riboPOOL Protocol Snapshot

Detailed protocol available at www.sitoolsbiotech.com/protocols.php

- ☐ 1. To 14 µl of RNA sample add 1 µl resuspended **RP** and add 5 µl **HB**. Vortex and briefly spin-down.
- ☐ 2. Incubate samples for 10 min at 68 °C and allow to cool slowly to 37°C (3°C/min).
- ☐ 3. Resuspend **SMB** by carefully vortexing tube at medium speed.
- ☐ 4. Transfer 90 µl of **SMB** per sample to a new tube. It is possible to aliquot beads for up to 12 samples (i.e. 1080 µl) in a single tube.
- ☐ 5. Place tube on magnet and wait 1 min. Discard supernatant. Add 80 µl per sample (e.g., 960 µl for 12 samples) of **DB** and agitate tube to resuspend beads.
- ☐ 6. Repeat step 5.
- ☐ 7. Briefly centrifuge the tube containing hybridized RNA (from step 2).
- ☐ 8. Add 80 µl of the prepared SMB to each sample. Mix by pipetting up and down.
- ☐ 9. Incubate samples at 37°C for 15 min, followed by 5 min at 50°C.
- ☐ 10. Place samples on magnet and wait for 2 min. Transfer supernatant to a new tube, without disturbing the beads pellet.
- ☐ 11. Place samples on magnet again for 2 min to remove potential trace amount of beads. Transfer 90 µl of rRNA-depleted RNA to a new tube. [Samples can be stored at -20°C overnight or -80°C for long-term storage].
- ☐ 12. Proceed to RNA purification with clean-up beads (option A) or ethanol precipitation (option B).

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Option A: Clean-up beads purification

- [ ] 1. Add 162 µl resuspended **CB** ● to each sample and mix by pipetting up and down 6-8 times. Incubate at room temperature for 5 min.
- [ ] 2. Place tubes on magnet and let beads separate for 5 min. Discard the supernatant.
- [ ] 3. Add 500-1000 µl of fresh 70% ethanol to each sample and incubate for 30 s at room temperature. Remove all ethanol without disturbing the pellet. **Repeat for a total of 3 washes, while keeping the tubes on the magnet.**
- [ ] 4. Let sample air-dry at room temperature for 8-10 minutes.
- [ ] 5. Add 30 µl of H_2O to each sample and resuspend beads by pipetting up and down several times. Elution volume can be modified if higher/lower RNA concentration is needed; but it's not recommended to eluate in less than 10 µl.
- [ ] 6. Incubate at room temperature for 2 min. Place samples on the magnet and wait 5 min or until beads have separated.
- [ ] 7. Carefully transfer the supernatant to a new tube.

Option B: Ethanol Precipitation

- [ ] 1. Add 10 ul of SA ○, 1 µl of LA ○ and 333 µl of 100% ethanol to each sample.
- [ ] 2. Vortex well and incubate for 30 min at -80°C or -20°C overnight.
- [ ] 3. Centrifuge at 11,000 g (or max speed) for 30 min at 4°C. Remove all supernatant without disturbing the pellet.
- [ ] 4. Add 200 µl of fresh 70% ethanol. Centrifuge at 11,000 g for 5 min at 4°C. Discard the supernatant.
- [ ] 5. Repeat step 4 for a total of 2 ethanol washes.
- [ ] 6. Air-dry pellet at room temperature for 5 min or until all traces of ethanol have evaporated.
- [ ] 7. Resuspend the pellet in 30 µl of H_2O or appropriate elution buffer. If required, elution volume can be decreased down to 10 µl.