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RNA extraction, cDNA synthesis and Taqman qPCR

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We use this protocol and it's working

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Abstract

RNA extraction, cDNA synthesis and TaqMan qPCR on samples.

RNA extraction

- 1 Cell pellets are snap-frozen using dry ice.
- 2 RNA is extracted using the Maxwell® RSC simply RNACells kit (Promega), and the Maxwell® RSC 48 instrument, following manufacturer instructions.
- 3 After RNA extraction, RNA concentration and quality are measured and assessed using a nanodrop.

cDNA synthesis

- 4 Up to 1 µg of RNA per sample is reverse-transcribed into cDNA using the High-Capacity cDNA Reverse Transcription kit (ThermoFisherScientific).

quantitative PCR

- 5 qPCR is performed using TaqMan™ Gene Expression Assay (Thermo Fisher Scientific) according to the manufacturer's instructions.
- 6 For each gene, TaqMan™ probes were used along with the TaqMan™ master mix, and sample cDNA following the manufacturer's protocol.
- 7 Samples, along with a minus reverse transcriptase control (-RT) were ran for each gene on the QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems).
- 8 The -RT served as a negative control, and the gene expression levels were normalised to the housekeeping gene GAPDH following the delta-delta Ct method. Gene expression values were expressed as the normalisation to their respective control sample.