



Experimental Design

- Definition of experimental and control groups
- Number within each group

Sample

- Description
- Microdissection or macrodissection
- Processing procedure
- If frozen - how and how quickly?
- If fixed - with what, how quickly?
- Sample storage conditions and duration (especially for FFPE samples)

Nucleic Acid Extraction

- Procedure and/or instrumentation
- Name of kit and details of any modifications
- Details of DNase or RNase treatment
- Contamination assessment (DNA or RNA)
- Nucleic acid quantification
- Instrument and method
- RNA integrity method/instrument
- RIN/RQI or Cq of 3' and 5' transcripts
- 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

qPCR Target

- Gene symbol
- Sequence accession number
- Amplicon length
- *In silico* specificity screen (BLAST, etc)
- Location of each primer by exon or intron (if applicable)
- What splice variants are targeted?

qPCR Oligos

- Primer sequences
- Location and identity of any modifications

qPCR Protocol

- Reaction volume and amount of cDNA/DNA
- Primer, (probe), Mg⁺⁺ and dNTP concentrations
- Polymerase identity and concentration
- Buffer/kit identity and manufacturer
- Additives (SYBR Green I, DMSO, etc.)
- Complete thermocycling parameters
- Manufacturer of qPCR instrument

qPCR Validation

- 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
- r² of standard curve
- Linear dynamic range
- C_q variation at lower limit
- 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 normalisation method
- Number and stage (RT or qPCR) of technical replicates
- Repeatability (intra-assay variation)
- Statistical methods for result significance
- Software (source, version)

Go to www.illumina.com/ecoqpcr to share your experiences with the MIQE guidelines and Real-Time PCR in general