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Reverse transcription of RNA to cDNA

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Protocol status: Working
We use this protocol and it's working

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Keywords: pipet 100 ng rna, rna 40x yellow sample buffer, cdna 100 ng rna, µl revertaid reverse transcriptase, ng template rna, µl ribolock rnase, revertaid reverse transcriptase, transcription of rna, rnase, yellow sample buffer to each reaction cdna, transcription, pcr programm, rna, yellow sample buffer random hexamer, completion of rt reaction, pcr stripe, rt reaction, µl 40x yellow sample buffer, mastermix of the following reagent, µl mastermix to each reaction, ng for rt, pcr, reaction cdna, mm of each nucleotide, following reagent, µl dntp mix, buffer dntp mix, rt, 5x rt, yellow sample buffer

Abstract

- 100 ng RNA
- 40x yellow sample buffer
- 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

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

Start

In PCR stripes, pipet in the following order:
100 ng template RNA
1 µL random hexamer primers
RNase-free H2O to 12,5 µL volume
Prepare a Mastermix of the following reagents:
4 µL 5x RT-buffer
0.5 µL RiboLock RNase-Inhibitor
2 µL dNTP Mix (10 mM of each nucleotide)
1 µL RevertAid Reverse Transcriptase
Add 7.5 µL Mastermix to each reaction
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 Programm

10 min 25 ˚C
60 min 42 ˚C
10 min 70 ˚C
After completion of RT reaction:
Add 60 µL 1,33 x yellow sample buffer to each reaction
cDNA can be stored at 4 ˚C for a short time, otherwise, freeze at -20 ˚C

Troubleshooting