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Protocol status: In development Protocol is working but we are continuing to optimize

(3) Isolating enriched fractions of nuclear and cytoplasmic RNAs from Arabidopsis flowers

Diep R Ganguly¹, Wil Prall¹, Susheel Sagar Bhat¹, Sean Hilgendorf¹, Brian D Gregory¹

¹University of Pennsylvania



Diep R Ganguly

University of Pennsylvania, The Australian National Universi...

ABSTRACT

This is an adapted protocol to isolate RNA fractions enriched for nuclear and cytosolic RNAs from frozen Arabidopsis flowers using the Norgen Biotek Cytoplasmic and Nuclear RNA Purification Kit (Product # 21000) alongside the RNase-Free DNase I Kit (Norgen, # 25710).

GUIDELINES

- 20-50 mg of Arabidopsis flowers yields approximately 20 μg cytosolic- and 5 μg nuclear-enriched RNA.
- Perform qRT-PCR on the resulting fractions with primers targeting mature mRNAs and pre-mRNAs (i.e. primers binding intron-exon junctions) to quantify enrichment. For example, we quantify pre-mRNA (nuclear-enriched) and mature mRNA (cytosol-enriched) of PP2AA3 (AT1G13320, mRNA: F CGACCAAGCGGTTGTGGAGA, R CACCATTCGTTGCTGTCTTCTTT; pre-mRNA: F GCTTTATGGGAAAGCTGTAAGG, R CAACCATATAACGCACACGCC).
- We also quantify long non-coding RNAs IncCOBRA3 (AT3G03435, F GTTGAGTCGCTTCGTCTATGT, R TGCCATCATAGGATCCTTCAATAA) and IncCOBRA5 (AT3G05655, F TGCACAAGTACTGGGACATC, R GATTCGGGTCGGGTCATAAG), which show a strong enrichment in the nucleus (Kramer et al 2022, Frontiers in Plant Science).

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MATERIALS

- Norgen Biotek Cytoplasmic and Nuclear RNA Purification Kit (Product # 21000)
- RNase-Free DNase I Kit (Norgen, # 25710)
- RNase-free filter tips and microcentrifuge tubes
- Refrigerated benchtop centrifuge
- 2-mercaptoethanol
- Ethanol
- Sodium acetate, RNase-free (3 M, pH 5.5)
- Glycogen or GlycoBlue (Invitrogen, AM9516)
- Nuclease free water or Tris-EDTA (10 mM Tris-Cl, 0.1 mM EDTA, pH 6.5)

SAFETY WARNINGS



2-mercaptoethanol is toxic, use in fume hood to avoid inhaling vapors and wear gloves.

BEFORE START INSTRUCTIONS

- Prepare an RNase-free environment (e.g. clean bench and pipettes, filter tips, nuclease-free solutions).
- Make sure plant material has been frozen and ground into a fine powder.
- Prepare 200 $\mu\text{L/sample}$ Lysis Buffer J containing 10 $\mu\text{L/mL}$ 2-mercaptoethanol (prepare before use).
- Prepare 600 μ L/sample Buffer SK containing 10 μ L/mL 2-mercaptoethanol (prepare before use).
- Ensure Wash Solution A contains ethanol.
- Ensure you have working aliquots of RNase-free DNase I (Norgen).

Lyse cells and fraction RNA

- 1 Grind frozen Arabidopsis flowers into a fine powder using a pestle. Keep samples frozen in liquid nitrogen until you are ready to add lysis buffer.
- 2 Remove samples from liquid nitrogen and immediately add Lysis Buffer J (200 μ L per 20-50 mg tissue).

3	Mix samples with gentle inversion until tissue is completely dissolved.
4	Incubate for 3 minutes at ambient temperature.
5	Centrifuge lysate for 10 minutes at max speed.
6	Transfer supernatant (cytoplasmic RNA) to a clean nuclease-free tube. Be careful not to disturb the pellet (nuclear RNA).
	Bind RNA onto column
7	Add 200 μL Buffer SK to cytoplasmic RNA.
8	Add 400 μL Buffer SK to nuclear RNA.
9	Mix all samples vigorously for 10 seconds.
10	Add 200 µL of 100% ethanol to all samples then mix vigorously for 10 seconds.

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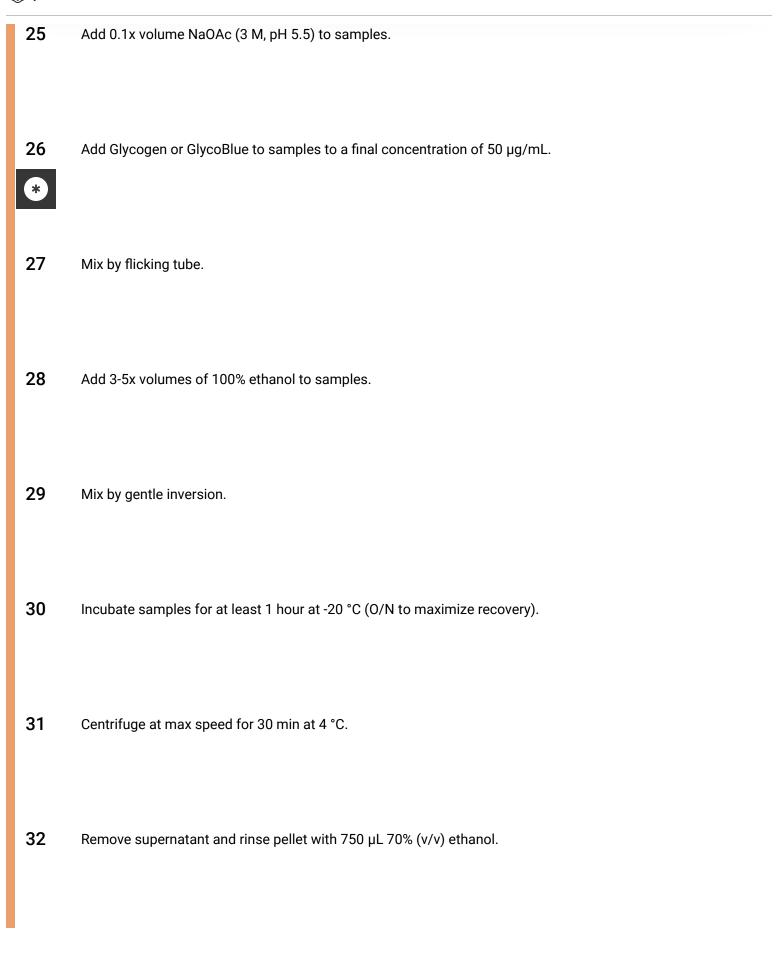
- Add 400 μ L of Wash Solution A to all columns, centrifuge for 1 minute at 18,000 rcf, then discard flow through.
- 19 Perform a final wash to all tubes, discard flow through, and reassemble spin column on collection tube.
- 20 Spin the column for 2 minutes at 18,000 rcf to thoroughly dry the resin.

Elution

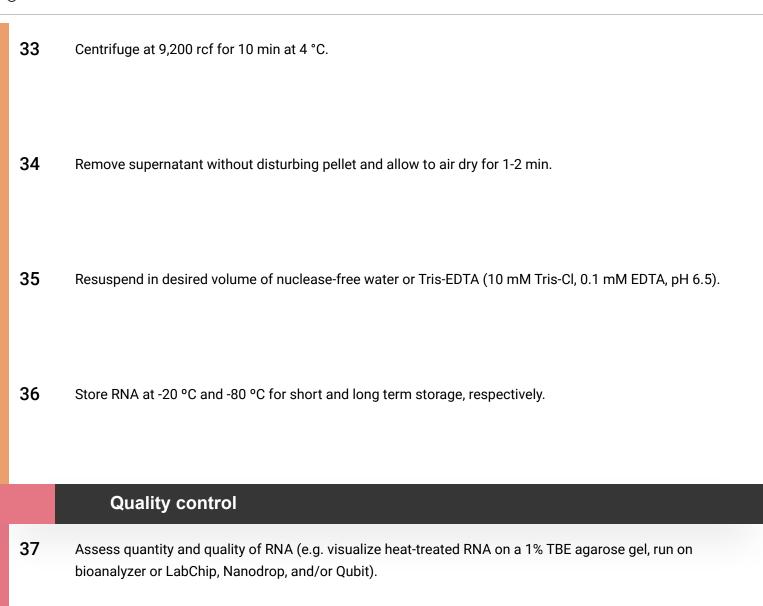
- Transfer column to clean nuclease-free tube (discard the collection tube).
- 22 Add 50 μ L of Elution Buffer E (or 10 mM Tris-Cl, 0.1 mM EDTA, pH 6.5).
- 23 Centrifuge for 2 minutes at 200 rcf, then 1 minute at 18,000 rcf.
- 24 Perform a second elution step for maximum RNA recovery.



Ethanol precipitation (optional)



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Perform qRT-PCR to test for level of enrichment by, for example, comparing mature mRNA and pre-mRNA

levels in each fraction (using primers spanning intron-exon junctions).

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