# RNA (and optional DNA) extraction from environmental samples (filters)

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## 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/).

Downstream RNA and DNA is used for molecular analyses such as tag sequencing (16S or 18S), (meta)genomic, or (meta)transcriptomic applications.

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

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## 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.

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
- 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
- 5mL centrifuge tubes, RNase-DNase free (additional UV-sterilization preferred)
- 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

## Protocol

**Step 1.**
Take frozen tubes out of the -80C freezer, keep on ice.

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

**Step 3.**
Vortex for 5 minutes to ensure beads disrupt filtera. Ensure filter remains in RLT+ buffer the whole timeb. Options to increase yield:i. Add pre-heated RLT+ buffer (heat for 2-3 minutes at 65C)ii. Place tube on Tissue lyser for bead beatingiii. Vortex for additional minutes

**Step 4.**
Once thoroughly lysed (note foam/bubbles may have appeared), transfer liquid lysate to new tube (avoid transferring filter).

**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.

**Step 7.**
New tube for each sample should only contain lysate.a. Optional step is to run this lysate through a Qiashredder for additional lysis.

**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).a. 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.

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

**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.

**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:
i. Pre-heat EB buffer water ahead of addition to DNA column
ii. 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).

a. For the Qubit load 2µl of product to the 198µl of buffer/dye solution for each sample

**Step 24.**