Bead Beating RNA extraction from 25 mm filter Version 2

Christa Smith

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).

Citation: Christa Smith Bead Beating RNA extraction from 25 mm filter. **protocols.io**
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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

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.

Materials

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

Protocol

Setup

Step 1.
Turn on 4°C centrifuge

Setup

Step 2.
Thaw internal standards on ice

Setup

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

Bead beating prep

Step 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

AMOUNT
400 µl Additional info:

PROTOCOL

. 3-bead mixture

CONTACT: Christa Smith
Step 4.1.
200 µl 0.1 mm zirconium beads

Step 4.2.
100 µl 0.4-0.6 mm glass beads

Step 4.3.
100 µl 0.5 mm zirconium beads

Bead beating prep

Step 5.
Add Ambion Denaturation Solution to each tube

AMOUNT
1 ml Additional info:

REAGENTS
Ambion Denaturation Solution AM8540G by Thermo Scientific

Bead beating prep

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

Bead beating prep

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

Bead beating and RNA extraction

Step 8.
Place sample tubes on vortex adaptor and beat for 5 min

DURATION

00:05:00

Bead beating and RNA extraction

Step 9.

Switch tube positions and beat another 5 min.

DURATION

00:05:00

Bead beating and RNA extraction

Step 10.

Centrifuge sample tubes for 1 min at 5000 rpm at 4°C

DURATION

00:01:00

Bead beating and RNA extraction

Step 11.

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

Bead beating and RNA extraction

Step 12.

Centrifuge RNA tubes for 5 min at 5000 rpm at 4°C

DURATION

00:05:00

Bead beating and RNA extraction

Step 13.

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

Bead beating and RNA extraction

Step 14.

Add 1 volume of 100% ethanol to the lysate

AMOUNT

1 ml Additional info:

REAGENTS

Ethyl alcohol, Pure 200 proof, for molecular biology E7023 by Sigma Aldrich

Bead beating and RNA extraction

Step 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

Bead beating and RNA extraction

Step 16.

Apply 700 µL of RNA lysate to RNeasy mini column

REAGENTS

RNeasy Mini Kit 74104 by Qiagen

Bead beating and RNA extraction

Step 17.

Close tube gently and centrifuge 15 sec at 13000 rpm

DURATION

00:00:15

Bead beating and RNA extraction

Step 18.
Discard flow-through.

Bead beating and RNA extraction

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

RNA purification

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

NOTES
Christa Smith 02 Nov 2015
Here we are continuing to following Qiagen RNeasy Mini kit (step5 of Qiagen manual); finishing with two sequential elutions and spins of 35 µ

RNA purification

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

DURATION
00:00:15

RNA purification

Step 22.
Add 500 µl Buffer RPE to RNeasy spin column.

RNA purification

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

DURATION
00:00:15

RNA purification

Step 24.
Add 500 µl Buffer RPE to RNeasy spin column.

RNA purification

Step 25.
Centrifuge for 2 min at 13000 rpm

DURATION
00:02:00

RNA purification

Step 26.
Place column in new 2.0 ml collection tube.

RNA purification

Step 27.
Centrifuge for 1 min at 13000 rpm

DURATION
00:01:00

RNA purification

Step 28.
Place column in a new 1.5 ml sterile tube

RNA purification

Step 29.

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

RNA purification

Step 30.

Incubate tube for 1 min at room temperature

DURATION

00:01:00

RNA purification

Step 31.

Centrifuge for 1 min at 13000 rpm.

DURATION

00:01:00

RNA purification

Step 32.

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

RNA purification

Step 33.

Incubate for 1 min at room temperature

DURATION

00:01:00

RNA purification

Step 34.

Centrifuge for 1 min at 13000 rpm

DURATION

00:01:00

RNA purification

Step 35.

Discard column and place tube with RNA on ice

RNA quantification

Step 36.

Quantify extracted RNA with Nanodrop (2 µl)

DNA removal

Step 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

REAGENTS

TURBO DNA-free™ Kit AM1907 by Thermo Scientific

DNA removal

Step 38.

Incubate for 20 min at 37 °C

DURATION

00:20:00

DNA removal

Step 39.

Add 3 µl more Turbo DNase to sample

DNA removal

Step 40.

Incubate an additional 20 min at 37 °C

DURATION

00:20:00

DNA removal

Step 41.

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

DNA removal

Step 42.

Vortex on and off for 4 min

DURATION

00:04:00

DNA removal

Step 43.

Spin for 1 min at 13000 rpm

DURATION

00:01:00

DNA removal

Step 44.

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

Quantification

Step 45.

Quantify DNased RNA with Nanodrop (2 µl)

Optional

Step 46.

Optional: Clean and concentrate DNased RNA using Zymo RNA Clean & Concentrator-5 according to manufacturer protocol

REAGENTS

RNA Clean & Concentrator™-5 R1015 by Zymo Research

Finish

Step 47.

Store RNA at -80 °C

Warnings

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