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IN DEVELOPMENT

Goga Lab RT-qPCR protocol: QuantStudio6 Machine V.2

This protocol is published without a DOI.

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COMMENTS 0

ABSTRACT

Guidelines for preparing RT-qPCR samples for QuantStudio 6 located in HSW7 lab space.

ATTACHMENTS

GogaLab-RTqPCR-Preparation.xlsx

PROTOCOL CITATION

Jeremy.williams 2022. Goga Lab RT-qPCR protocol: QuantStudio6 Machine. **protocols.io** https://protocols.io/view/goga-lab-rt-qpcr-protocol-quantstudio6-machine-ci9zuh76 Version created by Jeremy.williams

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GUIDELINES

*start with full tip boxes and use tips in coordination with your plate map, so you never get lost
*watch 1uL volumes in the pipet tip like a hawk - major source of variability
*cap and gently vortex your *mixture per primer set* every 12 replicates or so, sometimes things separate

MATERIALS TEXT

PowerUp SYBR Green Master Mix
Your generated cDNA samples
100uM single primer stocks

BEFORE STARTING

*Thaw on ice (leave time!) and keep all reagents on ice through preparation. Prepare the plate on ice.

ATTACHMENTS

GogaLab-RTqPCR-Preparation.xlsx

Prior to plate preparation:

1 Dilute stock IDT primers to 100uM (see note in Guidelines).

2 Use 'GogaLab-RTqPCR-Preparation' Excel spreadsheet to input your sample number and calculate reagent volumes.

3 Mix *total reagent volumes required* (see spreadsheet, green) for DI and PowerUP

4 Mix your forward and reverse primer pairs together, to a final dilution of 10uM forward and 10uM reverse. For example, add 10uL each of forward and reverse primers to 80uL PCR-quality DI for 100uL final volume.

5 Mix *mixtures per primer set* (blue) volumes together.

6 Pipet 1uL diluted cDNA into respective qPCR well, aiming for the sidewall of each respective well.

7 Add 19uL *mixture per primer set* into each respective well.

8 Seal plate, and spin down 1000rpm for 1 minute. Use bacterial, not tissue culture, centrifuge.

9 Load plate into centrifuge and proceed using QuantStudio software suite.