MIQE 2.0 (Bustin et al., Clinical Chemistry, 2025) essential criteria and displays a real-time compliance score.

3.2 Compliance Checklist

The MIQE panel checks the following criteria automatically:

- **NTC validation:** Are no-template controls present and clean?
- **Replicate consistency:** Do replicates have acceptable SD?
- **Reference gene stability:** Are reference genes stable (geNorm M, NormFinder ρ)?
- **Multiple reference genes:** Are at least 2 reference genes used?
- **Efficiency correction:** Is Pfaffl efficiency correction enabled?
- **Outlier handling:** Are outliers identified?

3.3 Scoring

Each criterion scores as **Pass**, **Warning**, or **Fail**. The overall compliance percentage reflects how many essential criteria your data meets. This score is included in generated PDF reports for publication transparency.

Note: MIQE compliance scoring reflects data-level checks only. Experimental design criteria (RNA quality, primer design, etc.) must be evaluated separately.

4. Setting Up Your Analysis

4.1 Choosing Reference Genes

Select your primary reference (housekeeping) gene from the dropdown in the left panel. This gene is used for ΔCq normalization. qPCR Guru auto-detects common reference genes (GAPDH, ACTB, 18S, etc.) and pre-selects one, but you should verify this matches your experimental design.

Tip: Using multiple reference genes improves normalization accuracy. Add additional reference genes in the **Reference Gene Validation** section. The geometric mean of all selected reference genes is used for normalization.

4.2 Calibrator Sample

The calibrator (control) sample is the baseline for ΔΔCq calculation. Typically this is your untreated control, time zero, or wild-type sample. The RQ of the calibrator is set to 1.0, and all other samples are expressed relative to it.

4.3 Sample Groups

For experiments with biological replicates, use **Sample Groups** to merge individual samples into groups. For example, group 'Control_1', 'Control_2', 'Control_3' into a single 'Control' group. This pools replicate data for statistical comparisons and simplifies chart presentation.

Note: Sample groups should represent **biological replicates**, not technical replicates. Technical replicates (same sample, same plate) are already averaged in the QC step.

4.4 Pfaffl Efficiency Correction

When primer efficiency data is available, enable **Pfaffl method** in the Primer Efficiency section. Enter the measured efficiency for each target gene (from standard curves). The Pfaffl method uses per-gene efficiencies rather than assuming 100% efficiency, producing more accurate RQ values when efficiencies differ between genes.

4.5 Multiple Reference Genes

Add additional reference genes in the Reference Gene Validation section. When multiple reference genes are selected, normalization uses the **geometric mean** of their Cq values (Vandesompele et al., 2002), which is more robust than using a single gene.

5. Understanding Your Results

5.1 The Data Table

The results table shows one row per sample-target combination with the following columns:

Column	Description
Sample	Sample or group name
Target	Target gene name
Mean Cq	Average Cq value across replicates
ΔCq	Cq(target) – Cq(reference): normalization to reference gene(s)
ΔΔCq	ΔCq(sample) – ΔCq(calibrator): relative to calibrator
RQ	Relative Quantification: 2^(–ΔΔCq) or Pfaffl ratio
RQ Min / Max	Error range based on propagated SD
p-value	Statistical significance vs. calibrator (when enabled)

5.2 Data Display Modes

Use the **Chart Data** selector to switch between:

- **RQ (Relative Quantification):** Fold change relative to calibrator. Default view.
- **ΔCq:** Normalized Cq difference. Useful for comparing raw expression levels.
- **Cq:** Raw mean Cq values. Useful for checking raw data distribution.
- **Copy Number:** Absolute quantification from standard curves (when available).

5.3 Interpreting Fold Change

An RQ of 1.0 means expression is the same as the calibrator. RQ > 1 indicates upregulation; RQ < 1 indicates downregulation. Common interpretation thresholds:

RQ Value	Interpretation
> 2.0	Upregulated (≥ 2-fold increase)
1.5 – 2.0	Mildly upregulated
0.67 – 1.5	No meaningful change
0.5 – 0.67	Mildly downregulated
< 0.5	Downregulated (≥ 2-fold decrease)

6. Charts & Visualization

6.1 Bar Charts

The main visualization is a bar chart showing RQ (or other data type) per sample-target combination. Bars are drawn from the baseline (0 for linear, 1 for log scale), with error bars representing propagated uncertainty.

6.2 Grouping

Charts can be grouped **by sample** (targets as bars within each sample group) or **by target** (samples as bars within each target group). Toggle this in Chart Options to find the view that best tells your story.

6.3 Error Bars

Three error bar modes are available:

- **Asymmetric (default):** Shows RQ Min and RQ Max directly. Reflects the true asymmetric distribution of 2^(−ΔΔCq).
- **Symmetric:** Uses the average of upper and lower bounds. Simpler visually, but less precise.
- **None:** Hides error bars entirely.

6.4 Overlay Options

Overlay individual data points on bars to show the underlying distribution:

- **Individual Points:** Jittered dots for each biological replicate.
- **Violin Plot:** Kernel density estimation showing data distribution shape.
- **Bean Plot:** Combination of individual points with density outline.
- **Box & Whisker:** Standard box plot showing median, quartiles, and whiskers.

6.5 Faceted Panels

Use the **Facet** option to split your chart into one panel per target or per sample. This is especially useful for experiments with many targets or samples. You can choose independent or shared Y-axes across panels.

6.6 Customization

Extensive chart customization is available:

- **Fonts:** Choose font family and size for all chart text.
- **Colors:** Custom colors per sample or target using hex color pickers.
- **Labels:** Custom X and Y axis labels, with bold/italic toggles.
- **Log scale:** Toggle $\log_2$ scale for fold-change visualization.
- **Label angle:** Rotate X-axis labels from 0° to 90° for long names.

7. Advanced Visualizations

7.1 PCA Plot

Principal Component Analysis (PCA) reduces your multi-gene expression data to 2–3 principal components, revealing clustering patterns among samples. Each point represents a sample, positioned by its overall expression profile. Samples that cluster together have similar gene expression patterns.

Use the axis selectors to view different PC combinations (PC1 vs PC2, PC1 vs PC3, etc.). The variance explained percentage indicates how much of the total variation each component captures.

7.2 Volcano Plot

The volcano plot shows statistical significance ($-\log_{10}$ p-value) vs. magnitude of change ($\log_2$ fold change) for each target gene. Genes in the upper corners are both significantly changed and biologically meaningful. Red dots indicate upregulated genes (FC ≥ 2, $p < 0.05$), blue dots indicate downregulated genes, and gray dots are not significant.

7.3 Correlation Scatter

The correlation plot shows the relationship between two selected samples or two selected targets. Each point represents a gene (sample mode) or sample (target mode). The R^2 value and linear regression line indicate the strength of correlation.

7.4 RQ Heatmap

The heatmap displays all RQ values as a color-coded matrix with samples on one axis and targets on the other. Optional hierarchical clustering (UPGMA algorithm with Euclidean distance) reorders rows and/or columns to group similar expression profiles together. Dendrograms show the clustering hierarchy.

Color scales available: diverging (blue-white-red centered on RQ=1) or linear (white to dark).

8. Statistical Analysis

8.1 Student's t-test

Two-sample t-tests compare each sample against the calibrator for each target gene. The test is performed on ΔΔCq values (log scale), producing a p-value indicating whether the expression difference is statistically significant. Results are shown in the data table and optionally as significance stars on charts.

8.2 Mann-Whitney U Test

A non-parametric alternative to the t-test, useful when data may not be normally distributed or when sample sizes are small. Select Mann-Whitney U in the Statistical Tests section to use this instead of the t-test.

8.3 One-Way ANOVA & Tukey HSD

When your experiment has 3 or more groups, one-way ANOVA tests whether any group differs significantly. If the ANOVA p-value is significant, Tukey's Honest Significant Difference (HSD) post-hoc test identifies which specific pairs of groups differ. Results appear in a collapsible summary panel below the chart.

8.4 Significance Stars & Brackets

Enable **Show significance** in Chart Options to display stars above bars:

Symbol	Meaning
*	$p < 0.05$
**	$p < 0.01$
***	$p < 0.001$
ns	Not significant ($p \geq 0.05$)

You can also add custom **brackets** between specific groups to highlight pairwise comparisons of interest.

8.5 FDR Correction

When performing multiple comparisons, p-values are automatically adjusted using the Benjamini-Hochberg procedure to control the false discovery rate. Both raw and adjusted p-values are reported.

9. Reference Gene Validation

Accurate normalization requires stable reference genes. qPCR Guru provides two complementary algorithms for assessing reference gene stability.

9.1 geNorm M Value

The geNorm algorithm (Vandesompele et al., 2002) calculates pairwise stability by comparing expression ratios between all candidate reference genes across all samples. The M value represents the average pairwise variation for each gene.

M Value	Interpretation
< 0.5	Excellent stability
0.5 – 1.0	Acceptable stability
> 1.5	Unstable — consider replacing this reference gene

Note: These are Guru recommended thresholds. The geNorm algorithm is by Vandesompele et al. (2002).

9.2 NormFinder ρ

NormFinder (Andersen et al., 2004) uses a model-based approach to estimate stability, measuring both intra- and inter-group variation independently. The ρ (rho) stability value represents the estimated systematic error introduced by using that gene for normalization.

Rho Value	Interpretation
< 0.3	Excellent stability
0.3 – 0.5	Acceptable stability
> 0.5	Unstable — consider replacing this reference gene

Note: These are Guru recommended thresholds. The NormFinder algorithm is by Andersen et al. (2004).

9.3 Interpreting Agreement

When both geNorm and NormFinder agree on the most stable gene, you have high confidence in your reference gene choice. When they disagree, investigate further — this may indicate group-specific effects or co-regulation that one algorithm is more sensitive to.

10. Standard Curves & Absolute Quantification

10.1 Entering Standards

In the **Standard Curves** section, enter known concentrations and corresponding Cq values for each target gene. qPCR Guru fits a linear regression to compute the slope, intercept, and R^2 value.

10.2 Efficiency from Slope

PCR efficiency is calculated from the standard curve slope:

$$E = 10^{(-1/\text{slope})}$$

An efficiency of 2.0 (100%) means perfect doubling per cycle. Acceptable range: 1.8–2.2 (90–110%). The efficiency value can be automatically applied to the Pfaffl method.

10.3 LOD / LOQ

When standard curves are available, qPCR Guru calculates:

- **LOD (Limit of Detection):** The lowest concentration reliably distinguished from a blank.
- **LOQ (Limit of Quantification):** The lowest concentration quantifiable with acceptable precision.

10.4 Copy Number Mode

Switch the chart data type to **Copy Number** to display absolute quantities calculated from the standard curve. Each sample's mean Cq is back-calculated through the regression to produce a copy number estimate.

11. Amplification & Melt Curves

11.1 Viewing Curves

If your data file includes amplification or melt curve data (common in exports from Bio-Rad CFX Maestro, QuantStudio, Roche LightCycler), qPCR Guru will automatically parse and display them. Use the checkboxes to show/hide individual samples or targets.

11.2 What to Look For

Amplification curves:

- Consistent curve shapes across replicates indicate good reproducibility.
- Curves that plateau at different levels may suggest varying starting quantities.
- Late or absent amplification in NTC wells confirms no contamination.

Melt curves:

- A single, sharp peak indicates a specific product.
- Multiple peaks suggest primer dimers, non-specific amplification, or SNPs.
- Shifted peaks between samples may indicate different amplicons or allele variants.

12. Guru Insights

Guru Insights provides an automated, expert-level review of your analysis, checking for common issues and highlighting important findings. Access it from the **Guru Insights** tab after running your analysis.

12.1 Automated Analysis Review

Guru Insights evaluates:

- Reference gene stability and whether additional reference genes are recommended.
- Significantly up- or downregulated targets.
- Outlier detection and quality issues.
- MIQE compliance status.
- Statistical test results and their interpretation.

12.2 How to Interpret Recommendations

Insights are categorized as:

- **Findings** (blue): Key observations about your data.
- **Warnings** (amber): Potential issues that may affect data quality.
- **Recommendations** (green): Suggested actions to improve your analysis.

Tip: Guru Insights requires login. Your work is automatically saved before redirecting to the login page.

13. Exporting Your Work

13.1 CSV Export

Export the full data table as a CSV file for further analysis in Excel, R, Python, or other tools. The CSV includes all columns: sample, target, mean Cq, ΔCq, ΔΔCq, RQ, RQ Min, RQ Max, and p-values.

13.2 Figure Export

Every chart can be exported as a publication-ready figure:

Format	Best For	DPI Options
JPEG	Presentations, web	96, 150, 300 DPI
TIFF	Journal submissions (lossless)	300, 600 DPI
PDF	Vector-quality documents	300, 600 DPI

The export bar appears above each chart. Select your desired format and DPI, and the figure is generated at full resolution.

13.3 PDF Reports

Click **Generate Report** to produce a comprehensive PDF report including your QC summary, MIQE compliance score, data table, charts, and Guru Insights (if available). This report is designed for lab notebooks, thesis appendices, or publication supplementary materials.

13.4 RDML Import & Export

RDML (Real-time PCR Data Markup Language) is the standardized format for qPCR data exchange. qPCR Guru can both import RDML files (v1.1–1.4) and export your data as RDML v1.4 for sharing with collaborators or importing into other software.

Tip: Exports (CSV, figures, reports, RDML) require a free registered account.

14. Saving & Loading Projects

14.1 Local Save

Click the **Save** button in the header to save your current analysis to your browser's local storage. This preserves your wells, format, filename, and instrument data so you can return to your analysis later.

Note: Saved data is stored locally in your browser. It will not transfer to other devices or browsers. Clearing your browser data will delete saved projects.

14.2 Loading Previous Work

On the upload screen, saved projects appear in the **Project History** section. Click any saved project to reload it instantly, restoring all your data and resuming where you left off. Up to 10 recent projects are retained.

References

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