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

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Protocol status: Working

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 $\Delta\Delta\text{CT}$ -method to analyze the expression data using the endogenous control genes and the reference experimental group.