rRNA Depletion

Wednesday, July 17, 2019 5:14 PM

Prepare Oligo Mix

1. Resuspend all biotinylated oligos in RNase-free water (or buffered water) at 100 µM.
2. Create a undiluted oligo mix by mixing equal aliquots of all 16S and 23S primers and double this for 5S primers. Since the 5S rRNA is so short, there are fewer opportunities to bind it and it seems having 2-fold the concentration of 5S primers improves depletion of the 5S. Generally, I make a mix with 5 µL of each of the 16S and 23S primers and 10 µL of each of the 5S primers and then split this mixture into a few aliquots for storage.
3. The undiluted oligo mix can then be diluted based on the quantity of rRNA to remove. This can be determined with using the calculator spreadsheet.

Prepare beads

Beads are not guaranteed RNase-free, but I have seen no evidence of RNase activity after following the standard washing protocols. Beads can also be washed in bulk and then split for individual reactions.

1. Aliquot calculated per reaction volume of Streptavidin beads into a 1.5 mL tube and place on magnetic rack.
2. Wash with an equal volume of 1x B&W buffer, letting beads settle on magnet for 1 min. Conduct a total of 3 washes. If conducting batch washing (for more than one sample), spilt beads into individual tubes after final wash.
3. Resuspend beads in 30 µL of 2x B&W buffer.
4. Add 1 µL of SUPERAse-In to each tube. Leave beads at room temperature until subtraction is conducted.

Probe annealing

Slow annealing on a thermocycler seems to get more replicable results than heating -> incubation at room temperature.

1. On ice, mix total RNA, 20x SSC, 30 mM EDTA, water and diluted oligo mix in calculated quantities. See below for sample protocol with
2. On a thermocycler, incubate 5 m at 70°C and 30s at each °C incrementing down by 1°C at a time to 25°C.

Subtraction & EtOH precipitation

1. Just before annealing is completed, resuspend beads by vortexing briefly at medium speed.
2. Add annealing reaction directly to beads, pipetting at least 20 times followed by medium-speed vortexing for 10 seconds.
3. Incubate 5m at room temperature.
4. Vortex at medium-speed for 10 sec and then place on heat block at 50°C for 5 minutes.
5. Remove reactions from heat block directly to magnetic rack and allow beads to settle (~1 minute).
6. Pipette up majority of reaction (ideally without magnetic beads!) and place supernatant on ice.
7. BTV 200 µL with RNase-free water (should add ~140 µL water assuming all of reaction was successfully pipetted up). Add 20 µL 3M NaOAc (pH 5.5), 2 µL glycogen, and 600 µL cold 100% EtOH.
8. Incubate at -20°C for at least 1 hour.
9. Spin reactions at maximum speed for 30m at 4°C
10. Wash with 800 µL cold 70% EtOH and respin for 5m at 4°C.
11. Resuspend in 10 µL of RNase-free water.

Estimate depletion

We've used two methods to compare 1 µL of ribosome depleted RNA to a small sample of pre-depletion RNA:
(1) Run out on a denaturing 6% TBE-Urea gel. Make sure you run short enough a time that you can see the 5S and tRNA. This enables you to use tRNA species as a loading control when comparing to the undepleted sample.
(2) Fragment analyzer.

Additional information

2x B&W Buffer (1 mL)

Reagent	[final]	added
1M Tris 7.0	10 mM (7.5 pH)	7 µL
1M Tris 8.0	10 mM (7.5 pH)	3 µL
0.5M EDTA	1 mM	2 µL
5M NaCl	2 M	400 µL
H2O	-	588 µL

Reagents

Reagent	Company	Product Code
Dynabeads MyOne Streptavidin C1	ThermoFisher	65002
SUPERase-In RNase inhibitor	ThermoFisher	AM2696
20x SSC	ThermoFisher	AM9770
0.5 M EDTA	ThermoFisher	AM9260G
3M NaOAc pH 5.5	ThermoFisher	AM9740
Glycoblue	ThermoFisher	AM9516
1M Tris 7.0	ThermoFisher	AM9850G
1M Tris 8.0	ThermoFisher	AM9855G
0.5M EDTA	ThermoFisher	AM9260G
5M NaCl	ThermoFisher	AM9760G