Promega

The MIQE guidelines:
Minimum Information for Publication of Quantitative Real-Time PCR Experiments[1]

Laboratory Notebook Checklist

Key: **Essential**, Desirable

Experimental Design

- ❑ **Definition of experimental and control groups**
- ❑ **Number within each group**
- ❑ Assay carried out by core lab or investigator's lab?
- ❑ Acknowledgement of authors' contributions

Sample

- ❑ **Description**
  - ☐ Volume/mass of sample processed
  - ☐ **Microdissection or macrodissection**
- ❑ **Processing procedure**
  - ☐ **If frozen - how and how quickly?**
  - ☐ **If fixed - with what, how quickly?**
- ❑ **Sample storage conditions and duration** (i.e. FFPE samples)

Nucleic Acid Extraction

- ❑ **Procedure and/or instrumentation**
  - ☐ **Name of kit and details of any modifications**
  - ☐ Source of additional reagents used
- ❑ **Details of DNase or RNAse treatment**
- ❑ **Contamination assessment (DNA or RNA)**
- ❑ **Nucleic acid quantification**
  - ☐ **Instrument and method**
  - ☐ Purity (A_{260}/A_{280})
  - ☐ Yield
- ❑ **RNA integrity method/instrument**
  - ☐ **RIN/RQI or Cq of 3' and 5' transcripts**
  - ☐ Electrophoresis traces
- ❑ **Inhibition testing (Cq dilutions, spike or other)**

Reverse Transcription

- ❑ **Complete reaction conditions**
  - ☐ **Amount of RNA and reaction volume**
  - ☐ **Priming oligonucleotide** (if using GSP) and concentration
  - ☐ **Reverse transcriptase and concentration**
  - ☐ **Temperature and time**
  - ☐ Manufacturer of reagents and catalogue numbers
- ❑ C_qs with and without RT
- ❑ Storage conditions of cDNA

qPCR Target Information

- ❑ **If multiplex, efficiency and LOD of each assay**
- ❑ **Sequence accession number**
- ❑ Location of amplicon
  - ☐ **Amplicon length**
  - ☐ ***In silico* specificity screen (BLAST, etc.)**
  - ☐ Pseudogenes, retropseudogenes or other homologs?
    - ☐ Sequence alignment
  - ☐ Secondary structure analysis of amplicon
- ❑ **Location of each primer by exon or intron (if applicable)**
  - ☐ **What splice variants are targeted?**

qPCR Oligonucleotides

- ❑ **Primer sequences**
- ❑ RT Primer DB Identification Number
- ❑ Probe sequences
- ❑ **Location and identity of any modifications**
- ❑ Manufacturer of oligonucleotides
- ❑ Purification method

qPCR Protocol

- ❑ **Complete reaction conditions**
  - ☐ **Reaction volume and amount of cDNA/DNA**
  - ☐ **Primer, (probe), Mg++ and dNTP concentrations**
  - ☐ **Polymerase identity and concentration**
  - ☐ **Buffer/kit identity and manufacturer**
  - ☐ Exact chemical constitution of the buffer
  - ☐ **Additives (SYBR Green I, DMSO, etc.)**
- ❑ Manufacturer of plates/tubes and catalog number
- ❑ **Complete thermocycling parameters**
- ❑ Reaction setup (manual/robotic)
- ❑ **Manufacturer of qPCR instrument**

qPCR Validation

- ❑ Evidence of optimization (from gradients)
- ❑ **Specificity (gel, sequence, melt, or digest)**
- ❑ **For SYBR Green I, C_q of the NTC**
- ❑ **Standard curves with slope and y-intercept**
  - ☐ **PCR efficiency calculated from slope**
  - ☐ Confidence interval for PCR efficiency or standard error
  - ☐ **r^2 of standard curve**
- ❑ **Linear dynamic range**
  - ☐ **C_q variation at lower limit**
  - ☐ Confidence intervals throughout range
- ❑ **Evidence for limit of detection**
- ❑ **If multiplex, efficiency and LOD of each assay.**

Data Analysis

- ❑ **qPCR analysis program (source, version)**
  - ☐ **C_q method determination**
  - ☐ **Outlier identification and disposition**
- ❑ **Results of NTCs**
- ❑ **Justification of number and choice of reference genes**
- ❑ **Description of normalization method**
- ❑ Number and concordance of biological replicates
- ❑ **Number and stage (RT or qPCR) of technical replicates**
- ❑ **Repeatability (intra-assay variation)**
- ❑ Reproducibility (inter-assay variation, %CV)
- ❑ Power analysis
- ❑ **Statistical methods for result significance**
- ❑ **Software (source, version)**
- ❑ C_q or raw data submission using RDML

To learn more, visit:
www.promega.com/qPCR

[1] Reproduced in cooperation with Michael W. Pfaffl. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Bustin et al., Clinical Chemistry 55:4 611-622 (2009)

We’re scientists!...ask us anything (well almost anything).

Stuff like:

Which kit is right for me?

How does this assay work?

Can we receive customized training?

Help! Something went wrong.

Is this the best experimental design?

Is this kit compatible with my sample type?

How do I interpret these research results?

Visit:

https://www.promega.com/contact

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