

Sep 20, 2023

Version 2

Co-extraction of RNA and DNA from soil and sediment samples V.2

DOI

dx.doi.org/10.17504/protocols.io.rm7vzb6m4vx1/v2



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Protocol Citation: Dominik Buchner, Lisa Wolany 2023. Co-extraction of RNA and DNA from soil and sediment samples. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.rm7vzb6m4vx1/v2> Version created by **Dominik Buchner**

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Protocol status: Working

We use this protocol and it's working



Created: September 19, 2023

Last Modified: September 20, 2023

Protocol Integer ID: 88003

Keywords: rneasy powersoil dna elution kit, rneasy powersoil total rna kit, extracting rna, sediment samples this protocol, dna from soil, sediment sample, rna, extraction, purelink hipure plasmid miniprep kit, sediment, dna, components of the kit, hipure columns from invitrogen, sample, soil, kit

Abstract

This protocol describes extracting RNA and DNA from soil and sediment samples.  2 g of soil or up to  5 g of sediment can be processed in one extraction. The protocol is based on the RNeasy PowerSoil Total RNA Kit but does not rely on any components of the kit. It also replaces the RNeasy PowerSoil DNA Elution kit. The JetStar 2.0 columns are replaced with the HiPure Columns from Invitrogen's PureLink HiPure Plasmid Miniprep Kit. A lot of the buffers can be found in the following patent <https://patents.google.com/patent/US7459548B2/en>.

Guidelines

Follow general lab etiquette. Wear gloves to prevent contaminating the samples. Clean the workspace before starting with 80% EtOH.

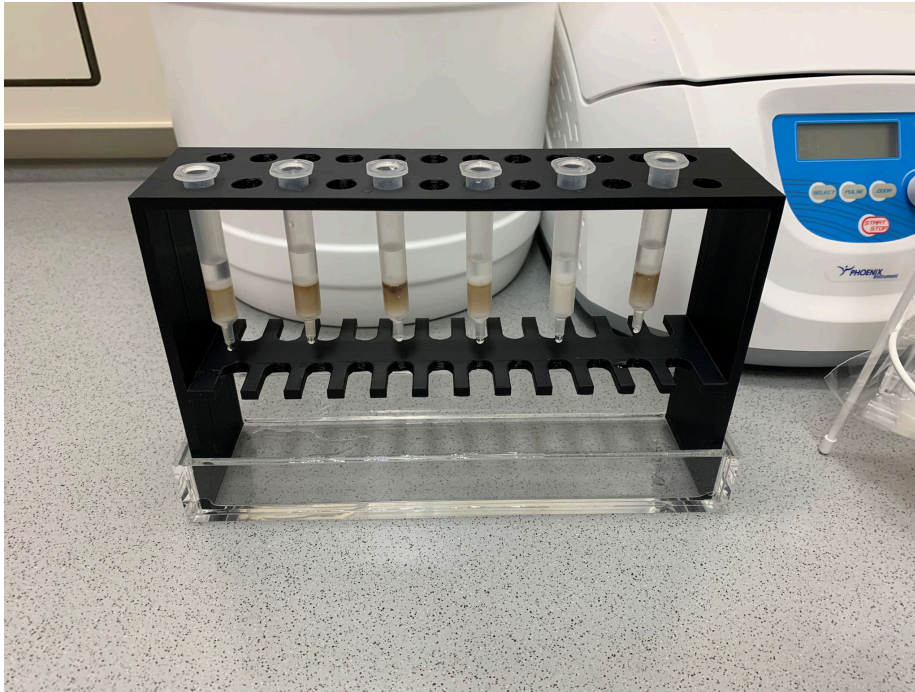
Consider working with two layers of gloves when working with phenol and change gloves often.

Materials


Materials required:

Below all materials needed for the protocol are listed. Vendors and part numbers are listed but interchangeable depending on the supply situation.

As an alternative to the PureLink Nucleic Acid Purification Rack from Thermo, we designed a 3D printable rack including a waste container. Both parts form the rack that can be used for purification, reducing the needed 15 mL tubes and corresponding waste immensely.



3D printed rack for up to 24 columns


 Column_Rack_PureLink_HiPure_Dr... 377KB

 Column Rack PureLink HiPure Bott... 1KB

Chemicals:

Sodium phosphate dibasic

 Sodium phosphate dibasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S0876-100G**

Guanidinium thiocyanate  Guanidinium thiocyanate **Fisher Scientific Catalog #10503345**

Sodium phosphate monobasic

 Sodium phosphate monobasic **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S0751-100G**

SDS ultrapure  Sodium dodecyl sulfate **Diagonal Catalog #A1112.0500**


Sodium chloride  Sodium chloride **Fisher Scientific Catalog #10616082**


Tris ultrapure 99.9%  Tris ultrapure 99.9% **Diagonal Catalog #A1086.1000**


Hydrochloric acid fuming 37%

 Hydrochloric acid fuming 37% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1003171011**

Aluminium ammonium sulfate dodecahydrate al

 Aluminium ammonium sulfate dodecahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A2140-500G**

Phenol/chloroform/isoamyl alcohol  8

 ROTI Phenol/Chloroform/Isoamyl alcohol **Carl Roth Catalog #A156.3**

Tri-Sodium citrate  tri-Sodium citrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1110371000**

Citric acid  Citric acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #251275-100G**

MOPS  MOPS **Carl Roth Catalog #6979.3**

Sodium hydroxide  Sodium Hydroxide Pellets **Fisher Scientific Catalog #S318**


PureLink HiPure Plasmid-MiniPrep-Kit


 PureLink HiPure Plasmid-MiniPrep-Kit **Thermo Fisher Scientific Catalog #K210002**

Labware:


15 mL centrifuge tubes, Ultra-High Performance

 Centrifuge tubes Ultra-High Performance **VWR International (Avantor) Catalog #525-1091**


0.1 mm glass beads  Glass Beads 0.1 mm dia **BioSpec Products Catalog #11079101**




0.5 mm glass beads  Glass Beads 0.5 mm dia **BioSpec Products Catalog #11079105**


1 mm zirconia/silica beads  Zirconia/Silica Beads 1 mm dia **BioSpec Products Catalog #11079110z**


2 mm zirconia beads  Zirconia Beads 2 mm dia **BioSpec Products Catalog #11079124zx**

Stock solutions:

 1 L SDS stock solution  10 Mass / % volume

- Add  100 g SDS ultrapure to a beaker
- Adjust volume to  1 L with ddH₂O
- Sterilize by filtering and store at  Room temperature

 1 L sodium chloride stock solution  5 Molarity (M)

- Add  292.2 g sodium chloride to a beaker



- Adjust volume to 1 L with ddH₂O
- Sterilize by filtering and store at Room temperature

1 L Tris stock solution 1 Molarity (M) pH 8

- Add 121.14 g Tris ultrapure 99.9% to a beaker
- Adjust volume to 800 mL with ddH₂O
- Adjust pH to pH 8 with HCl
- Adjust volume to 1 L with ddH₂O
- Sterilize by filtering and store at Room temperature

1 L Tris stock solution 1 Molarity (M) pH 8.5

- Add 121.14 g Tris ultrapure 99.9% to a beaker
- Adjust volume to 800 mL with ddH₂O
- Adjust pH to pH 8.5 with HCl
- Adjust volume to 1 L with ddH₂O
- Sterilize by filtering and store at Room temperature

500 mL trisodium citrate stock solution 300 millimolar (mM) pH 5

- Add 38.7 g tri-Sodium citrate to a beaker
- Adjust pH to pH 5 with citric acid
- Sterilize by filtering and store at Room temperature

500 mL MOPS stock solution






- Add 104.64 g MOPS to a beaker
- Adjust volume to 450 mL with ddH₂O
- Adjust pH to pH 7 with sodium hydroxide
- Sterilize by filtering and store at Room temperature






Working solutions:










500 mL bead-beating solution (180 millimolar (mM) sodium phosphate ,
 120 millimolar (mM) guanidinium thiocyanate) pH 8



- Add 12.8 g sodium phosphate dibasic to a beaker










- Add  7.1 g guanidinium thiocyanate
- Adjust volume to  490 mL with ddH₂O
- Adjust pH to  8 by adding sodium phosphate monobasic
- Adjust volume to  500 mL with ddH₂O
- Sterilize by filtering and store at  Room temperature







 500 mL lysis solution ( 150 millimolar (mM) sodium chloride ,  4 Mass / % volume SDS ,  500 millimolar (mM) Tris)  8






- Add  200 mL of  10 Mass / % volume SDS stock solution
- Add  15 mL of  5 Molarity (M) sodium chloride stock solution
- Add  250 mL of  1 Molarity (M) Tris stock solution  8
- Adjust volume to  500 mL with ddH₂O
- Sterilize by filtering and store at  Room temperature








 500 mL inhibitor removal solution ( 120 millimolar (mM) aluminium ammonium sulfate dodecahydrate)

- Add  27.2 g aluminium ammonium sulfate dodecahydrate to a beaker
- Adjust volume to  500 mL with ddH₂O
- Sterilize by filtering and store at  Room temperature

 500 mL precipitation solution ( 5 Molarity (M) sodium chloride ,  30 millimolar (mM) sodium citrate)  5

- Add  146.1 g sodium chloride to a beaker
- Add  50 mL of  300 millimolar (mM) tri-Sodium citrate stock solution  5
- Adjust volume to  500 mL with ddH₂O
- Sterilize by filtering and store at  Room temperature

 500 mL column equilibration buffer ( 500 millimolar (mM) sodium chloride ,  50 millimolar (mM) MOPS ,  15 % (v/v) isopropanol)  7

- Add  50 mL of  5 Molarity (M) sodium chloride stock solution to a beaker
- Add  25 mL of  1 Molarity (M) MOPS stock solution  7
- Add  75 mL isopropanol
- Sterilize by filtering and store at  Room temperature



🧪 500 mL RNA elution buffer ([M] 750 millimolar (mM) sodium chloride , [M] 50 millimolar (mM) MOPS , [M] 15 % (v/v) isopropanol) Ⓜ 7

- Add 🧪 75 mL of [M] 5 Molarity (M) sodium chloride stock solution to a beaker
- Add 🧪 25 mL of [M] 1 Molarity (M) MOPS stock solution Ⓜ 7
- Add 🧪 75 mL isopropanol
- Sterilize by filtering and store at 🌡 Room temperature

🧪 500 mL DNA elution buffer ([M] 1250 millimolar (mM) sodium chloride , [M] 50 millimolar (mM) Tris , [M] 15 % (v/v) isopropanol) Ⓜ 8.5

- Add 🧪 125 mL of [M] 5 Molarity (M) sodium chloride stock solution to a beaker
- Add 🧪 25 mL of [M] 1 Molarity (M) Tris stock solution Ⓜ 8.5
- Add 🧪 75 mL isopropanol
- Sterilize by filtering and store at 🌡 Room temperature

🧪 1 L elution buffer ([M] 10 millimolar (mM) Tris) Ⓜ 8.5

- Add 🧪 10 mL Tris stock solution Ⓜ 8.5 to a beaker
- Adjust volume to 🧪 1 L with ddH₂O
- Sterilize by filtering and store at 🌡 Room temperature

Troubleshooting



Safety warnings

❗ Phenol/chloroform/isoamyl alcohol is highly corrosive and a carcinogen. Do not inhale vapors, perform all steps under a fume hood, and control your gloves regularly for spills. Store the phenol/chloroform/isoamyl alcohol in a cool and dry space.

Always have PEG 400 ready when working with phenol to absorb small spills. Check the SDS before starting to work with any chemicals.

Buffers containing guanidine produce highly reactive compounds when mixed with bleach. Don't mix the extraction waste with bleach or solutions that contain bleach.

Reagents are potentially damaging to the environment. Dispose waste as mandated.

Before start

Make sure all buffers are prepared before starting.

Cell lysis

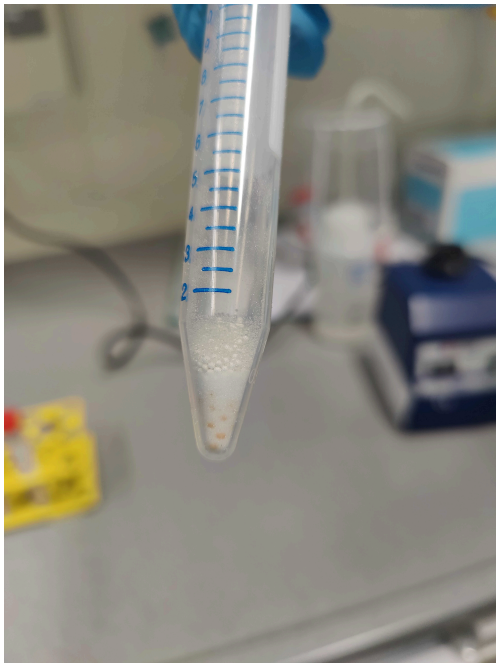
42m

- 1 Prepare one 15 mL conical centrifuge tube per sample by adding 0.5 g of a) 0.1 mm glass beads, b) 0.5 mm glass beads, c) 1 mm zirconia/silica beads d) 2 mm zirconia beads.

5m

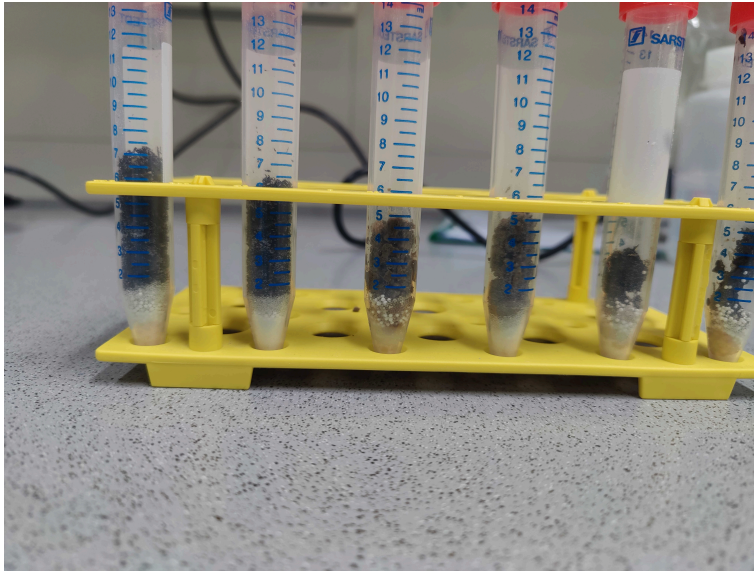
Note






Use high-quality centrifuge tubes for the bead-beating process and phenol/chloroform handling. See the materials section for a recommendation. Low-quality tubes may leak and contaminate the centrifuge or fume hood with phenol.



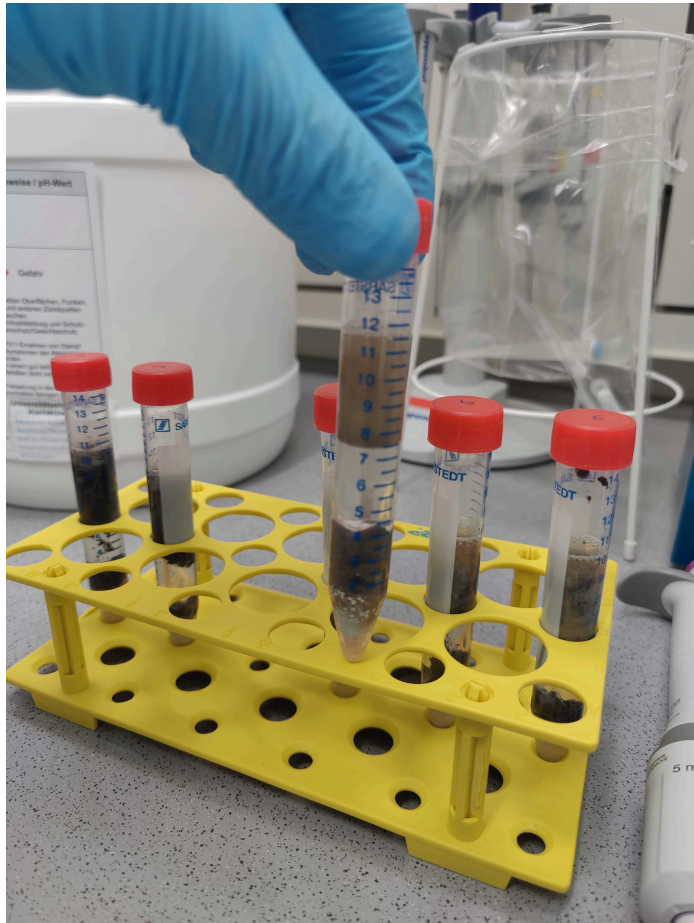
- 2 Add up to 2 g of soil or up to 5 g of the sediment sample.

5m




- 3 Add  2.5 mL bead-beating solution ,  0.25 mL lysis solution ,  0.8 mL inhibitor removal solution and  3.5 mL phenol/chloroform/isoamyl alcohol  8 . Two layers will appear in the centrifuge tube.

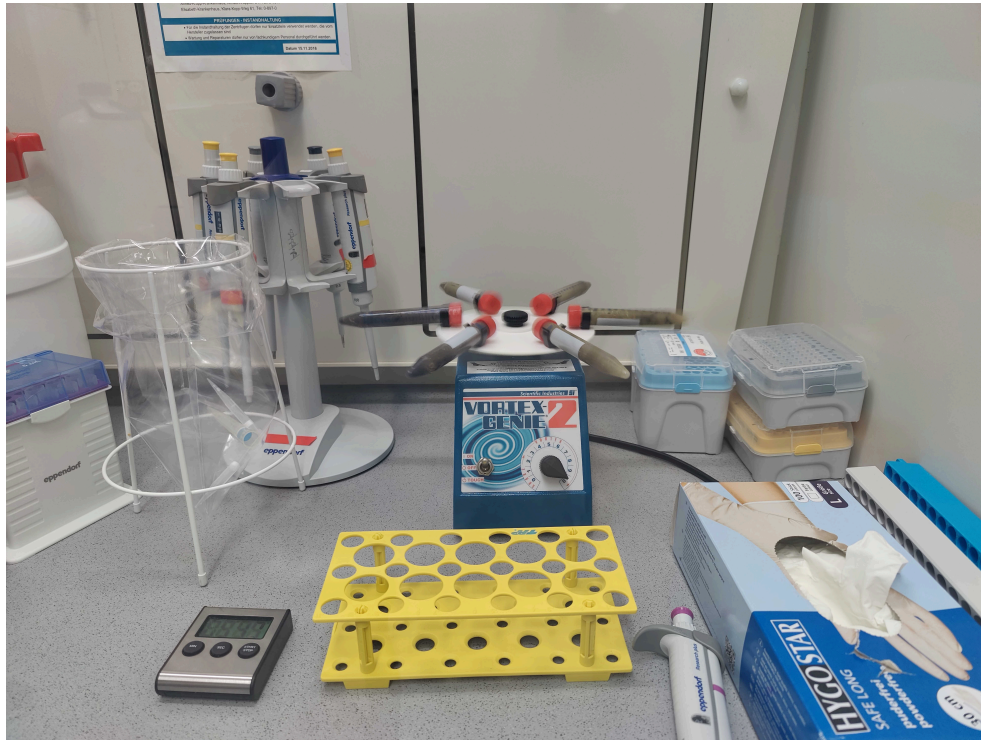
5m




- 4 Control that the cap is closed properly. Vortex until the biphasic layer disappears.
- 5 Place the samples on a Vortex adapter (e.g. Qiagen) and vortex at maximum speed for


 00:15:00 .

15m

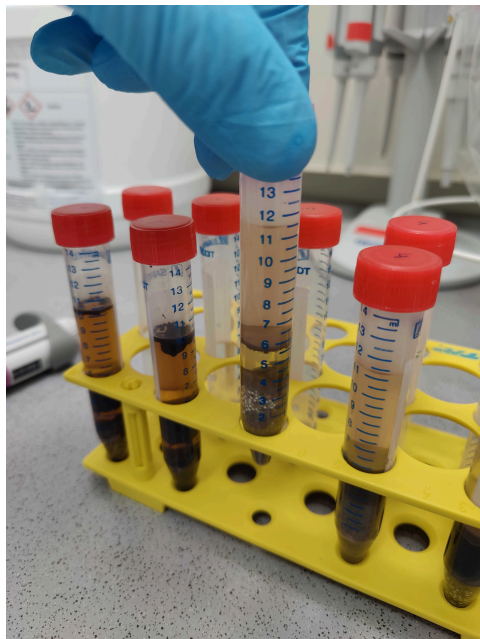
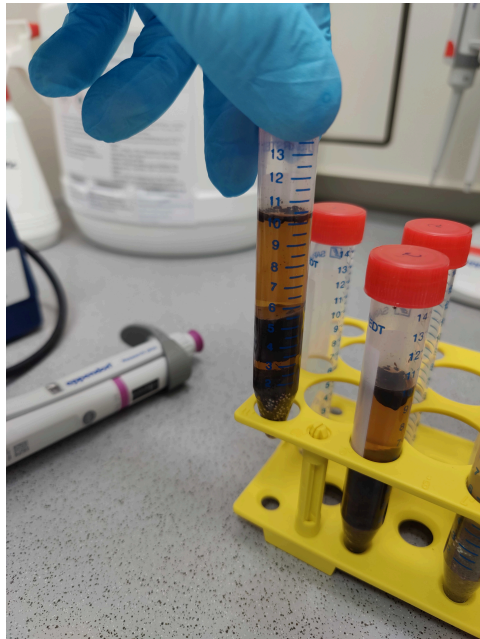


6  2500 x g, Room temperature, 00:10:00

10m

7 Transfer  3.5 mL of the upper aqueous phase to a new 15 mL centrifuge tube. Make sure not to pierce the biphasic layer and to not transfer any liquid from the lower phenol phase. If this happens by accident, recentrifuge the sample and repeat. In soil high in organic matter the biphasic layer will be much thicker than in sediment samples.

2m




Note

If you a 45° centrifuge rotor is used the biphasic layer will also be shifted by 45°. From this point on we use regular centrifuge tubes and have not encountered any issues by doing so.



Inhibitor removal

30m

- 8 Add  1.5 mL precipitation solution . The mixture will be cloudy at first but will clear up after vortexing for a few seconds.

5m



- 9 Incubate at  4 °C for  00:10:00 .




10m




10  2500 x g, Room temperature, 00:10:00 10m

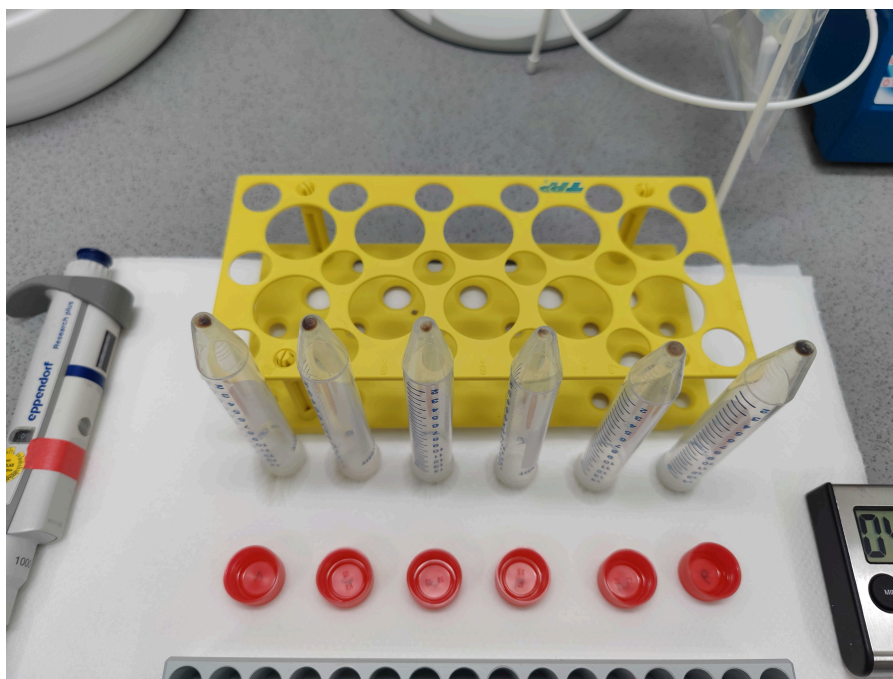
11 Without disturbing the pellet (if there is one), transfer the supernatant to a new 15 mL centrifuge tube. 5m

Nucleic acid precipitation 1h 5m

12 Add  5 mL isopropanol. Mix by vortexing and incubate at  Room temperature for  00:30:00 . 30m




13  2500 x g, Room temperature, 00:30:00 . 30m

14 After centrifugation, a brown pellet should form on the bottom of the tube. Decant the supernatant and invert the tube on a paper towel for  00:05:00 . 5m



Anion exchange clean-up 35m



15 Add  1 mL column equilibration buffer to the pellet and incubate the sample at  45 °C for  00:10:00 . Afterwards, dissolve the pellet by vortexing and/or pipetting.

10m

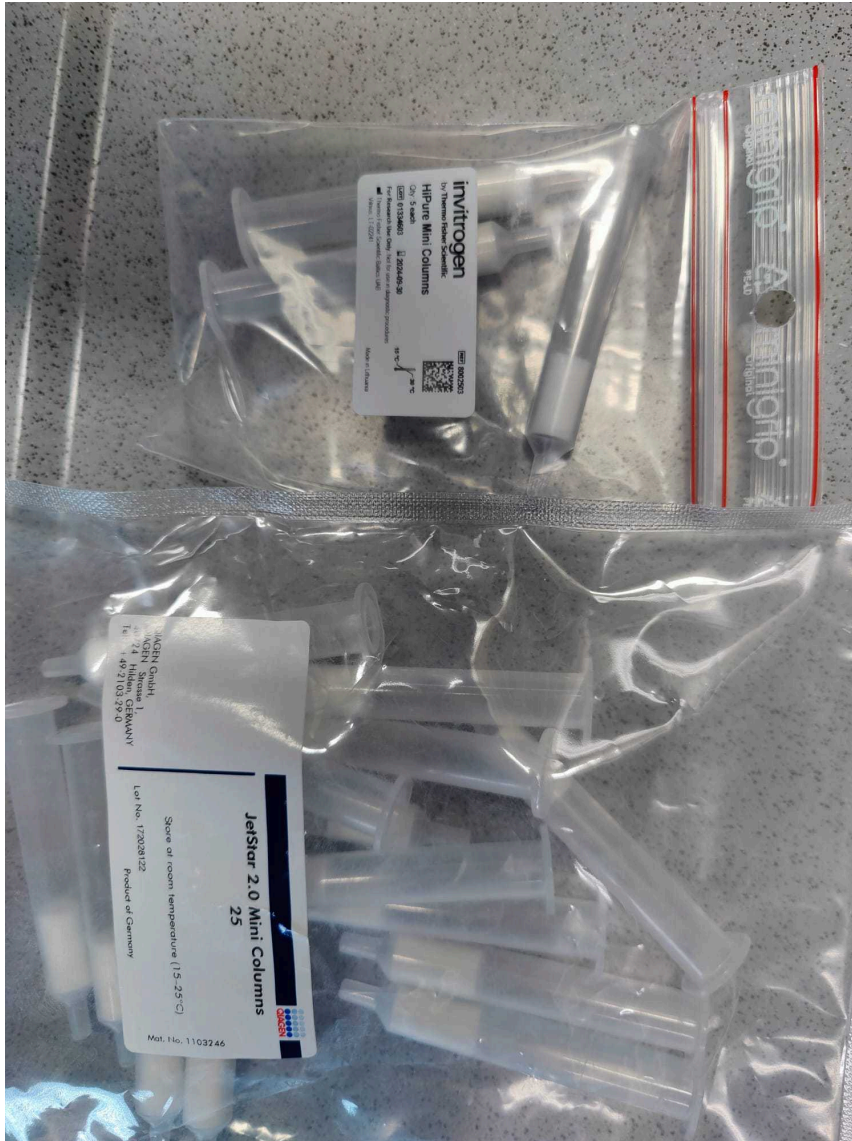
16 Place a HiPure or JetStar 2.0 column in a 15 mL centrifuge tube or a suitable rack.

Note





Racks can be bought at ThermoFisher or Qiagen but are really expensive considering it is only a piece of plastic. We plan on building our own rack for this protocol and publishing a 3D printable model.

Note

The columns seem to be identical. Unfortunately, they are not sold separately but the Kit with the HiPure mini columns is way more affordable than the original kit from Qiagen (approx. 120€ for 25 preps). We are working on a solution to clean and reuse these columns further reducing the costs of this protocol.







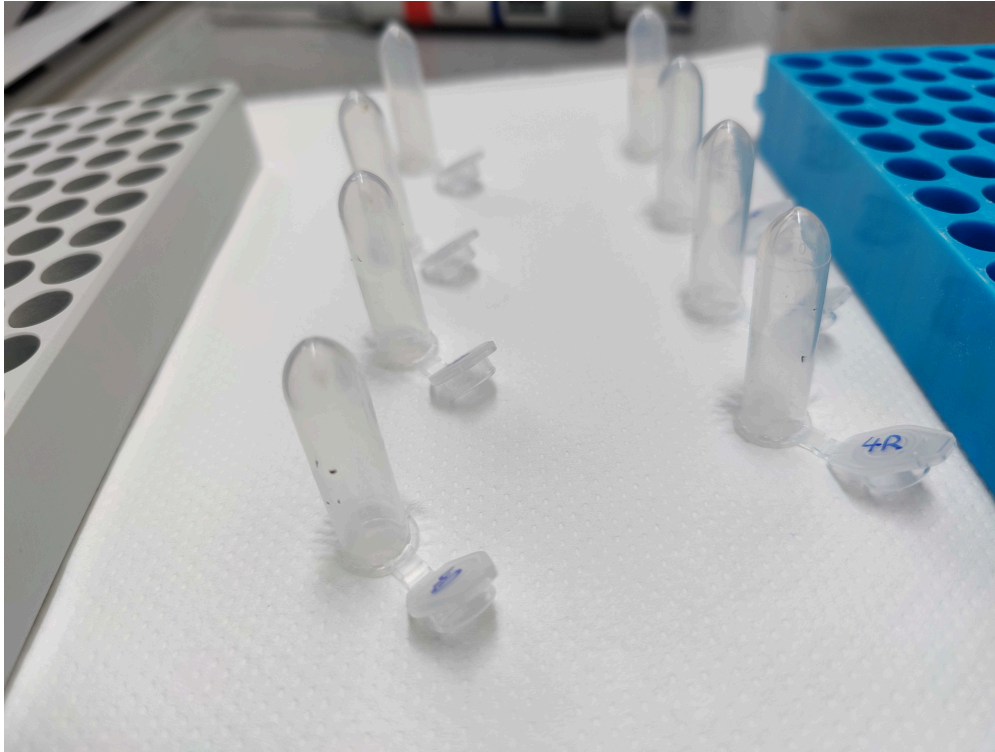



- 17 Equilibrate the column with  2 mL column equilibration buffer . Wait until all of the buffer volume has passed the column by gravity flow. 5m
- 18 Load the sample from step 15 on the equilibrated column. Make sure all that the pellet is dissolved properly or it might heavily reduce the flow-through and reduce the expected yield. Wait until all of the buffer volume has passed the column by gravity flow. 5m
- 19 Wash the column with  1 mL column equilibration buffer . Wait until all of the buffer volume has passed the column by gravity flow. 5m
- 20 Transfer the column to a new 15 mL centrifuge tube or place a 2 mL collection tube in the rack to collect the RNA.
- 21 Add  1 mL RNA elution buffer to the column. Wait until all of the buffer volume has passed the column by gravity flow. 5m
- 22 Transfer the column to a new 15 mL centrifuge tube or place a new 2 mL collection tube in the rack to collect the DNA
- 23 Add  1 mL DNA elution buffer to the column. Wait until all of the buffer volume has passed the column by gravity flow. 5m

Nucleic acid precipitation

50m

- 24 If a 15 mL centrifuge tube was used for elution, transfer the DNA/RNA sample to a 2 mL tube. 5m
- 25 Add 1 mL of isopropanol and incubate at  -20 °C for  00:15:00 15m
- 26  13.000 x g, Room temperature, 00:15:00 15m
- 27 Decant the supernatant and invert the tubes on a paper towel for  00:10:00 to dry the DNA/RNA pellet. The RNA pellet should be barely visible and white to transparent, while the DNA pellet is mostly brownish. 10m



- 28 Resuspend the DNA/RNA in  100 μ L elution buffer . Check the integrity of the DNA/RNA on an agarose gel. afterward

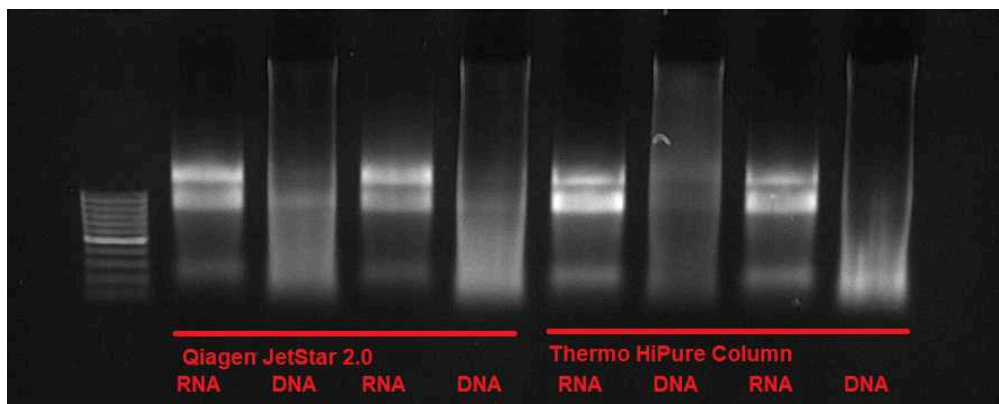
5m

Note

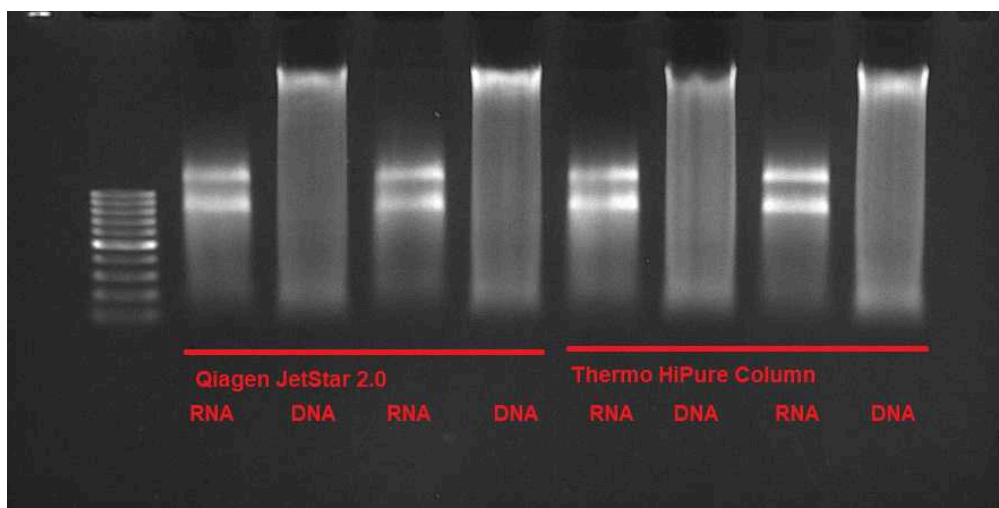
There is usually a low amount of DNA in the RNA sample, while there is always some RNA carry-over to the DNA sample. If RNA-free DNA is needed consider digesting it with RNase A and performing a bead-cleanup afterwards (e.g. **Bead-cleanup protocol for DNA**). We will also publish a similar protocol for the cleanup of RNA samples.

Expected result

In the gel picture, 2 clear RNA bands and an HMW DNA band should be visible. For ~ 5 g of sediment sample, the protocol typically yields ~ 5 μ g of DNA and RNA.



Exemplary gel picture after the extraction. 4 replicate samples were extracted to compare the JetStar and HiPure columns. Residual RNA can be seen in the DNA samples.



The same samples after RNase A digestion and bead-cleanup for DNA and RNA samples. Residual RNA in the DNA is gone, RNA bands look more distinct now.