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**Protocol status:** Working  
We use this protocol and it's working

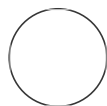
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## qRT-PCR sample preparation

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### ABSTRACT

Protocol for qRT-PCR sample preparation for analysis using a RotorGeneQ machine (Qiagen).

**Keywords:** ASAPCRN

- 1 Synthesise cDNA libraries from total RNA from each sample using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), using Oligo(dT)<sub>20</sub> primers.
- 2 Place the synthesised libraries on ice, and dilute the libraries 1:3 by adding  60 µL of DEPC-treated H<sub>2</sub>O to each sample, pipetting up and down gently to mix ~ 8 times.
- 3 Dilute primer stocks by adding  2 µL of the forward primer, and  2 µL of the reverse primer, to  46 µL of DEPC-treated H<sub>2</sub>O on ice to make a final combined forward and reverse primer stock of  4 micromolar (µM) forward primer and  4 micromolar (µM) reverse primer for each gene target to be analysed.
- 4 Thaw the 2x QuantiNova SYBR green master mix (Qiagen) on ice.
- 5 Depending on the number of samples being analysed, assemble a sample master mix (not containing the cDNA library) containing the following:

Reagent	Volume (1x)
2x SYBR Green Master Mix	5 uL
4 uM forward and 4 uM reverse primer master mix	1 uL
cDNA library (to be added individually to each tube)	4 uL
Total volume	10 uL

- 6 To qRT-PCR tubes sitting on ice (Qiagen – RotorGeneQ compatible), add  6 µL of the master mix for each target gene to the desired tubes, and add  4 µL of the appropriate cDNA library to each sample.

- 7 Tap the rack holder to ensure all solution is at the bottom of the tube before capping each sample.(NOTE: samples do not need to be mixed prior to analysis)
- 8 Load and run the samples on a RotorGeneQ (Qiagen) machine using the desired parameters for the gene targets you are analysing.