mRNA purification with Ambion Dynabeads

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1 Works for me

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ABSTRACT

The mRNA isolation that is usually used prior to RNAseq library preparation protocol

Adjust the volume of 2 ug of RNA to 36 uL with distilled water

1

Heat to 65°C for 2 minutes to disrupt secondary structures

2

Transfer 72 uL of well resuspended Dynabeads to microcentrifuge tube - place on magnet rack for 30 seconds or untill all Dynabeads migrate to the tube well

3

Discard the supernatant, remove tube from the magnet rack, and add 100 uL Binding Buffer to calibrate the beads. Put back on the magnet rack and remove the supernatant

4

Add 36 uL Binding Buffer to Dynabeads. Optimal hybridization required Binding Buffer added in 1:1 ratio relative to sample volume.

5

Add the total RNA to the Dynabeads / Binding Buffer suspension. Mix thoroughly and rotate on a mixer for 5 minutes at room temperature to allow mRNA to anneal to the oligo dT25 on the beads.

6

Place the tube on the magnet rack and wash mRNA-bead complex twice with 200 uL Washing Buffer B. Remove all the supernatant between each washing step with help of the magnet.

7

Elute the samples with 10-20 uL of 10 mM Tris-HCl pH 7.5. Heat to 65°C for 3 minutes and place the tube immediately on the magnet rack.

8

Transfer the eluted mRNA to a new RNAse free tube

9

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