WetLab-2 RT-qPCR procedure

Version 2

PLOS One

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ABSTRACT

Procedure used for RT-qPCR in the WetLab-2 study

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0183480

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Parra M, Jung J, Boone TD, Tran L, Blaber EA, Brown M, Chin M, Chinn T, Cohen J, Doebler R, Hoang D, Hyde E, Lera M, Luzod LT, Mallinson M, Marcu O, Mohamedaly Y, Ricco AJ, Rubins K, Sgarlato GD, Talavera RO, Tong P, Uribe E, Williams J, Wu D, Yousuf R, Richey CS, Schonfeld J, Almeida EAC, Microgravity validation of a novel system for RNA isolation and multiplex quantitative real time PCR analysis of gene expression on the International Space Station. PLoS ONE 12(9). doi: 10.1371/journal.pone.0183480

PROTOCOL STATUS

Working

BEFORE STARTING

- Use gloves
- RNase clean work surfaces (spray with RNase Zap, then 70% Ethanol)

qPCR

1 Preparation of qPCR reactions (per 25ul reaction):
- Promega GoTaq Probe Master Mix (2X): 12.5 ul
- Forward Primer (20X): 1 ul
- Reverse Primer (20X): 1 ul
- Hydrolysis Probe (20X): 1 ul
- DNA Template: varied from 1 pg to 1ug per reaction
- Molecular Biology Grade Water: to 25 ul total volume

RT-qPCR

2 Preparation of RT-qPCR reactions (per 25ul reaction):
- Promega GoTaq Probe Master Mix (2X): 12.5 ul
- GoScript RT Mix for 1-step RT-qPCR: 5 ul
- Forward Primer (20X): 1 ul
- Reverse Primer (20X): 1 ul
- Hydrolysis Probe (20X): 1 ul
- RNA Template: varied as listed (usually around 5ng)
- Molecular Biology Grade Water: to 25 ul total volume