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Supplementary Protocol

Enrichment of Poly(A)+ RNA in 200 µl plates

This protocol is used to enrich poly(A)+ RNA from total RNA using 200 µl strip tubes or 96-well plates. This protocol is optimized for RNA originating from all vertebrate species (human, mouse, rat, etc.) and can also be used with RNA originating from bacteria (e.g., isolated from the microbiome) as well as other species.

Important points before starting

- This protocol can be used with total RNA. For best results, use an isolation method described in the *QIAseq Stranded mRNA Select Kit Handbook*, Table 1. Ensure that the RNA has been eluted in RNase-free water.

Things to do before starting

- All buffers and reagents should be vortexed before use to ensure thorough mixing.
- Vortex Pure mRNA Beads for 3 min before first use or for 1 min before subsequent uses.
- Heat a water bath or heating block to 70°C, and then heat Buffer OEB to 70°C.
- Unless otherwise indicated, all protocol steps, including centrifugation, should be performed at room temperature (15–25°C).

Procedure

1. Vortex Pure mRNA Beads for 1 min to thoroughly resuspend.
2. Dilute 100–5000 ng of total RNA using RNase-free water to a final volume of 53 µl (RNA + water), and then add 1 µl RNase Inhibitor.
3. Add 71 µl Buffer mRBB and 25 µl of thoroughly resuspended Pure mRNA Beads to the diluted RNA. Vortex for 5 s, centrifuge for 5 s, and then incubate for 3 min at 70°C.

   Important: Prior to use, ensure that the Pure mRNA Beads are thoroughly resuspended.
4. Remove samples from 70°C and place at room temperature for 10 min.

5. Centrifuge briefly, and then place the tubes onto a magnetic rack. After the solution has cleared (~2 min), carefully discard the supernatant. Leave any residual liquid in the tube to minimize bead loss.
6. Resuspend the beads with 150 µl Buffer OW2. Vortex for 5 s (low setting), centrifuge for 5 s, and then place the tubes onto a magnetic rack. After the solution has cleared (~2 min), carefully discard the supernatant.
7. Repeat step 6 for a total of two washes with 150 µl Buffer OW2.
8. Add 50 µl Buffer OEB and vortex. Briefly centrifuge, and then incubate at 70°C for 3 min.
9. Remove the sample from 70°C and place at room temperature for 5 min.
10. Add 50 µl of Buffer mRBB, and then vortex. Briefly centrifuge, and then incubate at room temperature for 10 min.
11. Briefly centrifuge, and then place the tubes onto a magnetic rack. After the solution has cleared, carefully discard the supernatant. Leave any residual liquid in the tube to minimize bead loss.
12. Add 150 µl of Buffer OW2, and then vortex.
13. Place the tubes onto a magnetic rack. After the solution has cleared, carefully discard the supernatant.
14. Add 31 µl of Buffer OEB heated to 70°C to the bead pellet, and then vortex.
15. Briefly centrifuge, and then place the tubes onto a magnetic rack. After the solution has cleared, transfer 29 µl of the supernatant to a clean tube. The supernatant contains enriched poly(A)+ RNA.
16. Proceed to "Protocol: NGS Library Preparation" in the *QIAseq Stranded mRNA Select Kit Handbook*, or store the samples at –80°C.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.