

Simple Method for Efficient RNA Extraction From *Arabidopsis* Embryos

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Abstract

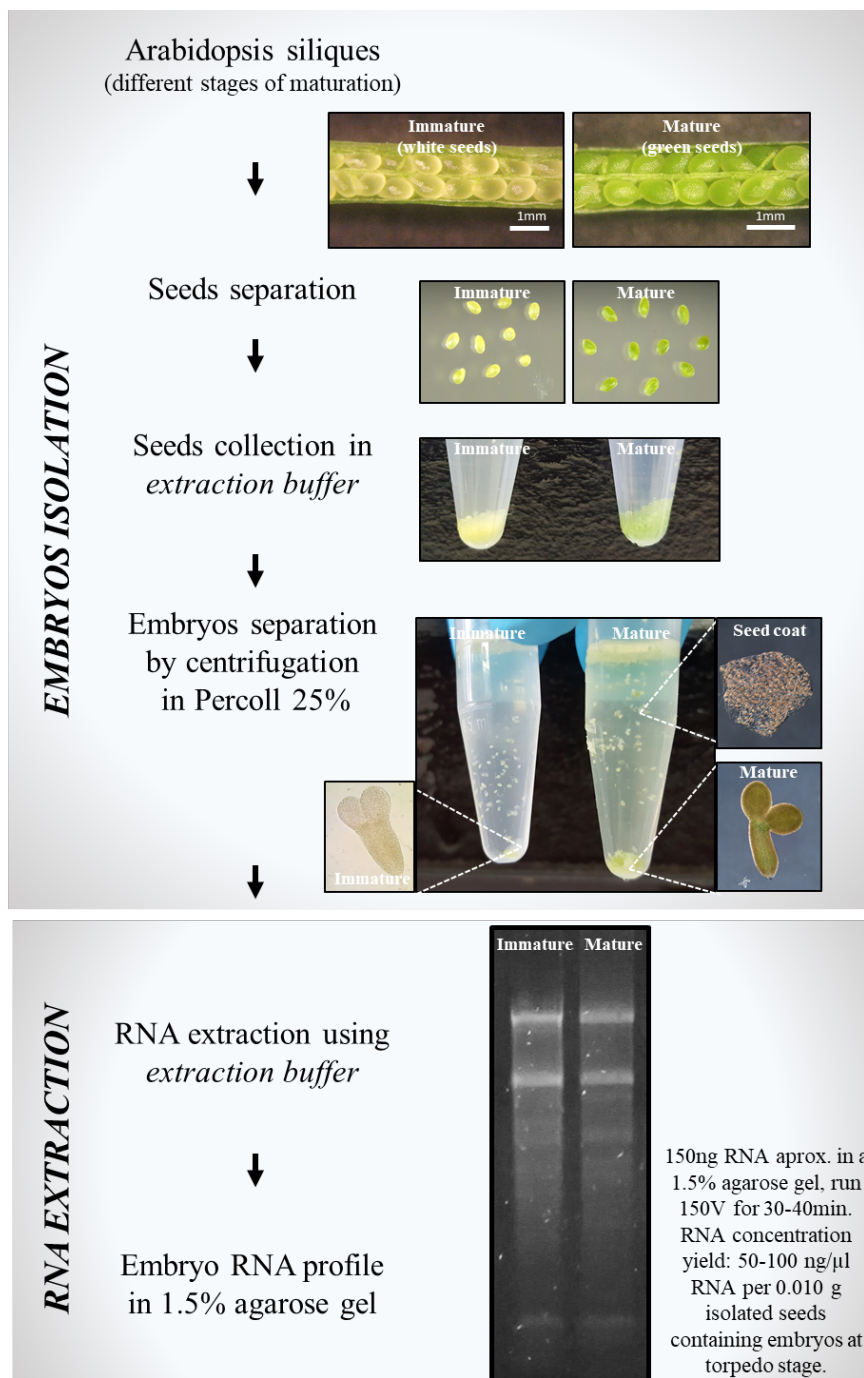
Plant embryos are contained within seeds. Isolating them is crucial when endosperm and seed coat tissues interfere with the study of mutant genetic functions due to differing genotypes between maternal and embryonic tissues. RNA extraction from plant embryonic tissue presents particular challenges due to the high activity of RNases, the composition of the seed, and the risk of RNA degradation. The developmental stage of the embryo is a key aspect of successful isolation and RNA extraction due to the size and amount of tissue. Proper handling during RNA extraction is critical to maintain RNA integrity and prevent degradation. While commercial kits offer various methods for RNA extraction from embryos, homemade protocols provide valuable advantages, including cost-effectiveness and accessibility for labs with limited funding. Here, we present a simple and efficient protocol for extracting RNA from isolated *Arabidopsis thaliana* embryos at the torpedo/cotyledon stage using a homemade extraction buffer previously reported for styles of *Nicotiana glauca*.

Key features

- This straightforward homemade protocol builds upon established methods for embryo isolation [1] and RNA extraction [2,3].
- It enables the extraction of high-quality RNA from *Arabidopsis* embryos at the torpedo stage.
- The protocol is designed to be both simple and cost-effective, making it an excellent option for labs with limited funding.

Keywords: Arabidopsis embryo, Embryo isolation, RNA extraction, Simple and efficient RNA extraction, Homemade protocol, Embryo development

Graphical overview



Simple method for RNA extraction from isolated *Arabidopsis* embryos using a homemade extraction buffer

Background

This protocol describes a straightforward two-step method for extracting RNA from isolated *Arabidopsis* embryos. Isolating RNA from plant tissues such as embryos, which are rich in RNases, poses significant challenges due to the risk of RNA degradation during sample preparation. To overcome these difficulties, commercial solutions such as RNeasy are commonly used to stabilize RNA by inactivating RNases, allowing for subsequent extraction using reagents like Trizol. In this protocol, we present a simple and effective homemade extraction buffer designed specifically for seed collection and RNA extraction. This buffer facilitates the isolation of high-quality RNA from *Arabidopsis* embryos. The method described here was successfully employed to extract RNA from developing embryos at the early torpedo and early cotyledon stages, from both immature (white) and mature (green) seeds. This approach offers an accessible and efficient alternative to commercial reagents, ensuring robust RNA recovery and preservation for downstream applications.

Materials and reagents

Biological materials

1. *Arabidopsis thaliana* ecotype Columbia (Col-0) wild type (WT) and embryo-lethal mutant plants from the *Arabidopsis* biological resource center (ABRC, <https://abrc.osu.edu/>)

Reagents

1. Tris [Tri(hydroxymethyl)aminomethane] (Genbiotech, catalog number: RU2510)
2. EDTA (ethylenediamine tetraacetic acid disodium salt) (Biopack, catalog number: 1604.07)
3. Urea (Sigma, catalog number: U5378)
4. Sodium dodecyl sulfate (SDS) (Anedra, catalog number: 7211)
5. Ammonium acetate (Fluka, catalog number: 09690)
6. Lithium chloride (LiCl) (Sigma, catalog number: L9650)
7. 2-Mercaptoethanol pure (Biopack, catalog number: 9545.05)
8. Diethyl pyrocarbonate (DEPC) (Sigma, catalog number: D5758)
9. Percoll (GE Healthcare, catalog number: 17-0891-01)
10. DEPC (diethylpyrocarbonate) (Sigma, catalog number: D5758)
11. SYBRTM Safe DNA gel stain (Invitrogen, catalog number: S33102)
12. Agarose (Gene Biotech LE-Agarose 1200, catalog number: RU1010)
13. DEPC water (1 mL DEPC/L water; stirred overnight and autoclaved)
14. Phenol:chloroform:isoamyl alcohol 25:25:1 (Sigma, catalog number: P3803)
15. Chloroform (Biopack, catalog number: 1651.08)
16. Isopropanol (Mallinckrodt, catalog number: 3032-08)

Solutions

1. Buffer Tris HCl pH 8, 1 M (see Recipes)

2. EDTA pH 8, 0.5 M (see Recipes)
3. SDS 10% (see Recipes)
4. Ammonium acetate, 10 M (see Recipes)
5. Lithium chloride, 8 M (see Recipes)
6. Extraction buffer (see Recipes)

Recipes

1. Buffer Tris-HCl pH 8, 1 M

Weigh 12.11 g of Tris, add 75 mL of pure sterile water, adjust to pH 8 with HCl, and fill with pure sterile water to a final volume of 100 mL.

2. EDTA pH 8, 0.5 M

Weigh 18.6 g of EDTA, add 75 mL of pure sterile water, adjust to pH 8 using 10 M NaOH, and stir until EDTA is completely dissolved. Solubility improves while alkalinity increases; complete with pure sterile water to a 100 mL final volume.

3. SDS 10%

Dissolve 10 g of SDS in 100 mL of pure sterile water.

4. Ammonium acetate, 10 M

Dissolve 7.78 g of ammonium acetate in 10 mL of DEPC water.

5. Lithium chloride, 8 M

Dissolve 3.39 g of lithium chloride in 10 mL of DEPC water.

6. Extraction buffer (10 mL)

Reagent	Final concentration	Quantity or Volume
Urea	7 M	4.2 g
EDTA	10 mM	200 µL
Tris-HCl (1 M, pH 8)	100 mM	1 mL
SDS 10%	1%	1 mL
2-Mercaptoethanol	1%	100 µL
H ₂ O	to 10 mL	*see note

**Note: Weigh urea in a 50 mL Falcon tube and dissolve urea in half volume (5 mL for a final volume of 10 mL) of water. Vortex the solution to help it dissolve. The bottom of smaller tubes hinders the full solubility of urea. Then, add the rest of the components and complete to final volume. Keep at room temperature. Cold will precipitate SDS in the extraction buffer. No risk for later RNA extraction.*

Laboratory supplies

1. Syringe, needles (13 × 0.3 mm) and pointy tips tweezers (Dumont, catalog number: 0103-2-PO)
2. Plastic grinding rod for Eppendorf tubes (SSibio, catalog number: 1005-39)

Equipment

1. Magnifying glass (LABKLASS, model: HG468405)
2. Table centrifuge (Eppendorf, model: centrifuge 5418)
3. Vortex (Vornado™, Benchmark Scientific, catalog number/model: BV101-B)
4. Agarose gel electrophoresis equipment (Bio-Rad, model: PowerPac™ Basic Power Supply, Horizontal Electrophoresis Systems)
5. NanoDrop™ One/One (Thermo Fisher, catalog number/model: ND-ONE-W)

Procedure

A. Embryo isolation

1. Collection of seeds
 - a. Add 100 µL of extraction buffer (see Recipes) in a 1.5 mL Eppendorf tube. Weigh the tube.
 - b. Using a needle under a magnifying glass, open mature or immature siliques to collect green and/or white seeds, respectively. Place the seeds inside the Eppendorf tube containing the extraction buffer. Collect seeds from approximately 25 siliques. Weigh the tube after collecting seeds. For a satisfactory RNA extraction, the minimum recommended amount for seed collection is 0.010 g of tissue.
 - c. Spin down the embryos in a table centrifuge at $1,700 \times g$ for 30 s. Carefully remove the extraction buffer by pipetting. Wash the embryos three times with 1 mL of DEPC water. Spin down at $1,700 \times g$ after each washing.
2. Embryo isolation from seed coat (from Perry and Wang [2])
 - a. Remove 750 µL of the DEPC water per tube.
 - b. Shake gently the lower part of the Eppendorf tube by hand (using fingers) just to let the seeds spread in the remaining water.
 - c. Use a plastic grinding rod for Eppendorf tubes (plastic sticks) to press softly the sample against the tube's wall to release embryos from seeds by applying soft pressure. Repeat three times with smooth movements.
 - d. Transfer the sample (250 µL) by pipetting to a new tube containing 500 µL of DEPC water and 250 µL of Percoll (25% v/v Percoll). To facilitate pipetting the sample, use a 200 µL yellow tip with the tip cut off.
 - e. Centrifuge at $72 \times g$ for 10 min.
 - f. Remove the seed coats of the upper layer together with the Percoll solution by pipetting.
 - g. Resuspend the embryos carefully in 250 µL of the remaining Percoll solution and transfer the sample by pipetting to a new tube containing 0.75 mL of 25% v/v Percoll solution.
 - h. Centrifuge at $72 \times g$ for 10 min.
 - i. Remove the seed coats from the upper layer and discard the remaining Percoll by pipetting.
 - j. Wash the embryos three times with 1 mL of DEPC water. Spin down at $72 \times g$ after each washing.

B. RNA extraction [2,3]

Before starting, prepare Eppendorf tubes containing:

- I. 500 µL of phenol:chloroform:isoamyl alcohol (25:24:1) + 500 µL of extraction buffer
- II. 0.5 mL of phenol:chloroform:isoamyl alcohol (25:24:1)

III. 0.5 mL of chloroform

IV. 0.1 mL of 10 M ammonium acetate

1. Remove washing water from the tube by pipetting. Add 100 μ L of extraction buffer and use a plastic grinding rod to crash the tissue against the tube wall. Crash embryos completely.
2. Add the sample to tube I and vortex immediately for 2 min.
3. Centrifuge at $18,000 \times g$ for 10 min at room temperature. Take the upper phase and transfer it to tube II. Vortex vigorously for 2 min.
4. Centrifuge at $18,000 \times g$ for 10 min at room temperature. Transfer the upper phase to tube III. Vortex vigorously for 2 min.
5. Centrifuge at $18,000 \times g$ for 10 min at room temperature. Transfer the aqueous phase to tube IV. Add 1 volume of cold isopropanol. Mix by inversion. Store at -20°C for any duration between 30 min and overnight.
6. Centrifuge at $18,000 \times g$ for 15 min at 4°C . Discard supernatant by inverting the tube. Dry pellet at room temperature.
7. Resuspend the pellet in 300 μ L of DEPC water. Maintain tubes on ice. Check that the pellet is well-solubilized. Heat sample at 55°C for 5 min to better solubilize the pellet (optional). Add 300 μ L of 8 M LiCl and mix by inversion. Place the tubes inside an ice bucket and leave on ice overnight inside the refrigerator to precipitate RNA.
8. Centrifuge at $18,000 \times g$ for 15 min at 4°C . Discard supernatant after centrifugation.
9. Resuspend the pellet in 300 μ L of DEPC water. Add 100 μ L of ammonium acetate. Fill the tube with 96% cold ethanol. Precipitate at -20°C for at least 30 min.
10. Centrifuge at $18,000 \times g$ for 15 min at 4°C . Wash pellet with 70% ethanol. Centrifuge at $18,000 \times g$ for 15 min at 4°C . Discard supernatant. Dry the pellet at room temperature and resuspend in 20 μ L of DEPC water.
11. Check RNA profile by loading 3 μ L of RNA (approximately 100–150 ng of RNA) in a 1.5% agarose gel; run at 150 V for 30–40 min. RNA concentration yield is approximately 50–100 ng/ μ L RNA per 0.010 g of isolated seeds containing embryos at the torpedo stage.
12. RNA can be stored at -20°C for short-term uses. Storage at -80°C is recommended for long-term use.

Validation of protocol

This protocol was developed by combining two existing protocols:

For RNA extraction:

- McClure et al. [2]. Self incompatibility in *Nicotiana glauca* involves degradation of pollen rRNA. Nature.
- Roldán et al. [3]. Molecular and genetic characterization of novel S-RNases from a natural population of *Nicotiana glauca*. Plant Cell Rep (using *extraction buffer*, no commercial kits).

For embryo isolation:

- Perry, S. E. and Wang, H. [1]. Rapid isolation of *Arabidopsis thaliana* developing embryos. BioTechniques. (using commercial stabilization solutions).

The innovation lies in the simultaneous application of both pre-existing protocols along with the incorporation of a homemade extraction buffer for RNA extraction from tissues with high RNase activity, such as *Arabidopsis* embryos, without relying on commercial kits. In our hands, the homemade extraction buffer effectively prevents RNA degradation from *Arabidopsis* embryos. The protocol presented here represents a valuable alternative for

two challenging steps when working with *Arabidopsis* seeds: first, the separation of embryos from seed coats (embryo isolation step), and second, RNA extraction from tissues that require RNase inhibitors and stabilization solutions to prevent RNA degradation (RNA extraction using homemade buffer step).

General notes and troubleshooting

General notes

This protocol is an alternative for RNA extraction from isolated *Arabidopsis* embryos using a homemade extraction buffer to prevent RNA degradation of tissues with high RNase activity.

Embryo developmental stages play a crucial role in obtaining the necessary amount of tissue. For example, mature seeds (light green) containing embryos at advanced developmental stages, such as early cotyledon or cotyledon stages, have larger embryos and provide higher amounts of tissue when collecting from *Arabidopsis* siliques. In contrast, immature seeds (white seeds) contain embryos at early torpedo and torpedo developmental stages, which are smaller than embryos found in mature seeds. However, 0.010 g of tissue yield around 100–150 ng/μL RNA, suitable for cDNA synthesis. Collection of 0.010 g can be performed in approximately 30 min. The more seeds collected, the more RNA can be obtained.

This protocol can be implemented not only for immature seeds containing embryos at the early torpedo stage but also for seeds at earlier developmental stages, for example, seeds that contain embryos at the heart stage. In those cases, collecting more tissue is required because embryos are smaller in size. In our hands, this protocol works great for the isolation of embryos at early stages of development, and RNA extraction succeeds. When collecting 0.010 g of immature seeds when working with embryos at the heart stage, the tissue yield is approximately 5–10 ng/μL RNA, also suitable for cDNA synthesis. However, when working with earlier embryo developmental stages, such as globular or early heart stages in which the embryonic amount of tissue is low (in relation to the whole seeds), this protocol can be applied by skipping the embryo isolation step to work with the whole seeds (embryo, endosperm, and seed coat), performing the step of RNA extraction straightforward with the homemade extraction buffer, which will prevent RNA degradation. When collecting 0.010 g of whole immature seeds, the yield is around 60–160 ng/μL RNA, which corresponds to RNA extracted from the entire seed and not only from isolated embryos as described above but also suitable for cDNA synthesis.

Measurements of RNA concentration were done using NanoDrop™ by absorbance at 260 nm and purity was estimated by the ratio 260/280 nm. RNA profile was analyzed by running (approximately) 150 ng of RNA in a 1.5% agarose gel. Note that RNA extracted from plant “white” tissue, such as embryo or root, lacks plastid RNA. In these cases, the RNA profile is composed mainly of two bands corresponding to cytoplasmic 28S and 18S rRNA, different from the RNA profile extracted from leaves in which smaller bands are present, corresponding to organellar rRNAs.

In summary, the protocol presented here is a great alternative to extract RNA from isolated *Arabidopsis* embryos as well as directly from seeds (when containing embryos in early developmental stages). It may be very useful when studying, for example, embryo-lethal mutants. Besides, using a homemade buffer suitable to prevent RNA degradation represents a valuable alternative when there is limited access to commercial stabilization solutions or kits.

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Competing interests

There are no competing interests.

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