Preparation of Tara Sample DNA From Iron-Chloride Precipitates

Matt Sullivan Lab

Abstract

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Guidelines

References to 'DNA Extraction of Viruses using Wizard Prep Columns' protocol

Needed:

- Filters
- Forceps (bleached)
- Aluminum foil squares
- 50cc tube
- Resuspension buffer (0.1M EDTA - 0.2M MgCl2 - 0.2M Ascorbate Buffer)
- Sterile applicator sticks
- 20 mL filter
- 2x 10mL filter
- 20L seawater
- Parafilm
- Aluminum foil
- Rotator
- Rubber bands
- 5mL pipet
- 15mL or 50 mL sterile tube
- Centrifuge @ 1000 rpm, 14rpm, 1,000g and 10,000g
- Stock DNase
- DNase buffer
- EDTA
- EGTA
- Amicon Ultra 100kDa centrifugal concentrator
- Wizard Prep Resin
- Wizard Prep Column
- 3mL Luer Lock Syringe
- 5mL Snap-cap tube
- Plunger
- Syringe barrel
- 80% Isopropanol
- 1.5 mL centrifuge tube

- 50-100 µL TE
- Pico Green assay

Materials

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

Protocol

Samples

Step 1.
Locate samples and record inventory number and number of filters (or portions) used

Samples

Step 2.
Turn the filters precipitate-side out with bleached forceps that have been rinsed with water and use aluminum foil squares as work surface, discarding after each filter

Samples

Step 3.
Remove filter from 50cc tube and refold so that precipitate side comes in contact with resuspension buffer

Samples

Step 4.
Return filter to tube using forceps and sterile applicator sticks

Samples

Step 5.
Keep filters dark and refrigerated until ready to suspend

ANNOTATIONS

Bonnie Poulos 11 Jan 2016
There should be moisture in the tube - do not let the filters dry out. Usually when the filters are put into the tube initially, they carry some residual seawater with them that serves this purpose. A milliliter of sterile molecular biology grade water can be added if necessary.

Resuspension of Iron Chloride Precipitates

Step 6.
Prepare 1x or 2x resuspension buffer.

NOTES

VERVE Team 07 Jul 2015
Check that pH of buffer is pH 6.0-6.5
VERVE Team 07 Jul 2015
Prepare about 20% more than needed
VERVE Team 09 Jul 2015
See 0.1M EDTA - 0.2M MgCl2 - 0.2M Ascorbate Buffer

Resuspension of Iron Chloride Precipitates

Step 7.
Prepare 20mL 1x or 10mL 2x per filter from 20L of seawater

Resuspension of Iron Chloride Precipitates

Step 8.
Add resuspension buffer to each filter

Resuspension of Iron Chloride Precipitates

Step 9.
Parafilm the tubes and wrap all of them in aluminum foil

Resuspension of Iron Chloride Precipitates

Step 10.
Put foil pack of tubes on rotator, in cold room, using rubber bands to secure, and rotate slowly overnight

DURATION
15:00:00

Resuspension of Iron Chloride Precipitates

Step 11.
To recover resuspended viruses, remove the liquid at the bottom using a 5mL pipet and transfer to a fresh 15mL or 50mL sterile, labeled tube

Resuspension of Iron Chloride Precipitates

Step 12.
Using bleached and rinsed forceps or sterile applicator sticks, pull edge of filter up and over lip of tube and secure with the lid

Resuspension of Iron Chloride Precipitates

Step 13.
Centrifuge 1000rpm, 5min, 18°C to recover liquid left on filter

DURATION
00:05:00

Resuspension of Iron Chloride Precipitates

Step 14.
Remove liquid and add more buffer if filter still has a lot of precipitate clinging to it

Resuspension of Iron Chloride Precipitates

Step 15.
Rotate for several more hours

DURATION
03:00:00

Resuspension of Iron Chloride Precipitates

Step 16.
Repeat removal of liquid

DNase Treatment

Step 17.
Dilute stock DNase 1:100 in 10x DNase buffer (concentration = 400 U/mL)

NOTES
VERVE Team 14 Jul 2015
See DNase Treatment Protocol for details

DNase Treatment

Step 18.

Add 1/10th volume of diluted DNase to each sample

NOTES

VERVE Team 24 Jun 2015

DNase now at 40 U/mL and in 1x reaction buffer

VERVE Team 14 Jul 2015

See DNase Treatment Protocol for details

DNase Treatment

Step 19.

Parafilm tubes, wrap them in aluminum foil and attach to tube rotator with rubber bands

NOTES

VERVE Team 14 Jul 2015

See DNase Treatment Protocol for details

DNase Treatment

Step 20.

Incubate by rotating slowly at room temperature for 2 hours

DURATION

02:00:00

NOTES

VERVE Team 14 Jul 2015

See DNase Treatment Protocol for details

DNase Treatment

Step 21.

Inactivate DNase by adding EDTA and EGTA to 0.1M final concentration each

NOTES

VERVE Team 14 Jul 2015

See DNase Treatment Protocol for details

DNase Treatment

Step 22.

Mix by inverting the tube several times

NOTES

VERVE Team 14 Jul 2015

See DNase Treatment Protocol for details

Concentration and DNA extraction

Step 23.

Add the DNase treated and inactivated sample to the top reservoir of Amicon Ultra 100kDA centrifugal concentrators

NOTES

VERVE Team 24 Jun 2015

Usually this will be in the 15mL size in 50mL centrifuge tubes

VERVE Team 14 Jul 2015

See DNA Extraction of Viruses using Wizard Prep Column for more details

ANNOTATIONS

Ievgeniia Prekrasna 21 Oct 2017

Are there other options to concentrate viruses instead of using Amicon Ultra centrifugal concentrators? Is it acceptable here to provide PEG precipitation?

Concentration and DNA extraction

Step 24.

Centrifuge the concentrators at 1000 *g* for 5 minute intervals at 18°C until samples are at less than 2mL each

DURATION

00:05:00

NOTES

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See DNA Extraction of Viruses using Wizard Prep Column for more details

Concentration and DNA extraction

Step 25.

Put 1mL of resin on one Wizard Prep column

AMOUNT

1 µl Additional info:

NOTES

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Use no more than 1mL sample per 1mL of Wizard Prep resin (0.5mL is ideal)

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See DNA Extraction of Viruses using Wizard Prep Column for more details

ANNOTATIONS

Bonnie Poulos 11 Jan 2016

This should state: use 1ml of resin per 0.5ml of sample for application onto one Wizard Prep column.

Concentration and DNA extraction

Step 26.

Thoroughly resuspend resin by shaking vigorously

NOTES

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See DNA Extraction of Viruses using Wizard Prep Column for more details

Concentration and DNA extraction

Step 27.

Mix 1mL per 0.5-1.0mL of DNA

NOTES

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See DNA Extraction of Viruses using Wizard Prep Column for more details

ANNOTATIONS

Bonnie Poulos 11 Jan 2016

Resin works best at a ratio of 1ml resin to 0.5ml sample. If there is more than 1ml sample per 1ml resin, DNA recovery will be reduced.

Concentration and DNA extraction

Step 28.

Add each 1mL of resin to a 3mL luer lock syringe attached to a Wizard column

NOTES

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See DNA Extraction of Viruses using Wizard Prep Column for more details

Concentration and DNA extraction

Step 29.

Push through into a 5mL snap-cap tube

NOTES

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See DNA Extraction of Viruses using Wizard Prep Column for more details

Concentration and DNA extraction

Step 30.

Save this tube until DNA quantification.

NOTES

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See DNA Extraction of Viruses using Wizard Prep Column for more details

Concentration and DNA extraction

Step 31.

Remove syringe from column

NOTES

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See DNA Extraction of Viruses using Wizard Prep Column for more details

Concentration and DNA extraction

Step 32.

Remove plunger

NOTES

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See DNA Extraction of Viruses using Wizard Prep Column for more details

Concentration and DNA extraction

Step 33.

Reattach syringe barrel to column

NOTES

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See DNA Extraction of Viruses using Wizard Prep Column for more details

Concentration and DNA extraction

Step 34.

Wash columns with 2mL 80% isopropanol pushing through with the syringe barrel into a waste container

NOTES

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See DNA Extraction of Viruses using Wizard Prep Column for more details

Concentration and DNA extraction

Step 35.

Put columns into 1.5mL centrifuge tube and centrifuge at 10,000 *g* for 2.5 minutes to remove residual alcohol

DURATION
00:02:30

NOTES
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See DNA Extraction of Viruses using Wizard Prep Column for more details

Concentration and DNA extraction

Step 36.
Discard tube

NOTES
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See DNA Extraction of Viruses using Wizard Prep Column for more details

Concentration and DNA extraction

Step 37.
Place column into a fresh 1.5mL centrifuge tube and pipet on 50-100µL of TE (0.02µm filtered and heated to 80°C)

NOTES
VERVE Team 14 Jul 2015
See DNA Extraction of Viruses using Wizard Prep Column for more details

Concentration and DNA extraction

Step 38.
Vortex gently (1400rpm) and let sit 1 minute

DURATION
00:01:00

NOTES
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See DNA Extraction of Viruses using Wizard Prep Column for more details

Concentration and DNA extraction

Step 39.
Centrifuge at 10,000 *g* for 1 minute

DURATION
00:01:00

NOTES
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See DNA Extraction of Viruses using Wizard Prep Column for more details

Concentration and DNA extraction

Step 40.
Transfer extracted DNA to Lo-bind DNA 0.5mL tubes

NOTES
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See DNA Extraction of Viruses using Wizard Prep Column for more details

Concentration and DNA extraction

Step 41.
Repeat elution above (steps 37-40) one more time.

NOTES

VERVE Team 14 Jul 2015

See DNA Extraction of Viruses using Wizard Prep Column for more details

DNA Quantification

Step 42.

Use 1-2µL of the first elution and 4-5µL of the second elution for the Pico Green assay to quantify the extracted DNA

Quant-iT dsDNA Pico Green assay kit (Invitrogen)

REAGENTS

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

DNA Quantification

Step 43.

Use the Excel spreadsheet to calculate the ng/µL DNA for each sample

DNA Quantification

Step 44.

Store in -80°C ultra-low freezer

DNA Quantification

Step 45.

Label tubes with date, station, depth and concentration and store in -80°C ultra-low freezer