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Purification of RNA from a DNA/RNA Extract V.2

In 1 collection

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Roey Angel[1], Eva Petrova[1]

[1]Soil and Water Research Infrastructure

Anaerobic and Molecular Microbiology Lab, Biology Centre CAS
Tech. support email: **eva.petrova@bc.cas.cz**

Roey Angel
Soil and Water Research Infrastructure

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We use this protocol and it's working

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Abstract

The following protocol is intended as a downstream application for our **Total Nucleic Acids Extraction from Soil** protocol. This protocol describes how to purify RNA from a DNA and RNA extract using **TURBO™ DNase** and **GeneJET RNA Cleanup and Concentration Micro Kit**. This protocol is a simplified and condensed version of the full protocols provided by the manufacturers.

Attachments

4393900B.pdf
281KB

MAN0012671_GeneJET_R...
276KB

THE RNA storage solu...
205KB

Materials

MATERIALS

- TURBO™ DNase (2 U/µL) **Thermo Fisher Scientific Catalog #**AM2238
- GeneJET RNA Cleanup and Concentration Micro Kit **Thermo Fisher Scientific Catalog #**K0841
- Ethanol, Absolute, Molecular Biology Grade **Thermo Fisher Scientific Catalog #**BP2818500
- RNase AWAY™ Surface Decontaminant **Carl Roth Catalog #**A998.4
- THE RNA Storage Solution **Thermo Fisher Scientific Catalog #**AM7000
- RNaseOUT Recombinant Ribonuclease Inhibitor **Thermo Fisher Scientific Catalog #**10777019

STEP MATERIALS

- TURBO™ DNase (2 U/µL) **Thermo Fisher Scientific Catalog #**AM2238
- RNaseOUT™ Recombinant Ribonuclease Inhibitor **Thermo Fisher Scientific Catalog #**10777019
- USB Dithiothreitol (DTT) 0.1M Solution **Thermo Fisher Scientific Catalog #**707265ML
- Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #**T143.1
- GeneJET RNA Cleanup and Concentration Micro Kit **Thermo Fisher Scientific Catalog #**K0841
- Ethanol, Absolute, Molecular Biology Grade **Thermo Fisher Scientific Catalog #**BP2818500
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Before start

1. For each sample prepare one Gene JET RNA Purification Micro Column tube and two RNase–free collection tubes (1.5 ml).
2. Add the required amount of ethanol to Wash Buffer 1 and Wash Buffer 2 (amount is dependent on kit size).

DNA digestion

45m

1 Prepare the following mixture in a 1.5 ml tube:

1. 10 µL to 42 µL of TNA extract (1 µg to 3 µg of DNA).
2. 5 µL TURBO DNase buffer 10x
3. 1 µL RNaseOUT
4. 1 µL 0,1M DTT
5. 1 µL Turbo DNase per up to 2 µg DNA
6. Complete to 50 µL with RNase-free water

TURBO™ DNase (2 U/µL) **Thermo Fisher Scientific Catalog #**AM2238

RNaseOUT™ Recombinant Ribonuclease Inhibitor **Thermo Fisher Scientific Catalog #**10777019

USB Dithiothreitol (DTT) 0.1M Solution **Thermo Fisher Scientific Catalog #**707265ML

Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #**T143.1

2 Incubate at 37 °C for 00:30:00 .

30m

STEP CASE

Extended DNA digestion

10 steps

If this procedure still leaves out undigested DNA (for example due to the presence of inhibitors), increase the incubation time (to 40–60 min) and add another equal dose of DNase half-way through.

RNA purification

7m

3 Add 250 µL Binding Buffer .

GeneJET RNA Cleanup and Concentration Micro Kit **Thermo Fisher Scientific Catalog #**K0841

4 Add 300 µL absolute ethanol .

Ethanol, Absolute, Molecular Biology Grade **Thermo Fisher Scientific Catalog #**BP2818500

5 Transfer the mixture to the Gene JET RNA Purification Micro Column preassembled with a collection tube. Centrifuge the column for 14000 x g, Room temperature, 00:01:00 . Discard the flow-through. Place the GeneJET RNA Purification Micro Column back into the collection tube. 1m

6 Add 700 µL Wash Buffer 1 (supplemented with ethanol) to the GeneJET RNA Purification Micro Column and centrifuge for 14000 x g, Room temperature, 00:01:00 . Discard the flow-through and place the purification column back into the collection tube. 1m

GeneJET RNA Cleanup and Concentration Micro Kit **Thermo Fisher Scientific Catalog #**K0841

7 Add 700 µL Wash Buffer 2 (supplemented with ethanol) to the GeneJET RNA Purification Micro Column and centrifuge for 14000 x g, Room temperature, 00:01:00 . Discard the flow-through and place the purification column back into the collection tube. 1m

GeneJET RNA Cleanup and Concentration Micro Kit **Thermo Fisher Scientific Catalog #**K0841

8 Repeat step 7. 1m

go to step #7

9 Centrifuge the empty GeneJET RNA Purification Micro Column for an additional 14000 x g, Room temperature, 00:02:00 to completely remove residual Wash Buffer. 2m

Note

This step is essential to avoid residual ethanol in the purified RNA solution. The presence of ethanol in the RNA sample may inhibit downstream enzymatic reactions.

10 Transfer the GeneJET RNA Purification Micro Column into a clean 1.5 ml Collection Tube tube.

11 Add 10 µL to 20 µL RNA storage solution or nuclease-free water to the GeneJET RNA Purification Micro Column. Centrifuge for 14000 rpm, Room temperature, 00:01:00 to elute the RNA. 1m

THE RNA Storage Solution **Thermo Fisher Scientific Catalog #**AM7000

12 Discard the purification column. Use the purified RNA immediately in downstream applications or store at -20 °C or -80 °C until use. Proceed to cDNA synthesis using superscript IV

Note

For prolonged storage (more than 1 month), storage at -80 °C is recommended.

Protocol

NAME

cDNA synthesis using SuperScript™ IV

CREATED BY

Roey Angel

PREVIEW

12.1 Prepare the following mixture in a PCR tube:

1. 1 µL to 4 µL purified RNA (10 pg - 5 µg ; usually 200 ng for soil extract)
2. 1 µL random hexamers (50 µM) or a gene-specific primer (2 µL)
3. 9.8 µL RNase free water

Random hexamers **Thermo Scientific Catalog #**N8080127

Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #**T143.1

12.2 Mix gently and spin down the solution.

12.3 Incubate the mixture at 65 °C for 00:05:00 in a thermocycler and chill On ice (or in the cycler at > 4 °C) for at least 00:01:00.

12.4 Prepare the following mixture and add to each tube:

1. 4 µL 5x Reaction buffer
2. 1 µL dNTP mix, 10 mM
3. 1 µL 0.1M DTT
4. 1 µL RNaseOUT™ (40 U/µl) *
5. 0.2 µL BSA (20 µg/µl)
6. 1 µL SuperScript™ IV RT (200 units/µl)

* Optional

USB Dithiothreitol (DTT) 0.1M Solution **Thermo Fisher Scientific Catalog #**707265ML

SuperScript™ IV First-Strand Synthesis System **Thermo Fisher Scientific Catalog #**18091050

RNaseOUT™ Recombinant Ribonuclease Inhibitor **Thermo Fisher Scientific Catalog #**10777019

Bovine Serum Albumin (BSA) **Thermo Fisher Scientific Catalog #**B14

12.5 Incubate the mixture in a thermocycler at 23 °C for 00:10:00 (only if using random hexamers, skip if using a specific primer) followed by 50 °C for 01:00:00 to 03:00:00 and 80 °C for 00:10:00. Chill On ice.

12.6 **For PCR templates > 1kb** remove the RNA by adding 1 µL (2 units) of E. coli RNase H and incubate at 37 °C for 00:20:00.

Ribonuclease H (RNase H) **Thermo Fisher Scientific Catalog #**18021071

12.7 Prepare the following mixture and add to each tube:

1. 1 µL DNA Polymerase I reaction buffer
2. 0.75 µL DNA Polymerase I
3. 0.2 µL RNase H
4. 3.05 µL RNase-free water
5. 5 µL Template cDNA

DNA Polymerase I (10 U/µL) **Thermo Fisher Scientific Catalog #**EP0041

Ribonuclease H (RNase H) **Thermo Fisher Scientific Catalog #**18021071

Nuclease-free autoclaved DEPC-treated water **Carl Roth Catalog #**T143.1

12.8 Incubate for at 15 °C for 02:00:00 followed by 00:10:00 at 75 °C for deactivation.

12.9 3. Purify the reaction through phenol/chloroform purification followed by ethanol precipitation or using a PCR purification kit.