Purification of polyA$^+$RNA from biological material.

Sample preparation:

1. Thaw the sample(s).
2. Spin 4000g 2 min and remove supernatant.
3. Add 200 µL GuSCN buffer. Vortex briefly.
4. Add quartz sand* and mix 2 min on ice using pestle for pulverizing the sample.
5. Spin the tube short (e.g. 60 s at 16100g or at max speed.) and remove the supernatant to a clean tube.
6. Heat the lysate supernatant 20 min. 65 °C on an Eppendorf Thermomixer (shaking 700 rpm).
7. Spin the tube short (e.g. 60 s at 16100g) and remove the supernatant to a clean tube.

Oligo-T pre-coating of streptavidin-coated magnetic particles

8. Pipette 60 µL of streptavidin-coated magnetic particles into a tube for each sample
9. Use the magnetic separator and remove the supernatant
10. Add 100 µL 0.5 µg/mL yeast tRNA diluted in TE.
11. Pre-block for 5 min at room temperature (RT).
12. Wash the particles in 100 µL TE.
13. Add 200 pmol biotinylated LNA oligo-T capture probe (product# 300100-B) to the pre-blocked streptavidin-coated particles in 100 µL binding buffer high salt
14. Incubate 5 min at 37 ºC (gentle shaking or rotating).
15. Wash the particles in 100 µL DEPC-treated H_2O and twice in binding buffer.
16. The particles are equilibrated in the adequate buffer used for binding the polyA$^+$RNA.

Purification protocol

17. The sample is added to the oligo-T pre-coated magnetic particles (particles without supernatant)
18. Incubate 5 min 37 °C (gentle shaking or rotating). The mRNA is bound to the beads.
19. The particles are washed in washing buffer low salt 3 times.
20. Beads are to Eppendorf tubes containing DEPC-H_2O.
21. The Eppendorf tubes are incubated at 65 °C 10 min for releasing the polyA$^+$RNA from the particles and quench on ice for 5 min.
22. Spin briefly (13.2 rpm 60 sec) and transfer the supernatant to a clean siliconezed eppendorf tube. To avoid any magnetic particles to disturb the absorbance measurement.

Quantification (optional)

23. Apply 1 µL of the polyA$^+$-selected RNA on a native 1-% agarose gel in 1x TAE buffer containing 1:10000 Gelstar.
24. Run the gel for ca. 60 min 7 V/cm
25. Scan the agarose gel on the Amersham Pharmacia Biotech Typhoon 9200 Imager and quantify the quality of the polyA$^+$-selected RNA.

Buffers:

GuSCN buffer (4 M GuSCN, 25 mM Na-citrate, pH 7.0, 0.5 % sodium N-lauroyl sarcosinate)

- Quartz sand e.g. sea sand (Merck euro lab cat no. 17712-1). The quartz sand is baked the same way as glassware is treated for an RNase free environment.

TE buffer : 10 mM Tris-HCl, 1 mM EDTA, pH 7.5

Binding buffer high salt: (20 mM Tris-HCl (pH 7.5), 0.5 M NaCl, 1 mM EDTA (pH 7.5) 0.1%(w/v) lauryl sarcosinate)

Binding/washing buffer low salt: (20 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 1 mM EDTA (pH 7.5) 0.1%(w/v) lauryl sarcosinate)

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