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© Removal of gDNA from totalRNA

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Iaem Dusseldorf¹

¹Heinrich-Heine Universität Düsseldorf



Igem Dusseldorf

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Protocol status: Working

We use this protocol and it's working



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Abstract

Removal of genomic DNA from RNA preparations

Guidelines

Always work with gloves and safety gear and work on ice.

Materials

- RNase-free H₂O
- DNasel-buffer (10X)
- DNasel
- 50 mM EDTA
- NaOAc (3M & pH 5,3) and 100% EtOH

Troubleshooting



Digestion of DNA

Calculate the volume required for 1 µg RNA

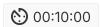
Com pon ent	amo unt
DNa sel buff er	1 μΙ
total RNA	1 μg
DNa sel	1 μΙ
H2O	to 10 μΙ

- 2 pipet components into a RNase-free 1.5 ml tube (following order: H2O, buffer, total RNA, DNasel)
- 3 incubate reaction at 37°C



Extraction of DNasel-digested RNA

4 add 1 μ l 50 mM EDTA and incubate at 65°C



- 5 add 1/10 Volume NaOAc and 3 Volumes 100% EtOH
- freeze at -20°C over night or at -80°C for at least 1h 6



7 centrifuge at 13000 rpm at 4°C



8 Discard supernatant

> RNA pellet may be invisible, make sure to align tube lid so you remember where the pellet is located

- 9 add 500 μl 70% EtOH
- 10 centrifuge at 13000 rpm at 4°C



- 11 discard supernatant, remove as much ethanol as possible
- 12 dry tubes at RT under the hood (~5min), do not overdry!
- 13 resuspend pellet in 20 µl RNase-free water
- 14 measure concentration

Quality control:

15 perform PCR on your DNA-free RNA. Include a positive control using gDNA as a template and primer that bind to it.

There should be no visible bands except for positive control!