Review

UHN Microarray Centre

MIQE Guidelines for qPCR

Summary of: Bustin SA, et al. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clinical Chemistry 2009, 55(4):611-622

Quantitative real-time PCR (qPCR) assays measure the number of copies of specific cDNA targets in a sample. While considered to be the gold standard for microarray data validation, qPCR is also recognised as a highly variable technique which is poorly standardised.

The Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE, pronounced *mykee*) guidelines describe the minimum information necessary for evaluating qPCR experiments that should be required for publication (1). The goal of the MIQE guidelines is to encourage better experimental practice and ensure the relevance, accuracy, correct interpretation, and repeatability of qPCR data (1). It is thought that by providing all relevant experimental conditions, investigators would be able to reproduce the results and reviewers would be able to assess the validity of the results. The MIQE guidelines were coordinated under the umbrella of MIBBI (Minimum Information for Biological and Biomedical Investigations; ref 2). Similar guidelines have already been proposed for DNA microarray analysis (3), proteomics experiments (4), and genome sequence specification (5), and are under discussion for RNA interference and metabolomics studies (6-8).

Briefly, the following items are considered essential by the MIQE checklist for authors, reviewers, and editors, and must be submitted with the manuscript and should be published either in abbreviated form or as an online supplement (1):

- Experimental design (definition of experimental and control groups, number within each group)
- Sample (full description, processing procedure, method of freezing/fixation and how quickly it was frozen/fixed, storage conditions and duration)
- Nucleic Acid extraction (procedure/ instrumentation, details of DNase/RNase treatment, contamination assessment, quantification method, RNA integrity, RIN/RQI or C_q of 3' and 5' transcripts, inhibition testing)
- Reverse transcription (complete reaction conditions, amount of RNA and reaction volumes, priming oligonucleotide, concentration of reverse transcriptase)
- qPCR target information (gene symbol, sequence accession number, amplicon length, in silico specification screen, location of each primer, identification of splice variant targeted)
- qPCR oligonucleotides (primer sequences)
- qPCR protocol (complete reaction conditions including buffer composition, enzyme concentrations, additives, and thermocycler parameters; reaction volumes and amount of cDNA/DNA, buffer/kit manufacturer)
- qPCR validation (specificity, C_q of the no-template control (NTC) for SYBR Green, calibration curve with slope and y intercept, PCR efficiency calculated from slope, linear dynamic range, C_q variation at limit of detection (LOD), evidence for LOD)
- Data analysis (qPCR analysis programme, method of Cq determination, outlier identification, results for NTC, justification of number and choice of references genes, description of normalization method, repeatability, statistical methods)

Additional information, which is listed as desirable in the MIQE checklist, should also be submitted if it is available. The full checklist is available at http://www.rdml.org/guidelines.php.

The report also discusses the need to standardise qPCR-related nomenclature to avoid confusion; for example, the abbreviation qPCR should be used for quantitative real-time PCR and RT-qPCR should be used for reverse transcription-qPCR, and genes used for normalisation should be referred to as reference genes instead of housekeeping genes. The report also proposes that investigators refrain from using commercially-derived terms like TaqMan® probes, instead referring to these as hydrolysis probes. In addition, the report proposes quantification cycle (C_q) be used to describe the PCR cycle used for quantification instead of threshold cycle (C_t), crossing point (C_p), and take-off point (TOP), which refer to the same value but were coined by different manufacturers of real-time instruments.

The report also explains some critical concepts that were considered during the development of the guidelines and also discusses the importance of providing detailed information about data normalisation and analysis.

References

1. Bustin SA, *et al*. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clinical Chemistry 2009, 55(4):611-622
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3. Brazma A, *et al.* Minimum information about a microarray experiment (MIAME) – toward standards for microarray data. Nat Genet 2001, 29:365
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5. Field D, *et al*. The minimum information about a genome sequence (MIGS) specification. Nat Biotechnol 2008, 26:541
6. Echeverri CJ, *et al.* Minimizing the risk of reporting false positives in large-scale RNAi screens. Nat Methods 2006, 3:777
7. Haney SA, *et al*. Increasing the robustness and validity of RNAi screens. Pharmacogenomics 2007, 8:1037
8. Sansone SA, *et al.* The metabolomics standards initiative. Nat Biotechnol 2007, 25:846