Isolation of RNA from Tissue

From fresh, flash frozen, or from tissue stored in RNAlater (note, rinse tissue in several changes of RNase-free PBS to remove excess RNAlater first)

1. Prep homogenizer: Rinse probe in ddH2O, 1% SDS, then fresh ddH2O
2. Place tissue (up to 100 mg) in Falcon 2059 tube (use forceps sprayed with RNAse-zap)
3. Add 1 ml Trizol reagent and homogenize 3X 20 sec. each time (rest sample between rounds on ice, and rinse homogenizer as above in between samples).
4. Incubate samples for 5-10 minutes at RT.
5. Spin 10 min. @ 12Krpm in Sorvall, transfer 1 mL (max) supe to 1.5 mL tube.
6. Add 200 uL chloroform, vortex 30 sec., spin @ max speed 20 min. at 4 degrees.
7. Transfer aqueous (top, clear) layer to new tube.
8. Add 0.7 volumes isopropanol (~500 ul) to each. Vortex.
9. Inc. at RT 10-15 minutes.
10. Spin at max speed (4 degrees), 20 min.
11. Wash RNA pellets with 1 mL 70% EtOH (RNAse-free).
12. Air dry pellets until most of EtOH gone.
13. Resuspend in 50 uL RNAse-free water.