

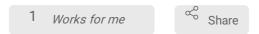


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RNA extraction, cDNA synthesis and Taqman qPCR

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ABSTRACT

RNA extraction, cDNA synthesis and TaqMan qPCR on samples.

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RNA extraction

1 Cell pellets are snap-frozen using dry ice.

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- 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

- qPCR is performed using TaqManTM Gene Expression Assay (Thermo Fisher Scientific) according to the manufacturer's instructions.
- 6 For each gene, TaqManTM probes were used along with the TaqManTM 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 QuantSudio 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.