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RT-qPCR

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We use this protocol and it's working

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Abstract

Gene expression levels by RT-qPCR

RT-qPCR

1 Phase separation

1.1 For gene expression analysis adherent cells were lysed using a mixture of TRIzol reagent (Invitrogen, Thermo Scientific, Germany) + 1% β-Mercaptoethanol (Sigma-Aldrich, USA) (100µL/per well in 96 well plate), snap frozen and stored at -80°C until further processing. After thawing, 200 µL of chloroform (Honeywell, US) were added to achieve for phase separation (centrifugation 13,000g at 4°C for 15min).

1.2 The upper phase was collected into a new eppendorf and RNA precipitation was performed by the addition of isopropyl alcohol (Sigma-Aldrich, USA) and glycerol (Thermo Scientific, Germany). The resulting mixture was incubated for 15 minutes on ice and then centrifuged for 15 minutes at 13,000g at 4°C.

1.3 The obtained RNA pellet was washed and centrifuged (5 minutes at 13,000g at 4°C) twice with ice-cold 75% EtOH and dissolved in 20 µL of Nuclease-free water (Invitrogen, Thermo Scientific, Germany).

1.4 Genomic DNA was removed using a DNase treatment and removal kit (Invitrogen, Thermo Scientific, Germany) following the user manual.

2 For the qPCR reaction, RNA samples at a concentration of 1 ng/µL were used. The RevTrans QPCR One-Step EvaGreen kit (Bio&Sell, Feucht, Germany) was used for cDNA synthesis and the subsequent qPCR reaction according to the user manual. The reaction mixtures were incubated for 15 minutes at 50°C for cDNA generation, followed by the qPCR reaction: 95°C for 5 minutes, 95°C for 15 seconds, 55°C for 20 seconds and 72°C for 30 seconds. For each gene, a reaction mixture without the total RNA template was run as a negative control.

2.1 The transcript data was analyzed using Agilent AriaMx 1.1 software (Agilent Technologies, Santa Clara, California, USA). The transcript level for the genes of interest was normalized to the transcription level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and acidic ribosomal phosphoprotein P0 (RPLP0) and presented as a logarithmic fold change to the healthy condition.

2.2 All primers were designed using Primer3 software. Specificity of the primers was analysed using the NCBI primer blast tool and in silico PCR tool with the UCSC genome browser.

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