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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).

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1 Synthesise cDNA libraries from total RNA from each sample using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), using Oligo(dT)$_{20}$ primers.

2 Place the synthesised libraries on ice, and dilute the libraries 1:3 by adding 60 µL of DEPC-treated H2O 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 H2O on ice to make a final combined forward and reverse primer stock of [M] 4 micromolar (µM) forward primer and [M] 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.