Dec 20, 2019

Tissue collection and extractions for RNA-seq

DOI
https://dx.doi.org/10.17504/protocols.io.basiiece

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Mimulus

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.basiiece

Protocol Citation: David Lowry 2019. Tissue collection and extractions for RNA-seq. **protocols.io** https://dx.doi.org/10.17504/protocols.io.basiiece

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

Protocol status: Working
We use this protocol and it's working

Created: December 20, 2019

Last Modified: December 20, 2019

Protocol Integer ID: 31274

Keywords: extractions for rna, rna, tissue collection, seq, extraction

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

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 foreceps 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&gclid=CjwKCAiA__HvBRACEiwAbViuU4EOr5CpJNBCqRf-923Uw8ZrDfSQ6bRXNHIhX4t6lKE7jA5AAHO5UxoCtP0QAvD_BwE