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Isolating enriched fractions of nuclear and cytoplasmic RNAs from Arabidopsis flowers

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Protocol is working but we are continuing to optimize

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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 - CACAATTCGTTGCTGTCTTCTTT; pre-mRNA: F - GCTTTATGGGAAAGCTGTAAGG, R - CAACCATATAACGCACACGCC).
- We also quantify long non-coding RNAs lncCOBRA3 (AT3G03435, F - GTTGAGTCGCTTCGTCTATGT, R - TGCCATCATAGGATCCTTCAATAA) and lncCOBRA5 (AT3G05655, F - TGCACAAGTACTGGGACATC, R - GATTCGGGTCGGGTCATAAG), which show a strong enrichment in the nucleus (Kramer et al 2022, Frontiers in Plant Science).

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)

Troubleshooting

Safety warnings

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

Before start

- 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 µL/sample Lysis Buffer J containing 10 µL/mL 2-mercaptoethanol (prepare before use).
- Prepare 600 µL/sample Buffer SK containing 10 µL/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 µL 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.

11 Transfer lysate to a spin column on a collection tube then centrifuge for 1 minute at 18,000 rcf.

12 Discard flow through and reassemble spin column on collection tube.

Washing RNA and on-column DNase treatment (nuclear RNA only)

13 Add 400 µL of Wash Solution A to the column and centrifuge 2 minutes at 14,000 rcf.

14 Discard flow through and reassemble spin column with collection tube.

15 For every on-column nuclear RNA sample, mix 15 µL DNase I with 100 µL enzyme incubation buffer from the RNase-free DNase I kit (Norgen Biotek, # 25710). Mix by gentle inversion.

16 Add 100 µL of RNase-free DNase I to columns with nuclear RNA and centrifuge for 1 minute at 18,000 rcf (ensure entire DNase I solution passes through filter).

17 Pipette flow through back onto column and incubate at 25 - 30 °C for 15 minutes.

18 Add 400 µL 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

21 Transfer column to clean nuclease-free tube (discard the collection tube).

22 Add 50 µL 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)

25 Add 0.1x volume NaOAc (3 M, pH 5.5) to samples.

26 Add Glycogen or GlycoBlue to samples to a final concentration of 50 µg/mL.

27 Mix by flicking tube.

28 Add 3-5x volumes of 100% ethanol to samples.

29 Mix by gentle inversion.

30 Incubate samples for at least 1 hour at -20 °C (O/N to maximize recovery).

31 Centrifuge at max speed for 30 min at 4 °C.

32 Remove supernatant and rinse pellet with 750 µL 70% (v/v) ethanol.

33 Centrifuge at 9,200 rcf for 10 min at 4 °C.

34 Remove supernatant without disturbing pellet and allow to air dry for 1-2 min.

35 Resuspend in desired volume of nuclease-free water or Tris-EDTA (10 mM Tris-Cl, 0.1 mM EDTA, pH 6.5).

36 Store RNA at -20 ºC and -80 ºC for short and long term storage, respectively.

Quality control

37 Assess quantity and quality of RNA (e.g. visualize heat-treated RNA on a 1% TBE agarose gel, run on bioanalyzer or LabChip, Nanodrop, and/or Qubit).

38 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).

Protocol references

https://norgenbiotek.com/product/cytoplasmic-and-nuclear-rna-purification-kit