RNA ISOLATION WITH TRIZOL

make up all solutions with 0.1% DEPC treated dH_2O
use filter tips if RNA/DNA is to be used for PCR

1. Add 500 µl TRIzol reagent to eppendorf tubes.

2. Grind tissue in pestle and mortar with liquid nitrogen and transfer a spatula full to the TRIzol containing eppendorf. Vortex.

3. Spin 5' at 12,000 x g at 4ºC to remove chunks of tissue.

4. Transfer supernate to new tube and leave at RT for 5'.

5. Add 200 µl $CHCl_3$ (no isoamylalcohol). Vortex and leave at RT for 5'.

6. Spin at 12,000 x g for 15' at 4ºC. The top aqueous phase contains the RNA. The interphase contains DNA, and the organic phase DNA and protein. Remove supernate (~ 600 µl) and add 500 µl isopropanol.

7. Let stand at room temperature for 10', mix by inverting and spin 10' at 12,000 x g at 4ºC. RNA should form a white pellet at bottom of tube.

8. Pour off supernate and wash with 1 ml 75 % EtOH by vortexing. Spin 5' at 7500 x g at 4ºC.

9. Let air dry for 10' and resuspend in 50 µl DEPC dH_20.