Ribominus selection

CAUTION: This is newly entered, and should be doublechecked before using.

Reagents required:

Ribominus Transcriptome isolation kit Yeast, Invitrogen, K1550-03

- Glycogen (Invitrogen 10814-010)
- NaOAC 3M, pH 5.2
- 100% EtOH
- 70% EtOH
- nuclease-free water
- Magnetic rack
- 37o C heating block
- Centrifuge
- -80oC (-20oC for 1h to o/n)

This protocol is a scaled-down version of the original Invitrogen protocol. I have found that it works well and gives enough rRNA-depleted material for making a sequencing library. You can also follow the exact Invitrogen protocol if you wish. Two rounds of purification however are essential to avoid saturation of your sequencing reads with rRNA sequences.

1. Make sure your RNA sample is at a concentration of 500ng/µl
2. Mix 5µg (10µl) total RNA with 0.4µl of the LNA probes and 18µl of Hybridization buffer.
3. Incubate at a 37o C heat block for 5min and place immediately in ice.
4. In the meantime, aliquot to a new tube 25µl of Streptavidin-coated magnetic Beads (supplied with the kit) for each sample. Place in magnetic stand for 1-2min until the liquid becomes clear and all magnetic particles are visibly on the side of the tube.
5. Remove liquid carefully and resuspend beads in 125µl Water (supplied with the kit). Place in magnetic stand for 1-2min until the liquid becomes clear once more, remove liquid, and repeat this step one more time.
6. Resuspend the beads in 10µl Hybridization buffer and keep them at the 37o C heat block until ready to use.
7. Add the 20µl of RNA waiting in ice to the beads, mix by pipeting up and down and leave at 37o C for 15 min
8. Place in magnetic stand for 1-2min until the liquid becomes clear and all magnetic particles are visibly on the side of the tube.
9. Transfer the liquid phase to a new tube and add 0.4µl of the NLA probes
10. Place in 37o C for 5min and put immediately in ice
11. In the meantime, aliquot to a new tube 25µl of Streptavidin-coated magnetic Beads and follow steps 4-5 as before.
12. At the end of the bead washes do not add hybridization buffer but add the RNA sample waiting on ice.
13. Mix the RNA/magnetic beads by pipeting and incubate at 37 C for 20min
14. Place the eppendorf in a magnetic stand as before and transfer the liquid to a new eppendorf containing ~5µg glycogen and 1/10th volume 3M NaOAC, pH5.2 followed by two volumes of 100% Ethanol
15. Place at -80C for 20min
16. Spin at maximum speed for 30min at 4o C and discard liquid
17. Add 100ul of 70% EtOH and spin for 5min at 4oC
18. Remove all traces of liquid carefully and let the pellet dry for 2-5min
19. Resuspend carefully with 6µl Nuclease-free water

Check your rRNA depletion by comparing 1µl of the sample before and after riboMinus at the Agilent RNA 6000 nano Bioanalyzer. Analyze at the mRNA II mode of the software.