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Nucleic acids preparations

In 1 collection

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1 *Works for me* dx.doi.org/10.17504/protocols.io.qwgdxbw

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ABSTRACT

This protocol describes the nucleic acids preparations for the *Tara* Oceans expedition and is part of Viral to metazoan marine plankton nucleotide sequences from the *Tara* Oceans expedition.

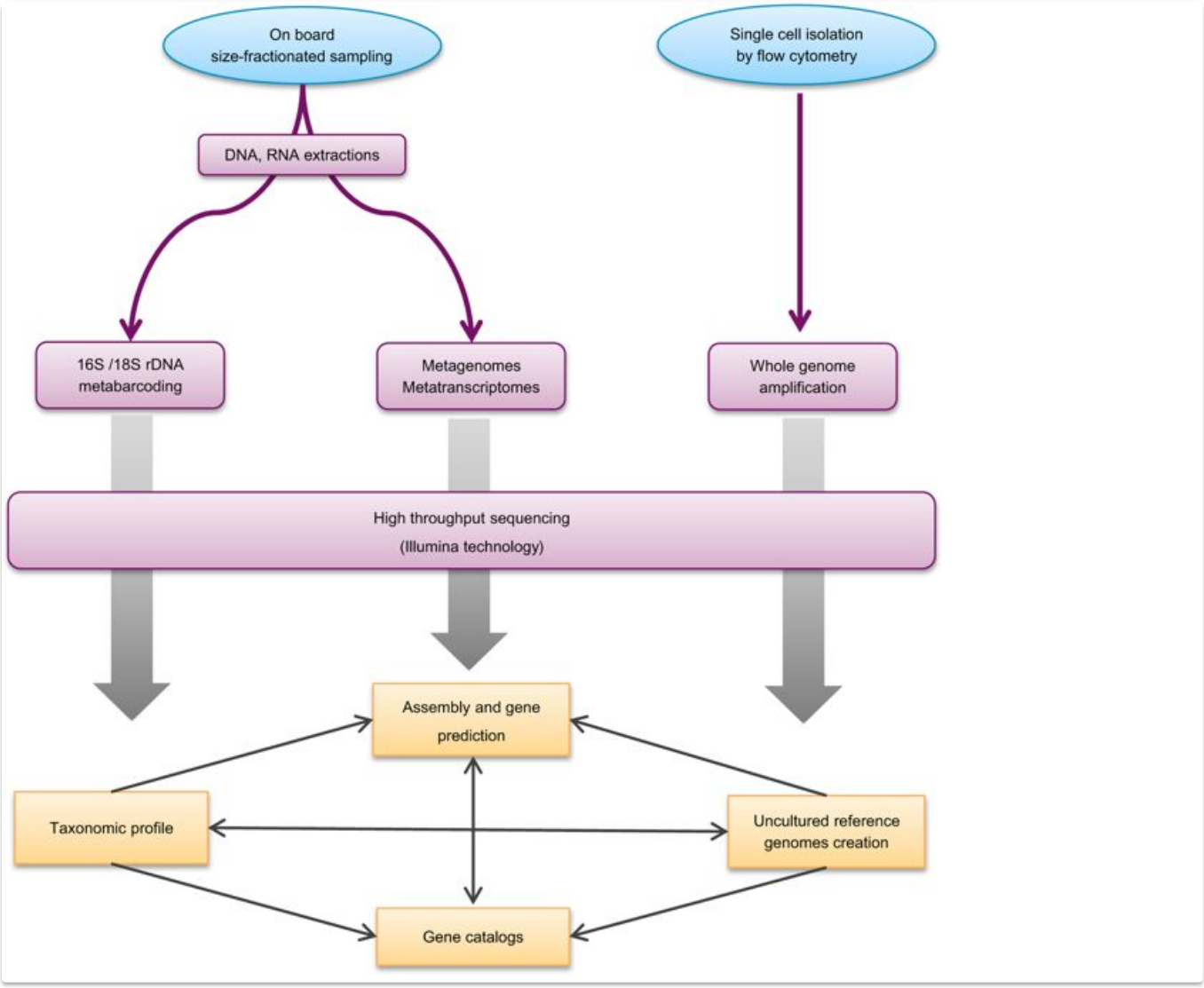

Figure 1: Overview of -omics analysis strategy applied on Tara Oceans samples.

EXTERNAL LINK

https://www.nature.com/articles/sdata201793#methods

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Alberti, A. (2017). Viral to metazoan marine plankton nucleotide sequences from the Tara Oceans expedition. *Scientific Data***4**, 170093 (2017)
doi: 10.1038/sdata.2017.93

ATTACHMENTS

Viral to metazoan marine plankton nucleotide sequences from the Tara Oceans expedition.pdf

GUIDELINES

The 'Nucleic acids preparations' section describes different nucleic acids extraction methods applied to obtain DNA and RNA from the different plankton groups sampled during *Tara* Oceans Expedition.

1. DNA/RNA extractions from size fractions 0.8–5 µm (or 0.8–3 µm), 0.8–2000 µm, 5–20 µm (or 3–20 µm), 3–2000 µm, 20–180 µm and 180–2000 µm (Method ID: Euk_ DNA_RNA_ext)

Plankton from these size fractions was collected on membrane filters and targeted unicellular eukaryotes (protists), usually <200 µm, and metazoans, usually >200 µm.

The protocol applied for nucleic acid extractions was based on simultaneous extraction of DNA and RNA by cryogenic grinding of cryopreserved membrane filters followed by nucleic acid extraction with NucleoSpin RNA kits (Macherey-Nagel, Düren, Germany) combined with DNA Elution buffer kit (Macherey-Nagel). This protocol was derived from optimization and validation experiments in the de Vargas laboratory at the Station Biologique de Roscoff (France). In particular, this preliminary work aimed principally to adapt the efficiency of the cell disruption and DNA/RNA extraction steps in order to efficiently capture nucleic acids from protists and metazoans collected from sea water filtering. Tests were conducted on a mock community composed of 26 monoclonal strains from the Roscoff Culture Collection (http://roscoff-culture-collection.org/) and natural filter samples collected in Roscoff (ASTAN, SOMLIT sampling). During these tests, the cell disruption step was optimized by applying a mechanical cryogrinding method to be sure that cells were efficiently disrupted, minimizing RNA and DNA degradation. Three cryogrinding protocols were tested using a 6,770 Freezer/Mill or 6,870 Freezer/Mill instrument (SPEX SamplePrep, Metuchen, NJ): 1 grinding cycle at 5 knocks per second for 1 min, 1 grinding cycle at 10 knocks per second for 1 min, and 2 grinding cycles at 10 knocks per second for 1 min. Best RNA and DNA quantities were obtained using 2 grinding cycles at 10 knocks per second for 1 min. Then, three simultaneous DNA /RNA extraction protocols were compared: Trizol method followed by RNeasy purification kit (Qiagen, Hilden, Germany), NucleoSpin RNA kit combined with DNA elution buffer set (Macherey-Nagel), and Nucleobond kit (Macherey-Nagel). Best quality results (DNA and RNA integrity conservation, ratios A260/280 and A260/230) were obtained with NucleoSpin RNA kit combined with DNA elution buffer set.

After validation, the procedure described herein was applied in the Genoscope laboratory on Tara Oceans filters from the size fractions cited above.

2. DNA/RNA extractions from size fractions 0.2–1.6 µm and 0.2–3 µm
Two different protocols were applied to these size fractions that mainly targeted prokaryotes. Viruses and giant viruses (giruses) were also recovered in these fractions although dedicated filters (e.g.,<0.22 µm) and specific extractions protocols (described in Sections 4 and 5) were allocated for their analysis.

3. DNA and RNA backups

After nucleic acids extractions, two RNA aliquots and three DNA aliquots were prepared for each sample. One aliquot was used for the library preparation and sequencing process, the second one was stored as a backup. The third DNA aliquot was used for a backup after a whole genome amplification (WGA) process described in the DNA and RNA backup section.

4. Viral particle concentration and DNA extractions from size fraction <0.22 µm (Method ID: virus_DNA_ext)
This protocol describes a technique to recover viruses from natural waters using iron-based flocculation and large-pore-size filtration, followed by resuspension of virus-containing precipitates in a pH 6 buffer.

Briefly, $FeCl_3$ precipitation was used to concentrate viruses from 20–60 l of 0.22 µm filtered seawater, which were then resuspended in ascorbate buffer (0.125 M Tris-base, 0.1 M sodium EDTA dehydrate, 0.2 M magnesium chloride hexahydrate, 0.2 M ascorbate). This Fe-based virus flocculation, filtration and resuspension method (FFR) is efficient (>90% recovery), reliable, inexpensive and adaptable to many aspects of marine viral ecology and genomics research. Data are also available from replicated metagenomes to help researchers' decisions on the impact of linker amplification methods from low input DNA, viral purification strategies, and library preparation and sequencing platform choices. Following resuspension, recovered viruses were treated with DNase I to remove free DNA, followed by the addition of 0.1 M EDTA and 0.1 M EGTA to halt DNase activity, and further concentrated to<1 ml using an Amicon 100 KDa filter (Sigma). DNA was extracted using the Wizard Prep DNA Purification system (Promega, Madison, WI). DNA concentration was assessed with PicoGreen (Thermo Fisher Scientific).

All detailed protocols are listed by name and are documented and available at https://www.protocols.io/groups/sullivan-lab.

5. DNA extractions from sizData availability e fractions 0.2–1.6 µm, 0.1–0.2 µm, 0.45–0.8 µm, 0.2–0.45 µm (Method ID: girus_DNA_ext)

6. Preparation of single cell amplified genomes (SAGs) (Method ID: SAGs_amplif)
Single amplified genomes (SAGs) were generated and their taxonomic assignments were obtained as in Martinez-Garcia et al. with the following modifications. Samples for heterotrophic (aplastidic) cells were stained using SYBR Green I. Samples for phototrophic (plasidic) cells were unstained. No attempt was made to identify mixotrophic cells. Several 384-well plates containing single cells of each type were prepared from each environmental sample. Backup plates were stored frozen at −80 °C. Single cell amplifications were validated by using

an aliquot for PCR with eukaryotic universal 18S primers. SAGs with positive 18S sequence were sent to Genoscope for whole genome sequencing. Upon arrival, 2.5 µl were removed from each well and used to generate an amplified DNA backup by WGA. The reactions were performed as described for DNA extractions (DNA and RNA backups) except that debranching reactions were omitted and instead amplified DNA was purified by QIAamp DNA Mini kit (Qiagen).

Table 1: Summary of libraries generated from Tara Oceans DNA and RNA samples and sequencing experiments performed on each type of library.

Size fractions (µm)	Mainly targeted organisms	Targeted genomic analysis	Sample storage laboratory	Sequencing laboratory	Method ID Nucleic acids preparation (Section)	Method ID Amplicons generation (Section)	Method ID Library preparation (Section)
< 0.2 µM	Viruses	Metagenomics	M. Sullivan lab (University of Arizona, AZ, US)	CEA, Genoscope, France	Virus_DNA_ext (2.4)		MetaG_virus (4.2)
0.2–1.6, 0.1–0.2, 0.45–0.8, 0.2–0.45	Giruses	Metagenomics	N. Grimsley lab (CNRS, Banyuls-sur -Mer, France)	CEA, Genoscope, France	Girus_DNA_ext (2.5)		MetaG (4.1)
0.2–1.6, 0.2–3	Viruses, Giruses, Prokaryotes, small Eucaryotes	16S metabarcoding	S.G. Acinas lab (ICM-CSIC, Barcelona, Spain)	CEA, Genoscope, France	Acinas_Prok_DNA_ ext (2.2)	16S_PCR (3.2)	MetaBar_16S (4.6)
		Metagenomics			Acinas_Prok_DNA_ ext (2.2)		MetaG (4.1)
		Metatranscriptomics by random priming			Acinas_Prok_RNA_ ext Genoscope_Prok_ RNA_ext (2.2)		RiboZero_SMART_strand (4.4)
0.8-inf, 3-inf, 0.8–5 (0.8–3), 5–20 (3–20), 20–180, 180–2,000	Protists and metazoa	18S metabarcoding	C. De Vargas lab (CNRS/UPMC, Roscoff, France)	CEA, Genoscope, France		18S_PCR (3.1)	MetaBar_18S (4.5)
		16S metabarcoding				16S_PCR (3.2)	MetaBar_16S (4.6)
		Metagenomics	P. Wincker lab (CEA, Genoscope, France)		Euk_ DNA_RNA_ ext (2.1)		MetaG (4.1)
		Metatranscriptomics on poly(A)$^+$ RNA			Euk_ DNA_RNA_ ext (2.1)		TS_RNA (4.4) TS_strand (4.4) SMART_dT (4.4)
Samples for SAGs	Protists	*De novo* sequencing	N. Poulton lab (Bigelow lab, ME, US)	CEA, Genoscope, France	SAGs_amplif (2.6)		MetaG_SAGs (4.3)

*Number of libraries with available readsets in public databases at the date of publication of the paper

STEPS MATERIALS

NAME	CATALOG #	VENDOR
1% β-mercaptoethanol	M6250	Sigma
NucleoSpin RNA Midi	740962.20	Macherey and Nagel
NucleoSpin RNA Midi	740962	Macherey and Nagel
70% Ethanol		
NucleoSpin RNA/DNA Buffer Set	740944	Macherey and Nagel
Qubit dsDNA HS (High sensitivity) Assays	Q32851	Thermo Fisher Scientific
NucleoSpin RNA	740955	Macherey and Nagel
NucleoSpin RNA	740955	Macherey and Nagel
Turbo DNA-free Kit	AM1907	Invitrogen - Thermo Fisher
RNA Clean & Concentrator-5 Kit	R1015	Zymo Research
RNeasy Mini Kit	74104	Qiagen

NAME	CATALOG #	VENDOR
PBS buffer		
0.5 mm glass beads		
0.1 mm Zirconia beads	11079101z	BioSpec Products
Buffer RLT 1% β-mercaptoethanol		
70% Ethanol		
142-mm-diameter Millipore polyethersulfone Express Plus membrane filter	GPWP14250	Merck Millipore
Turbo DNA-free Kit	AM1907	Invitrogen - Thermo Fisher
RNA Clean & Concentrator-5 Kit	R1015	Zymo Research
CTAB buffer		
liquid nitrogen		
Chloroform		
Isoamylalcohol		
Isopropanol		
NucleoSpin RNA Midi	740962	Macherey and Nagel
1% β-mercaptoethanol	M6250	Sigma
NucleoSpin RNA Midi	740962	Macherey and Nagel
NucleoSpin RNA Midi	740962	Macherey and Nagel
Ethanol 70%		
NucleoSpin RNA/DNA Buffer Set	740944	Macherey and Nagel
NucleoSpin RNA	740955	Macherey and Nagel
NucleoSpin RNA	740955	Macherey and Nagel
NucleoSpin RNA/DNA Buffer Set	740944	Macherey and Nagel
Lysozyme		
Sodium Dodecyl Sulfate		
Proteinase K		
Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v)		
Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v)		
chloroform:isoamyl alcohol (24:1)		
Centricon concentrator (Amicon Ultra-4 Centrifugal Filter Unit with Ultracel-100 membrane)		Millipore
Qubit dsDNA BR (Broad Range) assay	Q32850	Thermo Fisher Scientific
Qubit dsDNA HS (High sensitivity) Assays	Q32851	Thermo Fisher Scientific
NucleoSpin RNA	740955	Macherey and Nagel
NucleoSpin RNA	740955	Macherey and Nagel
Qubit RNA HS (High Sensitivity) assay	Q32852	Thermo Fisher Scientific

NAME	CATALOG #	VENDOR
Turbo DNA-free Kit	AM1907	Invitrogen - Thermo Fisher
RNA Clean & Concentrator-5 Kit	R1015	Zymo Research
Turbo DNA-free Kit	AM1907	Invitrogen - Thermo Fisher
RNA Clean & Concentrator-5 Kit	R1015	Zymo Research

SAFETY WARNINGS

Please refer to the SDS (Safety Data Sheet) for warnings and hazard information.

1 Please select nucleic acids extraction method.

Euk_ DNA_RNA_ext
DNA/RNA extractions from size fractions 0.8–5 µm (or 0.8–3 µm), 0.8–2000 µm, 5–20 µm (or 3–20 µm), 3–2000 µm, 20–180 µm and 180–2000 µm

DNA/RNA extractions from size fractions 0.2–1.6 µm

girus_DNA_ext
DNA extractions from sizData availability e fractions 0.2–1.6 µm, 0.1–0.2 µm, 0.45–0.8 µm, 0.2–0.45 µm

virus_DNA_ext
Viral particle concentration and DNA extractions from size fraction <0.22 µm

step case

Euk_ DNA_RNA_ext

Euk_ DNA_RNA_ext: filter disruption

2 Accommodate each filter into a grinding vial with 1 ml RA1 lysis buffer (included in the NucleoSpin RNA Midi kit) and 1% β-mercaptoethanol.

1% β-mercaptoethanol
by Sigma
Catalog #: M6250

1 ml RA1 lysis buffer

NucleoSpin RNA Midi
by Macherey and Nagel
Catalog #: 740962.20

[M]1 Mass Percent β-mercaptoethanol

3 Subject to the following grinding program using a 6,770 Freezer/Mill or 6,870 Freezer/Mill instrument :
- 2 min pre-cooling time
- first grinding cycle at 10 knocks per second for 1 min
- 1 min cooling time
- final grinding cycle at 10 knocks per second for 1 min.

6,770 Freezer/Mill or 6,870 Freezer/Mill instrumen (SPEX SamplePrep)

4 Resuspend the cryogrinded powder in 2 ml RA1 lysis buffer with 1% β-mercaptoethanol, transfer to a large capacity NucleoSpin Filter from RNA Midi kit and centrifuge for 10 min at 1,500 g.

2 ml RA1 lysis buffer with 1% β-mercaptoethanol

00:10:00 Centrifugation

NucleoSpin RNA Midi
by Macherey and Nagel
Catalog #: 740962

5 Add 1 ml RA1 lysis buffer with 1% β-mercaptoethanol.

1 ml RA1 lysis buffer with 1% β-mercaptoethanol

6 Recentrifuge the filter for 3 min at 1,500 g.

00:03:00 Centrifugation

7 Transfer the eluate to a new tube and add 1 volume of ethanol 70%. Mix well.

70% Ethanol

Euk_ DNA_RNA_ext: DNA purification

8 Elute DNA by three successive elutions each with 100 µl DNA elution buffer and store in sterile microtubes at −20 °C.

NucleoSpin RNA/DNA Buffer Set
by Macherey and Nagel
Catalog #: 740944

-20 °C Storage

100 µl DNA elution buffer

Euk_ DNA_RNA_ext: RNA purification

9 Immediately continue RNA purification on the previous NucleoSpin RNA Mini spin column by digesting residual genomic DNA. Prepare DNase reaction mixture by add 10 µL reconstituted rDNase to 90 µL Reaction Buffer for rDNase in a 1,5 ml tube. Mix by flicking the tube. Apply 95 µL DNase reaction mixture directly onto the center of the silica membrane of the column. Incubate at room temperature for 15 min.

10 µl rDNase

90 µl Reaction buffer for rDNase

NucleoSpin RNA
by Macherey and Nagel
Catalog #: 740955

10 Incubate column for 15 min at room temperature.

00:15:00 Incubation

11 Wash the column with RA2 and RA3 buffers according to the manufacturer's instructions.

NucleoSpin RNA
by Macherey and Nagel
Catalog #: 740955

12 Elute RNA in 60 µl RNase-free water and store in sterile microtubes at −80 °C.

60 µl RNase-free water

-80 °C Storage

Euk_ DNA_RNA_ext: DNA quality control

13 Quantify DNA by a dsDNA-specific fluorimetric quantitation method using Qubit 2.0 Fluorometer instrument with Qubit dsDNA BR (Broad range) and HS (High sensitivity) Assays.

Qubit dsDNA HS (High sensitivity) Assays
by Thermo Fisher Scientific
Catalog #: Q32851

Qubit 2.0 Fluorometer instrument Q33226
with Qubit dsDNA BR (Broad range) and HS (High sensitivity) Assays

14 Optional: check DNA quality in a sample subset by running 1 µl on 0.7% agarose gel for 60 min at 100 V.

01:00:00 Running 0.7% agarose gel

Euk_ DNA_RNA_ext: RNA quality control

15 Asses quantity of extracted RNA with RNA-specific fluorimetric quantitation on a Qubit 2.0 Fluorometer using Qubit RNA HS Assay.

Qubit 2.0 Fluorometer instrument Q33226
with Qubit RNA HS Assays

16 Assay quality of total RNA by capillary electrophoresis on a Bioanalyzer 2100 instrument (or equivalent), using the RNA 6,000 Pico LabChip kit.

Bioanalyzer 2100 instrument G2939BA
with RNA 6,000 Pico LabChip kit

Euk_ DNA_RNA_ext: additional DNAse treatment on purified RNA

17 In order to reduce as far as possible the risk of residual genomic DNA, apply a further DNase treatment.

The previous RNA extraction procedure includes an in-column DNase treatment. Based on previous experience with this method, this step is sometimes only partially effective and does not always preclude the presence of trace DNA in final RNA samples. DNA removal in RNA samples is essential to prevent the incorporation of any genomic material in the RNA-Seq library and consequently the misinterpretation of RNA-Seq data analyses.

18 Process the RNA samples further as follows: treat a quantity of 5 µg, or less, total RNA aliquots with Turbo DNA-free kit, according to the manufacturer's DNase treatment protocol.
Set up the reaction by mixing total RNA with 5 µL 10X TURBO DNase Buffer and 1 µl DNAse in final 50 µl volume. Mix by gently flicking the tube. Do not vortex.

5 µg Total RNA aliquots

Turbo DNA-free Kit
by Invitrogen - Thermo Fisher
Catalog #: AM1907

19 Incubate the reaction mixture for 30 min at 37 °C. (1/2)

37 °C Incubation

00:30:00 Incubation

20 Add 2 µL DNAse, mix by gently flicking the tube (no vortex) and incubate the reaction mixture for 30 min at 37 °C. (2/2)

37 °C Incubation

00:30:00 Incubation

Euk_ DNA_RNA_ext: RNA purification

21 Purify the reaction mixture with the RNA Clean and Concentrator-5 kit following the procedure described for retention of >17 nt RNA fragments.

RNA Clean & Concentrator-5 Kit
by Zymo Research
Catalog #: R1015

22 Elute RNA in 9–15 µl nuclease-free water by two elution steps in order to maximize recovery.

9 µl Nuclease-free water

Euk_ DNA_RNA_ext: RNA quality control

23 Quantify purified RNA with Qubit RNA HS Assay.

The efficiency of DNase treatment should be assessed by PCR. When dealing with large number of samples, this validation step should be done on a subset of samples in order to check the efficiency of the treatment.

DNA/RNA extractions from size fractions 0.2–1.6 µm

step case

DNA/RNA extractions from size fractions 0.2–1.6 µm

2 Two different protocols are applied to these size fractions that mainly target prokaryotes. Viruses and giant viruses (giruses) are also recovered in these fractions although dedicated filters (e.g.,<0.22 µm) and specific extractions protocols are allocated for their analysis.

Please select from the following protocols:

Acinas lab DNA extraction (Method ID: Acinas_prok_DNA_ext)

Acinas lab RNA extraction (Method ID: Acinas_prok_RNA_ext)

Genoscope lab DNA/RNA extraction (Method ID: Genoscope_prok_RNA_ext)

girus_DNA_ext

step case

girus_DNA_ext

2 These size fractions are used to target giant viruses (giruses). DNA is extracted using a modified CTAB protocol.

3 Crush filters in liquid nitrogen, incubate at 60 °C for one hour in a CTAB buffer.

CTAB buffer

liquid nitrogen

60 °C Incubation

01:00:00 Incubation

4 Purify DNA using an equal volume of chloroform/isoamyl alcohol (24:1) and a one-hour-long-RNase digestion step.

Chloroform

Isoamylalcohol

01:00:00 RNase digestion

5 Precipitate DNA with a 2/3 volume of isopropanol and wash with 1 ml of a solution containing 76% v/v ethanol and 10 mM ammonium acetate solution.

Isopropanol

1 ml Solution containing 76% v/v ethanol and 10 mM ammonium acetate solution

virus_DNA_ext

step case

virus_DNA_ext

2 Please follow steps of the following protocol:

Preparation of Virus DNA from Seawater for Metagenomics

step case

Acinas_prok_DNA_ext

Acinas lab DNA extraction

3 Cut half of the 0.22 µm 142-mm-diameter Millipore polyethersulfone Express Plus membrane filter into small pieces and soak in 3 ml lysis buffer (40 mM EDTA, 50 mM Tris-HCl, 0.75 M sucrose).

142-mm-diameter Millipore polyethersulfone Express Plus membrane filter
by Merck Millipore
Catalog #: GPWP14250

3 ml Lysis buffer

4 Add lysozyme (1 mg ml^{-1} final concentration) and incubate samples at 37 °C for 45 min while slightly shaken.

37 °C Incubation

00:45:00 Incubation

Lysozyme

5 Add sodium dodecyl sulfate (1% final concentration) and proteinase *K* (0.2 mg ml^{-1} final concentration) and incubate samples at 55 °C for 60 min while slightly shaking.

Sodium Dodecyl Sulfate

Proteinase K

55 °C Incubation

01:00:00 Incubation

step case

Acinas_prok_RNA_ext

Acinas lab RNA extraction

3 RNA isolation is performed using the RNeasy Mini kit with a modified protocol.

RNeasy Mini Kit
by Qiagen
Catalog #: 74104

4 Cut the filters into small pieces and wash with pre-chilled PBS buffer in order to eliminate the RNA Later.

PBS buffer

To avoid loss of any cell during the washing process, pass the washing solution through a glass cup filtration system including a filter of 0.22 µm pore-size.

5 Place the pieces of the filter and the extra filter in a 50 ml falcon tube with a mixture of beads (1 ml of 0.5 mm glass beads and 2 ml of 0.1 mm Zirconia beads) and 10 ml buffer RLT+1% β-mercaptoethanol.

0.5 mm glass beads

0.1 mm Zirconia beads
by BioSpec Products
Catalog #: 11079101z

Buffer RLT 1% β-mercaptoethanol

1 ml 0.5 mm glass beads

2 ml 0.1 mm Zirconia beads

10 µl Buffer RLT+1% β-mercaptoethanol

Genoscope_prok_RNA_ext: filter disruption

step case

Genoscope_prok_RNA_ext

Genoscope lab DNA/RNA extraction

3 Cut the membranes in many small pieces, dispatch equally in two vials.

This procedure is based on cryogenic grinding followed by DNA/RNA purification with Nucleospin RNA kit as previously described for protists- and metazoans- enriched nucleic acids isolation (Euk_DNA_RNA_ext section)

Modifications are applied in order to efficiently grind larger and thicker 0.2–1.6 µm and 0.2–3 µm filters.

4 Add 1 ml RA1 lysis buffer included in the NucleoSpin RNA Midi kit (Macherey-Nagel) and 1% β-mercaptoethanol (Sigma, St Louis, MO) to the grinding vials.

1 ml RA1 lysis buffer

NucleoSpin RNA Midi
by Macherey and Nagel
Catalog #: 740962

1% β-mercaptoethanol
by Sigma
Catalog #: M6250

5 Resuspend cryoground powders from each vial in 2 ml RA1 lysis buffer in the presence of 1% β-mercaptoethanol, then pool together and transfer to a single NucleoSpin Filter Midi.

2 ml RA1 lysis buffer with 1% β-mercaptoethanol

NucleoSpin RNA Midi
by Macherey and Nagel
Catalog #: 740962