Cesium Chloride Protocol for Phage

Forest Rohwer

Abstract

This protocol contains 4 sections:
1. Cesium Chloride Gradient to separate phage from bacteria. Marine viruses around 1.5g/ml
2. DNase I treatment of CsCl-purified phage
3. Formamide Extraction of DNA from phagE4.
4. CTAB

Citation: Forest Rohwer Cesium Chloride Protocol for Phage. **protocols.io**
dx.doi.org/10.17504/protocols.io.c37yrm
Published: 25 Jan 2016

Guidelines

Needed:

- Cesium solutions
- 0.02 µm filter
- CsCl
- Tubes
- Centrifuge @ 22,000 rpm, 4,000 rpm,
- 0.2 vol chloroform
- 10-15 µL DNase I
- RNase (if needed)
- Oak ridge tube
- 0.1 vol 2M Tris HCL (pH 8.5)
- 0.2M EDTA
- 100 µL 0.5M EDTA
- 10 Fl glycogen
- 2 vol 100% ethanol
- 70% ethanol
- Sorvall ST 8 Benchtop centrifuge
- 567 µL TE Buffer
- 30 µL 10% SDS
- 3 µL of 20 µg/mL proteinase K
- Incubator
- 100 µL 5M NaCl
- 80 µL CTAB/NaCl solution
- Microcentrifuge
- Phenol/Chloroform solution
- 0.7 vol Isopropanol
- 50 µL Sigma water

- Nanodrop

Materials

SW41 Ti (with Beckman Ultra) Swinging Bucket 331362 by Beckman Coulter

Sorvall ST 8 Benchtop Centrifuge 75007200 by Beckman Coulter

Protocol

Cesium Chloride Gradient to separate phage from bacteria. Marine viruses around 1.5g/ml

Step 1.

Filter cesium solutions with 0.02µm filter

NOTES

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Use filtered seawater to make solution. Mark top of first gradient layer with pen before adding next layers.

Cesium Chloride Gradient to separate phage from bacteria. Marine viruses around 1.5g/ml

Step 2.

Mark top of first gradient layer with pen before adding next layers

Cesium Chloride Gradient to separate phage from bacteria. Marine viruses around 1.5g/ml

Step 3.

Set up gradient as (from top to bottom):

- 1ml of 1.7 g/ml, mark top of layer
- 1ml of 1.5 g/ml, mark top of layer
- 1ml of 1.35 g/ml
- 8.5ml of 1.15 g/ml - this is phage concentrate.

Cesium Chloride Gradient to separate phage from bacteria. Marine viruses around 1.5g/ml

Step 4.

Add CsCl to your sample.

Cesium Chloride Gradient to separate phage from bacteria. Marine viruses around 1.5g/ml

Step 5.

Check balance of tubes.

Cesium Chloride Gradient to separate phage from bacteria. Marine viruses around 1.5g/ml

Step 6.

Centrifuge at 22,000 rpm for 2 hours at 4°C, approximately 60-80,000xg.

DURATION

02:00:00

Cesium Chloride Gradient to separate phage from bacteria. Marine viruses around 1.5g/ml

Step 7.

Pierce tube at 1.7/1.5 g/ml interface.

Cesium Chloride Gradient to separate phage from bacteria. Marine viruses around 1.5g/ml

Step 8.

Bevel up, and collect 1.5 mls (should include 1.5 g/ml step and 1.5 to 1.35 interface)

Cesium Chloride Gradient to separate phage from bacteria. Marine viruses around 1.5g/ml

Step 9.

Check with slides for virus particles, etc.

Cesium Chloride Gradient to separate phage from bacteria. Marine viruses around 1.5g/ml

Step 10.

Use 20 µl from [viral] fraction and 500 µl from "upper" layers.

NOTES

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Alternatively, can pierce at bottom of tube and take 0.5 ml fractions for first 3 mls, then make slides of each and extract from appropriate fraction.

Cesium Chloride Gradient to separate phage from bacteria. Marine viruses around 1.5g/ml

Step 11.

Store in fridge at 4°C until ready to extract.

DNase I treatment of CsCl-purified phage

Step 12.

Add 0.2 vol chloroform, mix and incubate 10 min. at room temperature

AMOUNT

0 µl Additional info:

DURATION

00:10:00

DNase I treatment of CsCl-purified phage

Step 13.

Spin at 4000 rpm in table Beckman for 10 minutes to separate phases.

DURATION

00:10:00

DNase I treatment of CsCl-purified phage

Step 14.

Save and transfer top phase to new 15 ml tubes.

DNase I treatment of CsCl-purified phage

Step 15.

Add 10-15 µl DNase I (1 mg/ml in H_2O) to phage sample (1.2-1.5ml)

AMOUNT

12 µl Additional info:

DNase I treatment of CsCl-purified phage

Step 16.

Incubate 15 minutes at 37°C. Treat with RNase if RNA is to be extracted.

DURATION

00:15:00

DNase I treatment of CsCl-purified phage

Step 17.

Inactivate for 15 minutes at 65°C.

DURATION

00:15:00

DNase I treatment of CsCl-purified phage

Step 18.

Transfer all to new "oak ridge" tube for faster centrifugation later.

Formamide Extraction of DNA from phage

Step 19.
Add 0.1 volume 2 M TrisHCL (pH 8.5)/0.2 M EDTA

Formamide Extraction of DNA from phage

Step 20.
Add 100 µl 0.5 M EDTA per 10ml

AMOUNT
100 µl Additional info:

Formamide Extraction of DNA from phage

Step 21.
Add 1 volume of formamide

Formamide Extraction of DNA from phage

Step 22.
Add 10 Fl glycogen

Formamide Extraction of DNA from phage

Step 23.
Incubate at room temperature for 30 minutes.

DURATION
00:30:00

Formamide Extraction of DNA from phage

Step 24.
Add 2 volumes of room temperature 100% ethanol.

Formamide Extraction of DNA from phage

Step 25.
Pellet in Sorvall (12,000 rpm for 20 minutes)

DURATION
00:20:00

Formamide Extraction of DNA from phage

Step 26.
Wash with 70% ethanol, two times.

Formamide Extraction of DNA from phage

Step 27.
Resuspend into 567 µl TE and continue with CTAB extraction.

AMOUNT
567 µl Additional info:

PROTOCOL

. TE Buffer

CONTACT: VERVE Team
Step 27.1.
Prepare 5ml of 1M Tris

AMOUNT
61 g Additional info:

PROTOCOL

. 1M Tris

CONTACT: VERVE Team

Step 1.1.
60.57 g of Tris (hydroxymethyl) aminomethane in 0.5L Milli-Qwater

Step 1.2.
Bring pH to 8.0 using HCl

Making 0.5M EDTA

Step 27.2.
Prepare 1ml 0.5M EDTA

AMOUNT
19 g Additional info:

PROTOCOL

. 0.5M EDTA

CONTACT: VERVE Team

Step 2.1.
Mix 18.6 g EDTA in 100ml Milli-Q water

AMOUNT
19 g Additional info:

Step 2.2.
Bring pH to 8.0 using NaOH

NOTES
VERVE Team 22 Jun 2015
EDTA will not be soluble until pH reaches 8.0–this will take time (hours)

Making 0.5M EDTA

Step 27.3.
Combine 5ml of 1M Tris and 1ml of 0.5M EDTA in 496ml dH_2O

Making 0.5M EDTA

Step 27.4.
Autoclave to sterilize.

NOTES
VERVE Team 22 Jun 2015
It is best to remove a working aliquot and not repeatedly access the stock

CTAB

Step 28.
Resuspend pellet in 567 µl TE.

AMOUNT
567 µl Additional info:

CTAB

Step 29.
Add 30 µl of 10% SDS and 3 µl of 20 µg/ml proteinase K. Mix.

AMOUNT

30 µl Additional info:

CTAB

Step 30.

Incubate 1 hour at 37°C - 56°C

DURATION

01:00:00

CTAB

Step 31.

Add 100 µl of 5 M NaCl and mix thoroughly.

AMOUNT

100 µl Additional info:

CTAB

Step 32.

Add 80 µl CTAB/NaCl solution. Mix.

AMOUNT

80 µl Additional info:

CTAB

Step 33.

Incubate 10 minutes at 65°C.

DURATION

00:10:00

CTAB

Step 34.

Add equal volume of chloroform; mix.

CTAB

Step 35.

Microcentrifuge for 2 minutes.

DURATION

00:02:00

CTAB

Step 36.

Transfer supernatant to separate tube.

NOTES

VERVE Team 21 Jan 2016

Your DNA is in the supernatant (the top).

CTAB

Step 37.

Add equal volume of phenol/chloroform to the supernatant fraction; mix.

NOTES

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Note: Your DNA is in the supernatant (the top)!

CTAB

Step 38.

Microcentrifuge for 2 minutes.

DURATION

00:02:00

CTAB

Step 39.

Transfer supernatant to separate tube.

CTAB

Step 40.

Add equal volume of chloroform to the supernatant fraction; mix.

CTAB

Step 41.

Microcentrifuge for 2 minutes.

DURATION

00:02:00

CTAB

Step 42.

Transfer supernatant to separate tube.

CTAB

Step 43.

Add 0.7 volume isopropanol to the supernatant fraction.

AMOUNT

1 µl Additional info:

CTAB

Step 44.

Mix gently until DNA precipitates.

CTAB

Step 45.

Centrifuge 15 minutes in cold.

DURATION

00:15:00

CTAB

Step 46.

Wash with 70% ethanol.

CTAB

Step 47.

Resuspend in 50 µl Sigma water

AMOUNT

50 µl Additional info:

CTAB

Step 48.

Check O.D. using the Nanodrop