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Removal of gDNA out of totalRNA

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ABSTRACT

Start

Calculate the volume required for 1 µg RNA

Digestion of DNA

Component	amount
DNaseI buffer	1 µl
total RNA	1 µg
DNaseI	1 µl
H ₂ O	to 10 µl

pipet components into a RNase-free 1.5 ml tube (following order: H₂O, buffer, total RNA, DNaseI)

incubate reaction at 37°C for 30 min

Extraction of DNaseI-digested RNA

add 1 µl 50 mM EDTA and incubate at 65°C for 10 min

add 1/10 Volume NaOAc and 3 Volumes 100% EtOH

freeze at -20°C over night or at -80°C for at least 1h

centrifuge at 13000 rpm for 10 min at 4°C

Discard supernatant (RNA pellet could be not visible, important to know where it should be; it is definitely there)

add 500 µl 70% EtOH

centrifuge at 13000 rpm for 10 min at 4°C

discard supernatant, remove as much ethanol as possible

dry tubes at RT under the hood (~5min), do not overdry!

resuspend pellet in 20 µl RNase-free water

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 We use this protocol and it's working

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measure concentration

Quality control:

perform PCR on your DNA-free RNA (using primers 538/747; 57°C, 653 bp). Include a positive control using gDNA as a template.

There should be no visible bands!