Streptavidin Pull-down of Biotinylated-HPDP-4SU RNA

From the Notes of Benjy

Materials:

- Streptavidin magnetic beads
- Magnetic Stand
- 5% Beta-mercaptoethanol
- Bead buffer (1M NaCl, 10mM EDTA, 100mM Tris-HCl @ 7.4)
- 1.5 ml eppendorfs

1. Take 200ul of bead buffer per sample and warm to 65C.
2. Label eppendorf and add 200ul beads.
3. Place tubes in magnetic rack and let collect for ~1.5min. Discard supernatant.
4. Wash the beads in 200ul of bead buffer. Mix with pipet. Place samples in magnetic stand and discard supernatant.
5. Resuspend in ~100ul of bead buffer. Make sure that total volume of RNA sample and bead buffer combined will equal ~200ul. If adding more than 50ug, make sure the volume of bead buffer to sample is at least 1:1. That means if you have 100ug in 200ul, add 200ul of the buffer to the 200ul of sample.
6. Add RNA sample.
7. Incubate RT for 20 min. Use vortex mixer for ~5 min on low setting and then give a quick spin in table-top centrifuge to pull droplets to bottom. Leave on bench for rest of 20 min.
8. Place samples in magnetic stand for ~2 min. Discard supernatant (unless interested in unbound mRNA).
9. Resuspend in 100ul bead buffer by pipetting up and down. Incubate RT for 5min. Collect beads in rack. Discard supernatant.
10. Resuspend in 100ul bead buffer by pipetting up and down. Incubate RT for 1 min. Collect beads in rack. Discard supernatant.
11. Resuspend in 100ul bead buffer (65C from earlier) by pipetting up and down. Let sit for 1 min and then collect beads in magnetic rack. Discard supernatant.
13. Resuspend beads in 20ul of 5% Beta-mercaptoethanol. Incubate for 10min at room temperature.
15. Repeat Beta-mercaptoethanol incubation at 65C with the beads from previous step, then pool that with the supernatant from previous step.
16. Precipitate RNA with
   a. 1/10 volume 5M NaCl
   b. At least 4ug glycogen (glycoblue) but 1ul undiluted is best.
   c. 1.1 volume Isopropanol
17. Incubate 10 min RT, spin down at max for 25min at 4C.
18. Discard supernatant and wash with 75% etOH. Spin down for 10min.
19. Resuspend pellet in RNAse free water in 5-10ul H2O.
20. Put in rack, transfer supernatant to new tube, quantify using Qubit Fluorometer.