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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.77chriw

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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