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## Tissue collection and extractions for RNA-seq

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Works for me

dx.doi.org/10.17504/protocols.io.basiiece

Mimulus

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Gould BA, Chen Y, Lowry DB. 2018. Gene regulatory divergence between locally adapted ecotypes in their native habitats. *Molecular Ecology*. 27: 4174-4188.

### GUIDELINES

A key aspect of collecting tissue for RNA-seq is that you want to make sure that all samples are collected during the same short period of the day to avoid effects of the circadian cycle. 1/3 to 1/2 of genes greatly change expression during the day. If you don't take this into account, your work may be unpublishable.

### SAFETY WARNINGS

Wear gloves during collections for safety from liquid nitrogen and also to prevent getting RNases from your hands onto the tissue or tubes.

### BEFORE STARTING

Get supplies together including a dewer containing liquid nitrogen, a container to pour the liquid nitrogen into, a large plastic spoon with holes in for removal of tubes from liquid nitrogen, and a cooler containing dry ice. Label all tubes beforehand. We have found that the Fisherbrand Premium Microcentrifuge tubes are most durable for collections of tissue into liquid nitrogen.

<https://www.fishersci.com/shop/products/fisherbrand-premium-microcentrifuge-tubes-2-0ml-9/05408140>. Clean area and equipment for collections with ethanol to destroy RNases before beginning collections.

- 1 Pour liquid nitrogen into a polystyrene cooler or another container that can safely hold liquid nitrogen.
- 2 Remove tissue from plant and place in a tube. Make sure to clean scissors or forceps with ethanol prior to collections.
- 3 Immediately plunge tube into liquid nitrogen (transcription profiles change rapidly, so do this as fast as possible).
- 4 Transfer tubes into a cooler containing dry ice for transport to a -80C freezer. Store at -80C until ready to extract.
- 5 Extract tissue with a Spectrum Plant Total RNA Kit (Sigma-Aldrich). The Lowry lab has achieved consistently high yields of RNA from *Mimulus guttatus* using this kit. [https://www.sigmaaldrich.com/catalog/product/sigma/strn50?lang=en&region=US&qclid=CjwKCAiA\\_HvBRACEiwAbViuU4EO5CpJNBCqRf-923Uw8ZrDfSQ6bRXNHlhX4t6lKE7jA5AAHO5UxoCtP0QAvD\\_BwE](https://www.sigmaaldrich.com/catalog/product/sigma/strn50?lang=en&region=US&qclid=CjwKCAiA_HvBRACEiwAbViuU4EO5CpJNBCqRf-923Uw8ZrDfSQ6bRXNHlhX4t6lKE7jA5AAHO5UxoCtP0QAvD_BwE)



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