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Gene expression analysis by quantitative Real-Time PCR (qPCR)

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We use this protocol and it's working

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Abstract

Quantitative Real-Time PCR (qPCR)

qPCR plate reagents

1 **Option 1: TaqMan qPCR**

1.1 Prepare Taqman Master mix (ul per well):
- 5ul: Taqman Gene Expression Master Mix (Applied Biosystems, # 4369016)
- 0.5ul Taqman Gene Expression Assays

1.2 Add 5.5ul of Taqman Master mix to each well with an automatic pipette (Multipette E3, Eppendorf)

1.3 Add 4.5ul of cDNA per well (10ng cDNA) in technical triplicates to each well of LightCycler 480 Multiwell 384-plate (#4729749001, Roche Diagnostics)

2 **Option 2: SYBR qPCR**

2.1 Prepare SYBR Master mix (ul per well):
- 5ul PowerUp SYBR Green Master Mix (#A25776, Applied Biosystem-ThermoFisher)
- 1ul Primers (Forward and Reverse, diluted in Nuclease-free water at 5µM)

2.2 Add 6ul of SYBR Master mix to each well with an automatic pipette (Multipette E3, Eppendorf)

2.3 Add 4ul of cDNA per well (10ng cDNA) in technical triplicates to each well of LightCycler 480 Multiwell 384-plate (#4729749001, Roche Diagnostics)

Thermocycling

3 Perform PCR using the following cycling conditions in a LightCycler® 480 System (Roche):
- 50˚C for 2min
- 95˚C for 2min
- 95˚C for 15s + 60˚C for 1min (x40cycles)

Data an

4 Using the LightCycler Software obtain the threshold cycles (CT) signals from each samples.

5 Perform ΔΔCT-method to analyze the expression data using the endogenous control genes and the reference experimental group.