RNA extraction, cDNA synthesis and Taqman qPCR

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Sep 20, 2022

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dx.doi.org/10.17504/protocols.io.36wgqj5rkvk5/v1

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ABSTRACT

RNA extraction, cDNA synthesis and TaqMan qPCR on samples.

DOI

dx.doi.org/10.17504/protocols.io.36wgqj5rkvk5/v1

PROTOCOL CITATION

gurvir.virdi 2022. RNA extraction, cDNA synthesis and Taqman qPCR. **protocols.io** https://protocols.io/view/rna-extraction-cdna-synthesis-and-taqman-qpcr-cgvstw6e

CREATED

Sep 20, 2022

LAST MODIFIED

Sep 20, 2022

PROTOCOL INTEGER ID

70290

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^{TM} Gene Expression Assay (Thermo Fisher Scientific) according to the manufacturer's instructions.

6 For each gene, TaqMan^{TM} probes were used along with the TaqMan^{TM} 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.

Citation: gurvir.virdi RNA extraction, cDNA synthesis and Taqman qPCR https://dx.doi.org/10.17504/protocols.io.36wgqj5rkvk5/v1