Preparation of Virus DNA from Seawater for Metagenomics

Matthew Sullivan Lab

Abstract

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Guidelines

Needed:

- 0.2 µm filtered seawater with iron chloride
- Mg-EDTA-Ascorbate (Oxalate) buffer
- DNase I
- EDTA
- EGTA
- Centrifugal concentrators
- Resuspension buffer
- Wizard prep column
- Wizard prep resin
- Quant-iT dsDNA Pico Green assay kit

Materials

Quant-iT dsDNA Pico Green assay kit (Invitrogen) P7589 by Life Technologies

Wizard Minicolumns A7211 by Promega

Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-100 membrane 910024 by Emd Millipore

Protocol

Step 1.
Precipitate 0.2µm filtered seawater with iron chloride and store the filters at 4°C

NOTES
VERVE Team 14 Jul 2015
See Ferric Chloride Precipitation of Viruses from Seawater protocol for more details

Step 2.
Resuspend the precipitated virus in Mg-EDTA-Ascorbate (or Oxalate) buffer just prior to DNA extraction *(also see Ferric Chloride Resuspension Buffer protocol)*

Step 3.
Treat the resuspended virus preparation with DNase I for 2 hours at room temperature

DURATION
02:00:00

Step 4.
Inactivate the enzyme with EDTA and EGTA

NOTES
VERVE Team 16 Jun 2015
Previous work in the Tucson Marine Phage Lab has shown that it is not necessary to perform CsCl gradient centrifugation on the virus preparation prior to DNA extraction. The iron chloride precipitation followed by DNAse I treatment removes the majority of bacterial DNA that could contaminate the final metagenomic sequence. CsCl may be used, but for deep or other samples where viruses are limiting, the recovery of DNA may be significantly diminished by performing this step.
VERVE Team 14 Jul 2015
See DNAse I Treatment Protocol for more details.

Step 5.
Concentrate the resuspended virus preparation using centrifugal concentrators down to 3-5 ml total volume if performing CsCl cleanup or to 1-2 ml total volume if proceeding directly to DNA extraction

REAGENTS
Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-100 membrane 910024 by Emd Millipore

NOTES
VERVE Team 16 Jun 2015
The Sullivan Lab recommends Amicon Ultra, 100kDa MWCO, either 15 ml or 5 ml capacity (Millipore UFC910024 or UFC810024), following manufacturer's directions.

Step 6.
Remove the concentrate to a fresh tube

Step 7.
Rinse the membrane with an additional 0.5-1 ml resuspension buffer by pipetting up and down along the membrane

PROTOCOL

. Resuspension Buffer P1

CONTACT: Bonnie Poulos
Step 7.1.
Dissolve 6.06g Tris Base in 800 mL MilliQ water

REAGENTS
Tris Base BP152-1 by Fisher Scientific

ANNOTATIONS
Chris Upton 12 Oct 2015
Isn't this usually made from stock Tris and EDTA solutions?

Bonnie Poulos 12 Oct 2015
It can be made either way depending on what you have available in the lab. Some find it easier to use stock solutions, but it is not necessary as long as final pH is adjusted.

Protocol

Step 7.2.
Add 3.72g EDTA disodium salt, dihydrate to the 800 mL Tris base and stir to dissolve

AMOUNT
6 g Additional info:

REAGENTS
EDTA, disodium salt, dihydrate S312-500 by Fisher Scientific

Protocol

Step 7.3.
Adjust the pH to 8.0 with HCl

Protocol

Step 7.4.
Adjust the volume to 1 liter with MilliQ water

Protocol

Step 7.5.
Add 100mg RNase A per liter of buffer P1

AMOUNT
100 mg Additional info:

Step 8.
Pool with the concentrated sample.

Step 9.
At this point, either layer this onto premade CsCl gradients and isolate the virus fraction according to the protocol *Cesium Chloride Purification of Viruses* (or alternatively, *Cesium Chloride DNA Extraction of Viruses using Wizard Prep Columns)* or proceed directly to Wizard Prep purification of DNA.

Step 10.
Extract the DNA using Wizard Prep columns and resin

NOTES
VERVE Team 14 Jul 2015
See Cesium Chloride DNA Extraction of Viruses using Wizard Columns protocol for more details

Step 11.
Calculate the amount of DNA recovered using Quant-iT dsDNA Pico Green assay kit following manufacturer's directions

REAGENTS
Quant-iT dsDNA Pico Green assay kit (Invitrogen) P7589 by Life Technologies

NOTES
VERVE Team 26 Jun 2015
The community virus DNA is now ready for metagenomic library preparation or for linker amplification if more DNA is needed for sequencing (see Linker Amplification protocol and Bar-Coded Oligos for LA if needed).
VERVE Team 14 Jul 2015
See Quant-iT Pico Green dsDNA Assay Protocol for more details