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Reverse transcription of RNA to cDNA

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1 Works for me dx.doi.org/10.17504/protocols.io.8bjhskn

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ABSTRACT

Synthesizing cDNA (for qPCR)

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0012757_RevertAid_Reverse_Transcriptase_UG.pdf,
https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0012904_DyNAmo_ColorFlash_SYBR_Green_qPCR_F416L_UG.pdf

GUIDELINES

Always work with gloves and safety gear and work on ice

MATERIALS TEXT

- 100 ng RNA
- 40x yellow sample buffer (from DyNAmo Color Flash Kit)
- random hexamer primers
- 5x RT-buffer
- dNTP Mix (10 mM of each nucleotide)
- RevertAid Reverse Transcriptase
- **You will require 100 ng for RT and 100 ng for -RT control (include a -RT control for each sample!)**

Preparation of 1.33X yellow buffer

1 33,25 µL 40x yellow sample buffer + 966,75 µL H2O

optional: This was used to be able to see the pipetting scheme for qPCR more easily

Start

2 In PCR stripes, pipet in the following order:

100 ng template RNA
1 µL random hexamer primers
RNase-free H2O to 13 µL volume

3 Prepare a Mastermix of the following reagents:

4 µL 5x RT-buffer
2 µL dNTP Mix (10 mM of each nucleotide)
1 µL RevertAid Reverse Transcriptase

4 Add 7 µL Mastermix to each reaction

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5 For your -RT control, pipet 100 ng RNA in PCR stripes, leave out buffer and everything, just add H2O to a final volume of 20 µL. Mark as -RT so you can distinguish it from your actual cDNA!

PCR

6

PCR protocol:

10 min	25 ˚C
60 min	42 ˚C
10 min	70 ˚C

7 Add 60 µL 1,33 x yellow sample buffer to each reaction

optional: This was used to be able to see the pipetting scheme for qPCR more easily

8 cDNA can be stored at 4 ˚C for a short time, otherwise, freeze at -20 ˚C

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