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VERSION 2

NOV 14, 2022



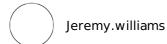
IN DEVELOPMENT

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.xl SX

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.

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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.