RNaseH Depletion of rRNA

Hybridize Probes

1. Combine the following and mix well

	Volume
5x Hybridization Buffer	2 ul
100 uM Pooled Antisense rRNA Oligos	0.7 ul
Total RNA (~ 1 ug)	7.3 ul
Total	**10 ul**

2. Hybridize using the following program in a thermocycler with heated lid set to 105C

95C	2 minutes
Ramp from 95C to 22C	-0.1 C/s
22C	5 minutes

3. Transfer immediately to ice

RNase H Digestion

1. Prepare the RNase H Mix on ice and mix well. A master mix may be prepared, but the mix must be used immediately, not prepared ahead of time.

	Volume
RNase H (5U/ul)	4 ul
10x RNase H Reaction Buffer	2 ul
H2O	4 ul
Total	**10 ul**

2. Add 10ul of RNase H Mix to each hybridized sample and mix well.
3. Incubate at 37C for 30 minutes
4. Spin down, transfer immediately to ice, and then proceed with DNase I digestion

DNase I Digestion

1. Prepare the DNase I Mix on ice and mix well. A master mix may be prepared, but the mix must be used immediately, not prepared ahead of time.

	Volume
10x DNase I Reaction Buffer	20 ul
DNase I (2U/ul)	8 ul
H2O	152 ul
Total	**180 ul**

2. Add 180 ul of DNase I Mix to each RNase H digested sample
3. Incubate at 37C for 35 minutes
4. Spin down, transfer immediately to ice, and then proceed with cleanup

Clean Up: Zymo RNA Clean & Concentrator-5 kit.

All centrifugation steps should be performed at 10,000 – 16,000 x g.

1. Move the DNaseI reactions to labeled ependorf tubes
2. Add 400ul (2 volumes) **RNA Binding Buffer** to each sample and mix.
3. Add 600ul (equal volume) of **ethanol** (95-100%) and mix.
4. Transfer 500ul of the the sample to the **Zymo-SpinTM IC Column** in a **Collection Tube** and centrifuge for 30 seconds. Discard the flow-through. Transfer the remaining 500ul to the spin column, centrifuge for 30 seconds, and discard the flow-through.
5. Add 400 µl **RNA Prep Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
6. Add 700 µl **RNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through.
7. Add 400 µl **RNA Wash Buffer** to the column and centrifuge for 30 seconds. Discard the flow-through and centrifuge for an additional 2 minutes to remove any residual wash buffer. Transfer the column carefully into a labelled RNase- free tube (not provided).
8. Add 7 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge for 30 seconds.
9. Pipet the 7 µl eluate from the tube back to the column matrix and centrifuge for 30 seconds.
10. **Stop here for the day!** Be sure your tubes are well labelled (include group letter) and bring them to the wet lab staff for overnight storage at -20C