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 We use this protocol and it's working

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Tissue collection and extractions for RNA-seq

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Mimulus

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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 START INSTRUCTIONS

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/stn50?lang=en®ion=US&gclid=CjwKCAiA_HvBRACEiwAbViuU4EOr5CpJNBCqRf-923Uw8ZrDfSQ6bRXNHlhX4t6lKE7jA5AAHO5UxoCtP0QAvD_BwE