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RNA Isolation from Plant Tissue

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ABSTRACT

Methods for RNA isolation

These methods were originally included in Appendix S1 of "Evaluating Methods for Isolating Total RNA and Predicting the Success of Sequencing Phylogenetically Diverse Plant Transcriptomes" Marc T. J. Johnson et al. PLOS ONE, November 21, 2012. https://doi.org/10.1371/journal.pone.0050226

Many of the protocols share elements or combine components from several methods. For each method, we describe the reagents and procedures used, and identify the researchers or institute that implemented the protocol.

Due to the potential for contamination and degradation by RNase enzymes, as well as health concerns in handling some substances and chemicals, best practices in aseptic wet lab techniques must be practiced at all times during RNA isolation. Chief among these are the critical need to avoid contamination of samples by using extreme care when moving liquids and opening and closing tubes to avoid aerosols. Because of the risk of degradation by RNase enzymes, it is essential to use sterile RNase-free equipment, disposable plastics and solutions.

RNase degradation and contamination can be avoided by keeping samples constantly frozen at low temperature (< -80 °C) prior to adding buffers that denature or immobilize RNase. Treating equipment with RNase denaturants (e.g. RNase Zap, Ambion, Austin, TX) and solutions with diethylpyrocarbonate (DEPC) can also prevent contamination and/or degradation of samples, but it can have some negative effects on samples[1]. Many additional helpful tips for successful RNA isolation are available in Sambrook and Russell[1] and in Appendix A of Qiagen's RNeasy Mini Handbook downloadable from www.qiagen.com.

[1] Sambrook, J. & Russell, D.W. Molecular Cloning: A Laboratory Manual, 3rd ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001).

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Appendix S1 of "Evaluating Methods for Isolating Total RNA and Predicting the Success of Sequencing Phylogenetically Diverse Plant Transcriptomes" Marc T. J. Johnson et al. PLOS ONE, November 21, 2012. https://doi.org/10.1371/journal.pone.0050226

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MATERIALS TEXT

Standard equipment used

Although the reagents vary among protocols, most methods used the same basic equipment, which include:

- Ceramic or porcelain mortar and pestle for tissue homogenization, or some other equipment that can homogenize frozen tissue (e.g. bead mill)
- Water bath or heating block, capable of holding temperatures up to 70°C
- Non-refrigerated microcentrifuge (with rotor for 2 ml tubes)
- Refrigerated microcentrifuge (with rotor for 2 ml tubes) – only for protocols where noted
- Liquid nitrogen (and associated thermos or dewer)
- Sterile, RNase-free disposable tips with filter barriers
- Sterile, RNase-free disposable microcentrifuge tubes (2 ml and 15 ml were most commonly used, but sizes vary among protocols)
- Pipettors – (capable of pipetting various volumes between 0.1–1000µl)
- Stainless steel spatulas
- RNase-free water
- Sterile razor blades
- Analytical balance
- Glass beakers, flasks and graduated cylinders

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

Collection protocols