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Bead Beating RNA extraction from 25 mm filter V.1

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Abstract

This protocol is for extracting RNA from 25 mm filters and can be used with filters stored in RNAlater for preservation. This protocol has been tested with 0.22 µm pore size Durapore filters. Custom synthesized RNA transcript standards are added at the time of extraction and are recovered post-sequencing for quantitative metatranscriptome analysis (Satinsky et al., 2013).

Guidelines

Reference:

Satinsky BM, Gifford SM, Crump BC, Moran MA (2013) Use of internal standards for quantitative metatranscriptome and metagenome analysis. Meth Enzymol, ed DeLong EF (Academic, Burlington, MA), Vol 531, pp 237–250

Materials

MATERIALS

- 0.1 mm Zirconia/Silica Beads **Bio Spec Products Inc.** **Catalog #**11079101z
- 0.5 mm Zirconia/Silica Beads **Bio Spec Products Inc.** **Catalog #**11079105z
- Glass beads, acid-washed, 425-600 µm (30-40 U.S. sieve) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**G8772-100G
- Ambion Denaturation Solution **Thermo Scientific** **Catalog #**AM8540G
- Ethyl alcohol, Pure 200 proof, for molecular biology **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**E7023
- RNeasy Mini Kit **Qiagen** **Catalog #**74104
- TURBO DNA-free™ Kit **Thermo Scientific** **Catalog #**AM1907
- RNA Clean & Concentrator™-5 **Zymo Research** **Catalog #**R1015

STEP MATERIALS

- Ambion Denaturation Solution **Thermo Scientific** **Catalog #**AM8540G
- Ethyl alcohol, Pure 200 proof, for molecular biology **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**E7023
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- TURBO DNA-free™ Kit **Thermo Scientific** **Catalog #**AM1907
- RNA Clean & Concentrator™-5 **Zymo Research** **Catalog #**R1015

Protocol materials

- 0.5 mm Zirconia/Silica Beads **Bio Spec Products Inc.** **Catalog #**11079105z
- Ethyl alcohol, Pure 200 proof, for molecular biology **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**E7023
- Ambion Denaturation Solution **Thermo Scientific** **Catalog #**AM8540G
- TURBO DNA-free™ Kit **Thermo Scientific** **Catalog #**AM1907
- 0.1 mm Zirconia/Silica Beads **Bio Spec Products Inc.** **Catalog #**11079101z
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Safety warnings

Proper sterile technique when handling samples and reagents is critical at every step to prevent introducing RNases that will degrade RNA samples.

Before start

Have the following on hand in addition to materials: forceps, 2.0 and 1.5 ml RNase free tubes, temperature controlled microcentrifuge, vortex with 2.0 ml adaptors, 21g1 needls, and 3 ml syringes.

Setup

1 Turn on 4°C centrifuge

2 Thaw internal standards on ice

3 Set up 2.0 ml tube adaptor on Mo Bio Vortex Genie 2.

Bead beating prep

4 For each sample, prepare a 2.0 ml tube with the following 3-bead mixture: 200 µl 0.1 mm zirconium beads, 100 µl 0.4-0.6 mm glass beads, 100 µl 0.5 mm zirconium beads

400 µL

Protocol

NAME

3-bead mixture

CREATED BY

Christa Smith

Preview

4.1 200 µl 0.1 mm zirconium beads

4.2 100 µl 0.4-0.6 mm glass beads

4.3 100 µl 0.5 mm zirconium beads

5 Add Ambion Denaturation Solution to each tube

1 µL

Ambion Denaturation Solution **Thermo Scientific** **Catalog #**AM8540G

6 To each bead tube, add internal standards to reach ~0.5% of expected RNA yield

7 Place one 25 mm sample filter into each bead tube using RNase-free forceps

Bead beating and RNA extraction

8 Place sample tubes on vortex adaptor and beat for 5 min
00:05:00

9 Switch tube positions and beat another 5 min.
00:05:00

10 Centrifuge sample tubes for 1 min at 5000 rpm at 4°C
00:01:00

11 Transfer as much of the RNA lysate as possible to a new 1.5 ml centrifuge tube (Can carryover some beads)

12 Centrifuge RNA tubes for 5 min at 5000 rpm at 4°C
00:05:00

13 Transfer lysate to new 2.0 ml tube (Do not carry over any beads).

14 Add 1 volume of 100% ethanol to the lysate
1 µL
Ethyl alcohol, Pure 200 proof, for molecular biology **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7023

15 Draw the RNA lysate up into a 3.0 ml syringe with a 21g1 gauge needle and pass it back out 8 times to shear RNA

16 Apply 700 µL of RNA lysate to RNeasy mini column
RNeasy Mini Kit **Qiagen Catalog #**74104

17 Close tube gently and centrifuge 15 sec at 13000 rpm
00:00:15

18 Discard flow-through.

19 Repeat steps 16-18 until entire sample has been applied to column.

RNA purification

20 Add 700 µl Buffer RW1 to RNeasy Mini spin column.

> Note
>
> Here we are continuing to following Qiagen RNeasy Mini kit (step5 of Qiagen manual); finishing with two sequential elutions and spins of 35 µ

21 Centrifuge for 15 sec at 13000 rpm. Discard the flow-through.
00:00:15

22 Add 500 µl Buffer RPE to RNeasy spin column.

23 Centrifuge for 15 sec at 13000 rpm. Discard flow-through.
00:00:15

24 Add 500 µl Buffer RPE to RNeasy spin column.

25 Centrifuge for 2 min at 13000 rpm
00:02:00

26 Place column in new 2.0 ml collection tube.

27 Centrifuge for 1 min at 13000 rpm
00:01:00

28 Place column in a new 1.5 ml sterile tube

29 Add 35 µl RNase-free water directly onto filter and close the lid.

30 Incubate tube for 1 min at room temperature
00:01:00

31 Centrifuge for 1 min at 13000 rpm.
00:01:00

32 Add another 35 µl RNase-free water into same column/tube

33 Incubate for 1 min at room temperature
00:01:00

34 Centrifuge for 1 min at 13000 rpm
00:01:00

35 Discard column and place tube with RNA on ice

RNA quantification

36 Quantify extracted RNA with Nanodrop (2 µl)

DNA removal

37 Mix the following in a 1.5 ml tube:

	component	amount
	extracted sample	50 µl
	RNase-free water	40 µl
	DNase reaction buffer	10 µl
	Turbo DNase	3 µl

TURBO DNA-free™ Kit **Thermo Scientific Catalog #**AM1907

38 Incubate for 20 min at 37 °C
00:20:00

39 Add 3 µl more Turbo DNase to sample

40 Incubate an additional 20 min at 37 °C
00:20:00

41 Add 20 µl inactivation solution to sample (make sure solution is well mixed)

42 Vortex on and off for ~4 min
00:04:00

43 Spin for 1 min at 13000 rpm
00:01:00

44 Carefully, without disturbing inactivation reagent, remove supernatant (~100 µl) into a new 1.5 ml tube and place on ice

Quantification

45 Quantify DNased RNA with Nanodrop (2 µl)

Optional

46 **Optional:** Clean and concentrate DNased RNA using Zymo RNA Clean & Concentrator-5 according to manufacturer protocol
RNA Clean & Concentrator™-5 **Zymo Research Catalog #**R1015

Finish

47 Store RNA at -80 °C