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We use this protocol and it's working

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Co-extraction of RNA and DNA from plant tissue

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ABSTRACT

This protocol describes how to co-extract RNA and DNA from plant tissue samples. Samples are homogenized and simultaneously lyzed by bead-beating. Cell debris is then caught with a pre-filter column, the DNA is then subsequently bound to a silica column, while the RNA passes the membrane. The RNA in the flow-through is then precipitated with 100% ethanol and bound to a second silica column. Both, DNA and RNA are washed with different wash buffers to remove remaining proteins and other contaminants and finally eluted in separate tubes. If the user is just interested in the RNA, the DNA spin-column can just be discarded.

GUIDELINES

Follow general lab etiquette. Wear gloves to prevent contamination of samples. Clean the workspace before starting and after finishing with 80% EtOH.

MATERIALS

Materials required:
Below all materials needed for the protocol are listed. Vendors and part numbers are listed but interchangeable depending on the supply situation.

Chemicals:
Guanidinium thiocyanate
Guanidinium thiocyanate **Fisher Scientific Catalog #10503345**
Tris ultrapure 99.9% Tris ultrapure 99.9% **Diagonal Catalog #A1086.1000**
Hydrochloric acid fuming 37%
Hydrochloric acid fuming 37% **Sigma Aldrich Catalog #1003171011**
Pre-filter columns
Pre Filter Columns - 850 µl **Biopolymer Isolation Technologies Catalog #M 01P-100**
Guanidinium chloride
Guanidine hydrochloride **Fisher Scientific Catalog #10543325**
Ethanol absolute
Ethanol absolute 99.8% **Fisher Scientific Catalog #11994041**

Labware:

2 mL screwcap tubes 2 mL screwcap tube **Sarstedt Catalog #72.693**

2 mm zirconia beads

Zirconia Beads 2 mm dia **BioSpec Products Catalog #11079124zx**

0.1 mm glass beads

Glass Beads 0.1 mm dia **BioSpec Products Catalog #11079101**

EconoSpin mini spin column

EconoSpin mini spin clumn with lid **Epoch Life Science Catalog #1920-050**

Stock solutions:

1 L Tris stock solution [M] 1 Molarity (m) pH 7.5

- Add 121.1 g Tris ultrapure 99.9% to a beaker
- Adjust volume to 800 mL with ddH_2O
- Adjust pH to pH 7.5 with HCl
- Adjust volume to 1 L with ddH_2O

1 L sodium chloride stock solution [M] 5 Molarity (m)

- Add 292.2 g sodium chloride to a beaker
- Adjust volume to 1 L with ddH2O
- Sterilize by filtering and store at Room temperature

1 L Tris stock solution [M] 1 Molarity (m) pH 8.5

- Add 121.1 g Tris ultrapure 99.9% to a beaker
- Adjust volume to 800 mL with ddH_2O
- Adjust pH to pH 8.5 with HCl
- Adjust volume to 1 L with ddH_2O

1 L DNA wash buffer 2 stock solution [M] 50 millimolar (mM) Tris pH 7.5

- Add 50 mL of [M] 1 Molarity (m) Tris stock solution pH 7.5 to a beaker
- Adjust volume to 1 L with ddH2O
- Sterilize by filtering and store at Room temperature

Working solutions:

1 L GITC lysis buffer ([M] 4 Molarity (m) Guanidinium thiocyanate , [M] 10 millimolar (mM) Tris) pH 7.5

- Add 472.6 g guanidinium thiocyanate to a beaker
- Add 10 mL of [M] 1 Molarity (m) Tris stock solution pH 7.5

- Adjust volume to 1 L with ddH_2O
- Stir until the GITC is completely dissolved (heating will speed this up)
- Sterilize by filtering and store at Room temperature

1 L RNA wash buffer 1 ([M] 900 millimolar (mM) Guanidinium thiocyanate , [M] 10 millimolar (mM) Tris , [M] 20 % (v/v) Ethanol absolute) pH 7.5

- Add 106.3 g guanidinium thiocyanate to a beaker
- Add 10 mL of [M] 1 Molarity (m) Tris stock solution pH 7.5
- Add 200 mL Ethanol absolute
- Adjust volume to 1 L with ddH_2O
- Sterilize by filtering and store at Room temperature

1 L RNA wash buffer 2 ([M] 100 millimolar (mM) sodium chloride , [M] 10 millimolar (mM) Tris , [M] 80 % (v/v) ethanol absolute) pH 7.5

- Add 20 mL of [M] 5 Molarity (m) sodium chloride stock solution
- Add 10 mL of [M] 1 Molarity (m) Tris stock solution pH 7.5
- Adjust volume to 200 mL with ddH_2O
- Adjust volume to 1 L with ethanol absolute
- Sterilize by filtering and store at Room temperature

1 L DNA wash buffer 1 ([M] 2.5 Molarity (m) Guanidinium chloride , [M] 10 millimolar (mM) Tris , [M] 57 % (v/v) Ethanol absolute) pH 7.5

- Add 238.9 g guanidinium chloride to a beaker
- Add 10 mL of [M] 1 Molarity (m) Tris stock solution pH 7.5
- Adjust volume to 430 mL with ddH_2O to dissolve the GuHCl
- Adjust volume to 1 L with Ethanol absolute
- Sterilize by filtering and store at Room temperature

1 L DNA wash buffer 2 ([M] 10 millimolar (mM) Tris , [M] 80 % (v/v) ethanol absolute) pH 7.5

- Add 200 mL DNA wash buffer 2 stock solution to a beaker
- Adjust volume to 1 L with Ethanol absolute
- Sterilize by filtering and store at Room temperature

1 L elution buffer [M] 10 millimolar (mM) Tris pH 8.5

- Add 10 mL of [M] 1 Molarity (m) Tris stock solution pH 8.5 to a beaker
- Adjust the volume to 1 L with ddH_2O

- Sterilize by filtering and store at Room temperature

SAFETY WARNINGS

Buffers containing guanidine produce highly reactive compounds when mixed with bleach. Don't mix the extraction waste with bleach or solutions that contain bleach.
Reagents are potentially damaging to the environment. Dispose waste as mandated.

BEFORE START INSTRUCTIONS

Make sure all buffers are prepared before starting.

Sample preparation and lysis

5m

1 For each sample prepare one 2 mL screwcap tube pre-filled with approximately 400 mg of 2 mm zirconia beads and 0.1 mm glass beads.

2 Add up to 200 mg of plant tissue to the prepared tube.

Note

For samples with a high RNA content less starting material might lead to better results. For most sample types 50 mg of starting material will yield a sufficient amount of DNA and RNA for downstream analysis.

3 Add 800 µL GITC lysis buffer to the sample tube.

Note

For complete inactivation and destruction RNAses of 2-Mercaptoethanol can be added in addition. We usually don't because then the samples have to be handled under a fume hood until all lysate has been handled and discarded appropriately.

4 Immediately bead beat for 00:05:00 at maximum speed. 5m

Note

Depending on the bead beater used in this step the time might have to be adjusted. We'd recommend to bead beat the sample until the material is completely homogenized.

Lysate clearing and pre-filtering 10s

5 Room temperature, 00:00:10 , at maximum speed 10s

6 Transfer 700 µL of the crude lysate to a pre-filter column.

7 Room temperature, 00:10:00 , at maximum speed 10m

DNA binding

8 Transfer 700 µL of the flowthrough from step 7 to a silica spin column to bind the DNA in the lysate. **Keep the flow-through. Mark the spin column as the DNA column.**

Note

The protocol will work with all kinds of silica spin columns. See materials section for what we use.

RNA precipitation and binding

15s

9 Add 350 µL Ethanol absolute to the flow-through from step 8 to adjust the binding conditions to bind RNA to the silica column.

10 Vortex the samples to mix the lysate with the ethanol. Do not centrifuge.

11 Load the mixture on a second spin column. **Mark this column as the RNA spin column.** 15s
11000 x g, Room temperature, 00:00:15 and discard the flow-through.

Note

Two loading steps will be necessary to pass the complete volume through the spin column.

Washing steps

15s

12 Add 700 µL RNA wash buffer 1 to the **RNA spin column**, 15s
11000 x g, Room temperature, 00:00:15 and discard the flow-through.

13 Add 500 µL RNA wash buffer 2 to the **RNA spin column**, add 15s
500 µL DNA wash buffer 1 to the **DNA spin column**,
11000 rpm, Room temperature, 00:00:15 and discard the flow-through.

14 Add 500 µL RNA wash buffer 2 to the **RNA spin column**, add 500 µL DNA wash buffer 2 to the **DNA spin column**, 11000 rpm, Room temperature, 00:00:15 and discard the flow-through. 15s

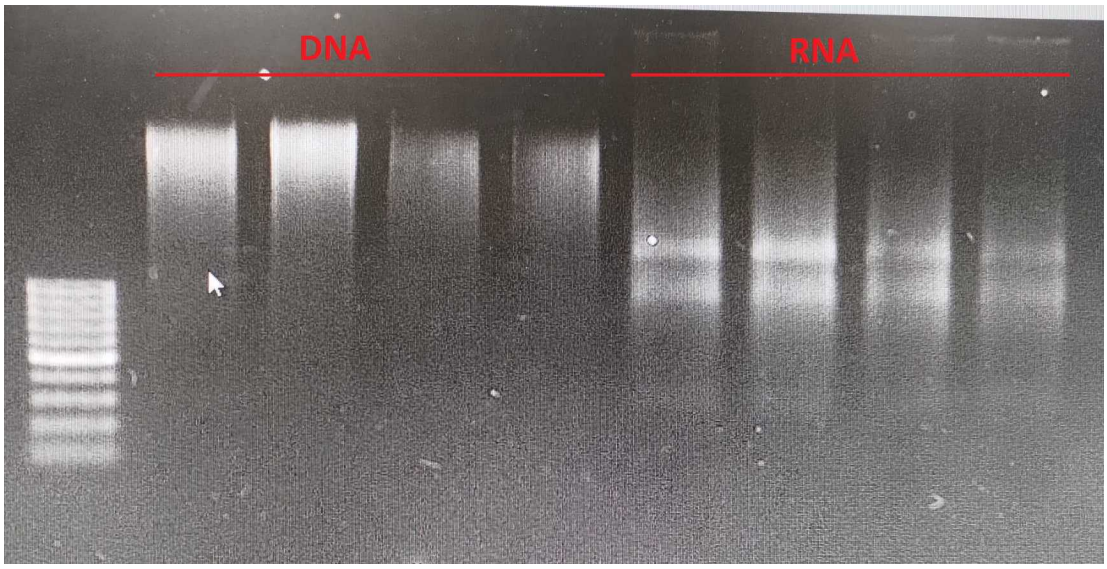
Column drying and elution 4m

15 11.000 rpm, Room temperature, 00:01:00 to dry the silica membrane of the spin columns. Transfer the spin column to a fresh 1.5 mL microcentrifuge tube. 1m

16 Add 100 µL elution buffer directly to the silica membrane. Incubate the column for 00:03:00 at Room temperature 3m

17 11.000 rpm, Room temperature, 00:01:00 , store the eluted RNA at -80 °C and the eluted DNA at -20 °C 1m

Expected result



Expected result of the described protocol. Extraction was carried out in 4 replicates, left part of the gel picture shows the DNA fraction of the sample, while the right part shows the RNA fraction.