Total RNA Extraction and Clean-up with Trizol

\*Protocol modified from Marie Strader by Hannah Aichelman, Brooke Benson, Sarah Davies

Note: keep the samples as cold as possible throughout the extraction.

1. Homogenize tissue in 500L of Trizol + beads for 2 min at 6 m/s in bead blaster.
   1. Use the scoopula to transfer beads, clean scoopula with ethanol before using.
2. Let stand for 5 minutes *on ice*.
3. Spin at 12,000 x g for 10 minutes at 4
4. Remove and save supernatant in a fresh tube; discard pellet. Add 100 L *cold* chloroform to supernatant.
5. Invert rapidly for 15 seconds and let stand for 2-3 minutes *on ice*.
6. Spin at 12,000 x g for 15 minutes at 4, put tubes on ice immediately after removing from the centrifuge.
7. Pull off ~200-250L aqueous fraction, transfer to a fresh tube, add 250 L of *ice cold* isopropanol and invert 3 times.
8. Precipitate in -20 (freezer) for 30 minutes, then spin at 12,000 x g and 4 for 30 minutes.
9. Discard supernatant. Wash pellet in 250L 80% *ice cold* ethanol and spin at 12,000 x g for 5 minutes at 4.
10. Repeat wash in 80% cold ethanol.
11. Air dry pellet for 10 minutes.
12. Resuspend pellet in 30 L of 65 RNAse free (volume can be changed based on amount of RNA in your samples.
    1. Note: heat aliquot of DNAse/RNAse free in the heat block.
    2. Also: if continuing straight on to Zymo clean-up, best to re-suspend in 50uL (plus a little extra if running gels beforehand).
13. Store in -80 freezer.