Removal of gDNA out of totalRNA

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1 Works for me

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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
H2O	to 10 µl

pipet components into a RNase-free 1.5 ml tube (following order: H_2O, 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 definetly 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
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!

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