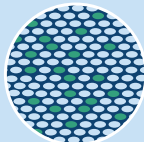


Your mental checklist for reproducible gene expression analysis

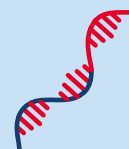
Quick MIQE check: Have you asked yourself these questions for your PCR experiments?

Assay design



- Does my assay cover alternative splicing variants?
- In my one-step workflow, does RNA structure impact primer binding for reverse transcription?
- Are secondary structures in my amplicon sequence impacting PCR?

Reverse transcription (RT)



- Am I within the dynamic range of my reaction?
- Are inhibitors affecting my RT efficiency?
- In my two-step workflow, was my cDNA diluted enough to avoid PCR interference?

Reference genes



- Did I use a sufficient number of reference gene?
- Is my choice of reference genes biasing my results?
- Is there empirical data to justify the use of the selected reference genes?
- Are these reference genes stably expressed in my samples and experimental conditions?

Additional considerations



- Apply appropriate normalization methods and statistical analyses to ensure accurate conclusions when comparing samples.
- For real-time PCR experiments, guidelines discourage the use of the $\Delta\Delta C_q$ method and instead recommend calculating PCR efficiency-corrected target quantities to determine fold-changes.
- For digital PCR experiments, target quantities are directly measured for direct use in determining fold-changes.

For more recommendations on sample handling, assay design and validation, quality control and data analysis, refer to the (d)MIQE guidelines:

Bustin SA, et al. MIQE 2.0: Revision of the minimum information for publication of quantitative real-time PCR experiments guidelines. *Clin Chem.* 2025;71(6):634–651

dMIQE Group. The digital MIQE guidelines update: Minimum information for publication of quantitative digital PCR experiments for 2020. *Clin Chem.* 2020;66(8):1012–1029.



More information on using www.qiagen.com/dPCR-gene-expression



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