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Co-extraction of RNA and DNA from animal tissue

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We use this protocol and it's working

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Abstract

This protocol describes how to co-extract RNA and DNA from animal tissue samples. Samples are homogenized and simultaneously lyzed by bead-beating. Cell debris is pelleted by centrifugation, 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 70% 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% **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**1003171011
Pre-filter columns Pre Filter Columns - 850 µl **Biopolymer Isolation Technologies** **Catalog #**MC-01P-100
Guanidinium chloride Guanidine hydrochloride **Fisher Scientific** **Catalog #**10543325
Ethanol absolute Ethanol absolute 99.8% **Fisher Scientific** **Catalog #**11994041
Antifoam solution (optional): Silicon-Antischaumemulsion 30 **Carl Roth** **Catalog #**0734.1

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 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

Troubleshooting

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

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.

Note

Generally, we just add a small spoon of each type of beads to the tube. As long as the tissue is fully homogenized after bead-beating, the amount of beads is sufficient.

2 Add up to 30 mg of animal 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 10 mg of starting material will yield a sufficient amount of DNA and RNA for downstream analysis.

3 Add 1000 µL GITC lysis buffer to the sample tube.

Note

For complete inactivation and destruction RNAses 10 µL 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.

If you experience a lot of foam formation after bead-beating consider adding [M] 30 Parts per Million (PPM) silicone antifoam to the lysis buffer when preparing it. See materials for a recommendation.

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 recommend to bead beat the sample until the material is completely homogenized.

Lysate clearing

10s

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

DNA binding

6 Transfer 700 µL of the cleared lysate from step 5the 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 the materials section for what we use.

If you are only interested in RNA: If only RNA is of interest the DNA spin column can be discarded at this point in the protocol.

RNA precipitation and binding

15s

7 Add 700 µL 70% Ethanol to the flow-through from step 6 to adjust the binding conditions for RNA to bind to the silica column.

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

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

Note

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

Washing steps

15s

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

Note

For less experienced users: If you are concerned about needing to much time to process both fractions at the same time and risk RNA degradation it is fine to first finish the RNA extraction until safe storage and then finish the DNA fraction.

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

12 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 x g, Room temperature, 00:00:15 and discard the flow-through. 15s

Column drying and elution

4m

13 11.000 x g, 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

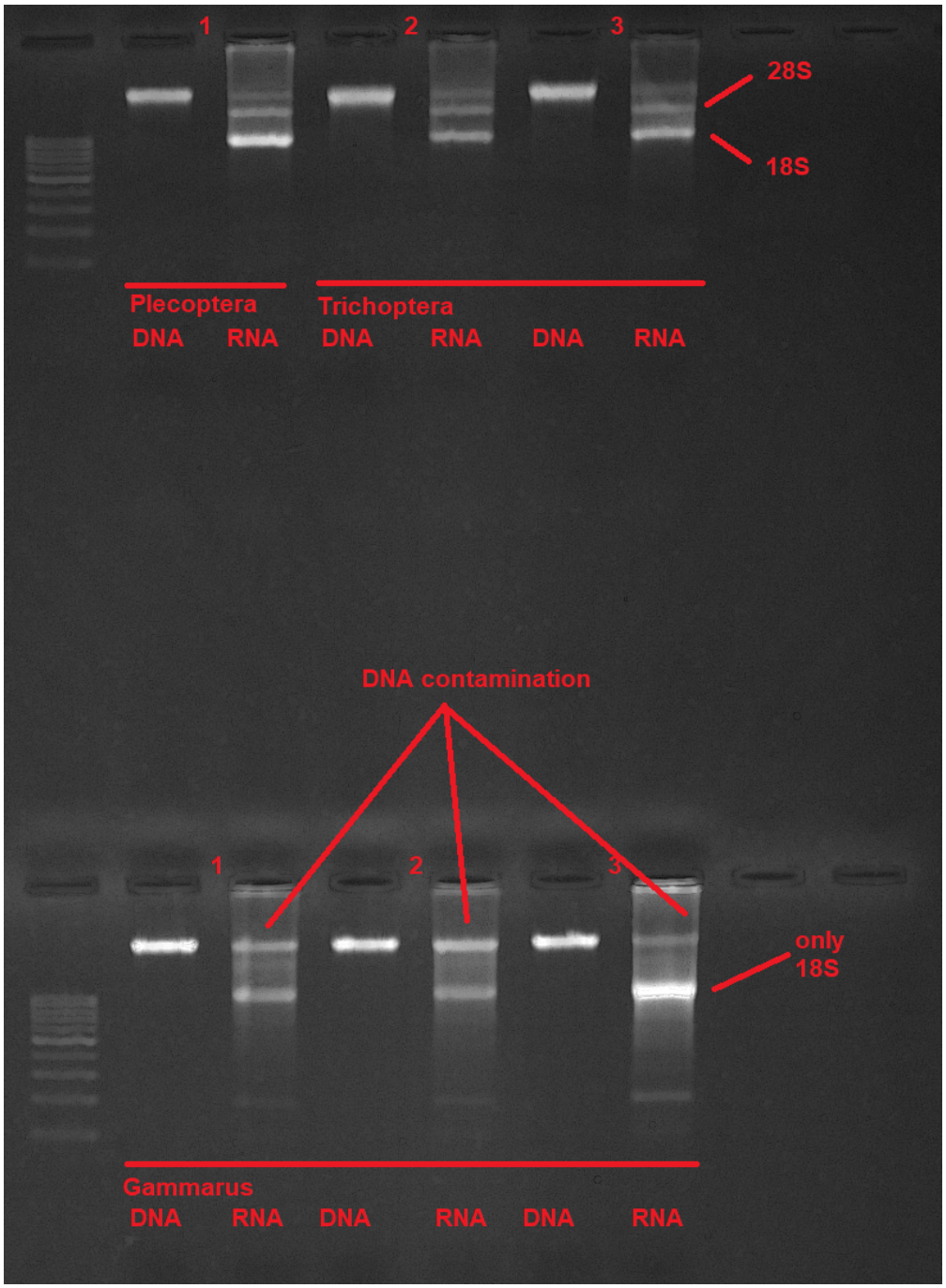
14 Add 100 µL elution buffer directly to the silica membrane. Incubate the column for 00:03:00 at Room temperature 3m

Note

The volume of the elution buffer can be adjusted in this step if a higher concentration or higher volume is required for downstream analysis. Usually, every volume in the range from 30 µL to 200 µL is fine.

15 11.000 x g, Room temperature, 00:01:00 , store the eluted RNA at -80 °C and the eluted DNA at -20 °C 1m

Expected result



The described protocol was tested with different kinds of invertebrate samples, we expect it to work with all animal tissue.
Top row: Plecoptera sample and two Trichoptera samples.
Lower row: Three Gammarus samples.

28S/18S bands are clearly visible and should have a clear band. Genomic DNA is free from RNA contamination. There is some DNA contamination in the RNA extracts. If DNA-free RNA is needed for downstream analysis consider treating the RNA samples with DNase and cleaning them up with an RNA cleanup protocol afterward (see **RNA Cleanup with magnetic beads**).