RNA (and optional DNA) extraction from environmental samples (filters) Version 2

Sarah Hu

Abstract

This protocol describes the extraction process of RNA and/or DNA from marine environmental samples. Samples are assumed to be collected via filtering sample seawater onto filters. Filters are then flash frozen in the first lysis buffer. The protocol describes the steps used to lyse and separate material from filters and use the lysate for RNA and/or DNA extraction using Qiagen extraction kits (e.g. https://www.qiagen.com/us/shop/Sample-Technologies/Combined-Sample-Technologies/).

Using this protocol, RNA and DNA has been used for molecular analyses such as tag sequencing (16S or 18S), (meta)genomic, or (meta)transcriptomic applications

See other Caron lab protocols that detail field work sampling and downstream PCR amplification/libray prep for microbial eukaryotic-specific tag sequencing (18S rRNA gene).

List of materials needed before starting is in **Guidelines.**

Citation: Sarah Hu RNA (and optional DNA) extraction from environmental samples (filters). **protocols.io** dx.doi.org/10.17504/protocols.io.hk3b4yn
Published: 24 May 2017

Guidelines

These steps for RNA (& DNA) extraction are specific to samples of seawater filtered onto GF/F, nitex mesh, or polycarbonate filters*. This protocol focuses on additional steps taken for lysis and collection of material from filters before using a Qiagen DNA/RNA Allprep kit.

See other Caron lab protocols that detail field work sampling and downstream PCR amplification/libray prep for microbial eukaryotic-specific tag sequencing (18S rRNA gene).

Considerations:

- *Please confirm/test that protocol chemistry is compatible with filter of your choice. The beginning of this protocol assumes that collected filters were flash frozen into RLT+ Buffer (Qiagen)
- This protocol is designed to also be compatible with other Qiagen extraction protocols. Please read all steps of the protocol ahead of time to confirm other Qiagen components are interchangeable.
    - Ensure loading material is within the range of extraction kit (see Qiagen manual)
- When working with RNA please minimize any exposure to non-RNase DNase safe environments and prior to lysing, ensure samples remain frozen at -80C.

Materials:

- Qiagen extraction kit, can be from a variety of kits
- RNase-free DNase set from Qiagen (cat # 79254)
- Ice
- Heat block (65C)
- RNase-DNase clean pipets and filter pipet tips
- gloves and RNase/DNase-free bench space
- 5mL sterile syringe, 1 needed for each filter* (if using filter extraction)
- 5mL centrifuge tubes, RNase-DNase free (additional UV-sterilization preferred)
- 0.5 mm Silica beads (e.g. Biospec products), made RNase-DNase free. Beads can be baked in oven to make RNase/DNase-free for 2 hrs at 550F.
- vortex
- Microcentrifuge, both desktop mini and larger centrifuge to fit 1.5mL tubes
- 96-100% EtOH
- 70% EtOH (Freshly made in sterile water)
- Sterile forceps (or disposable forceps)

Before start

- Please read through all of the warnings, steps, and considerations for Qiagen extraction kit
- Ensure that Buffer RLT+ (or RLT) has beta mercaptoethanol added, as per the Qiagen instructions
- Consider filter type (or however you are collecting cellular material) and compatibility to reagents (RLT+ buffer)

Protocol

Lysis steps

Step 1.

Take frozen tubes out of the -80C freezer, keep on ice.

See other Caron lab protocol for collecting environmental samples!

Step 2.

1. While filters are thawing, add RNase/DNase-free* 0.5mm Silica beads to each sample tube
2. If RLT+ buffer (with beta mercaptoethanol) was not added previously, add it here.

*Clean your silica beads

Step 3.

Vortex for 5 minutes to ensure beads disrupt filter, but ensure filter remains in RLT+ buffer the whole time

Options lysing (especially if you're working with difficult tissue types):

1. Add pre-heated RLT+ buffer (heat for 2-3 minutes at 65C)
2. Place tube on Tissue lyser for bead beating
3. Vortex for additional minutes
4. Alternate freezing and thawing
5. QIA shredder, see step 8

Step 4.

Once thoroughly lysed (note foam/bubbles may have appeared), transfer liquid lysate to new tube (avoid transferring filter).

*If you did not use a filter (i.e. spun samples down from pure culture, etc). Ignore steps 5-7.

Step 5.
Using sterile forceps, transfer the filter carefully into a 5mL sterile syringe.

Step 6.

Squeeze out excess lysate from filter through the syringe into the new tube of lysate. This step ensures that you've collected all the lysate possible from the filter - removing the filter from lysate. You shouldn't have filters in the lysate during the downstream placement of lysate through Qiagen extraction columns.

Step 7.

New tube for each sample should only contain lysate.

Optional step is to run this lysate through a Qiashredder for additional lysis.

RNA extraction (with Qiagen kit)

Step 8.
**Start with Qiagen extraction steps here (following Qiagen DNA/RNA All prep kit): Transfer the lysate

to the AllPrep DNA spin column. Centrifuge for 30 seconds at > 10,000 rpm.

Step 9.

Transfer flow-through (filtrate) into a new tube for RNA purification. This DNA column can now be stored in the fridge until DNA extraction (4C)

- If multiple spins of the lysate are required, continue this until all lysate has been passed through the DNA column and all flow-through has been obtained in a new tube.
- Add 1 volume of 70% EtOH to the RNA flow-through product, mix by pipetting. Do not centrifuge.

Step 10.
Add 1 volume of 70% EtOH to the RNA flow-through product, mix by pipetting. Do not centrifuge.

Step 11.
Transfer up to 700µl of sample (including any precipitate) to an RNeasy spin column. Centrifuge for 30 seconds at > 10,000 rpm. Discard the flow-through.

Step 12.
Add 350µl of Buffer RW1 to RNeasy spin column. Centrifuge for 15 seconds at > 10,000 rpm. Discard the flow-through.

Step 13.
Make up DNase I and Buffer RDD stock mix for DNase digestion. For each sample add 10µl of DNaseI stock to 70µl of Buffer RDD, mix solution and centrifuge briefly.

Step 14.
Add DNaseI mix (80µl) directly to RNase spin column. Incubate at room temperature for 15 minutes.

Step 15.
Again, add 350µl of Buffer RW1 to RNeasy spin column. Centrifuge for 15 seconds at > 10,000 rpm. Discard the flow-through.

Step 16.
Add 500µl of Buffer RPE to RNeasy spin column. Centrifuge for 15 seconds at > 10,000 rpm. Discard the flow-through.

Step 17.
Again, add 500µl of Buffer RPE to RNeasy spin column. Centrifuge for 2 minutes at > 10,000 rpm. Discard the flow-through.

Step 18.
Option to place RNeasy spin column into a new 2mL collection tube and centrifuge at full speed for 1 minute – this will eliminate any possible carry over of Buffer RPE.

Elution of total RNA

Step 19.

Place RNeasy spin column into a new 1.5ml collection tube, add 30-50µl RNase-free water. Centrifuge for 1 minute at > 10,000 rpm to elute the RNA.
Options to increase yield:

- Pre-heat RNase-free water ahead of addition to RNeasy column
- Let RNase free water sit on RNeasy column for 1-2 minutes before centrifugation
- Transfer eluted RNA back into the RNeasy column and re-centrifuge to increase concentration.Genomic DNA purification

DNA extraction (using Qiagen kit)

Step 20.
Grab the DNA spin columns out of the fridge. Add 500µl Buffer AW1 to AllPrep DNA spin column. Centrifuge for 15 seconds at > 10,000 rpm. Discard the flow-through.

Step 21.
Again add 500µl of Buffer AW2 to the DNA column. Centrifuge for 2 minutes at full speed. Discard the flow-through.

Elution of DNA

Step 22.

Place DNA column into a new 1.5ml collection tube, add 50-100µl of Buffer EB to the column. Let sit at room temperature on the column for 1 minute. Centrifuge for 1 minute at > 10,000 rpm.

Options to increase yield:

- Pre-heat EB buffer water ahead of addition to DNA column
- Transfer eluted DNA back into the RNeasy column and re-centrifuge to increase concentration.

Step 23.

QC both DNA and RNA product using both Qubit fluorometer (concentration) and Agilent Bioanalyzer (quality).

- For the Qubit load 2µl of product to the 198µl of buffer/dye solution for each sample (recommend you use Qubit High sensitivity assay for both DNA and RNA)

Next steps

Step 24.

See other Caron lab protocol for amplifying the V4 hypervariable region for downstream sequencing applications.