DNA Extraction of Cesium Chloride-Purified Viruses using Wizard Prep Columns

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Abstract

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This protocol decribes the extraction of DNA from viral particles using Wizard Prep Resin and Columns from Promega.

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Guidelines

This protocol is part of a larger collection of Cesium-Chloride related protocols. This is number (3) of (4):
1. Cesium Chloride Gradients
2. CsCl Step Gradient to Purify Phage
3. Cesium Chloride and DNA Extraction of Viruses using Wizard Prep Columns
4. Cesium Chloride Dialysis for Viruses

Needed:

- 1 mL DNA Purification Resin
- 0.5 mL CsCl purified virus
- Minicolumn
- 3ml or 5ml sterile syringe with plunger removed
- 80% isopropanol
- 1.5 mL centrifuge tube
- Mico-centrifuge @ 10,000g
- TE Buffer (10mM Tris-Cl, pH8.0, 1mM EDTA)
- Quant-iT PicoGreen dsDNA assay kit

To prepare a CsCl solution of a particular density, the percent by weight of CsCl can be calculated by the formula:
% wt/wt = 137.48 - 138.11/p
where 'p' is the desired density. For example, for p = 1.7 g/mL, use 56.24g CsCl and 43.76 mL H2O.

Materials

Wizard® PCR Preps DNA Purification Resin A7181 by Promega

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

Wizard Minicolumns A7211 by Promega

Protocol

Step 1.
Prepare 2 labeled 1.5ml microfuge tubes per sample and make sure resin is at room temperature

Step 2.
Shake resin vigorously to resuspend particles

Step 3.
Mix 1ml DNA Purification Resin with 0.5ml CsCl sample

AMOUNT

1 ml Additional info:

REAGENTS

Wizard® PCR Preps DNA Purification Resin A7181 by Promega

NOTES

Bonnie Poulos 16 Jun 2015

Note: if you have more than 1ml of CsCl sample, you can use more Wizard columns, or you can concentrate prior to DNA extraction using Amicon Ultra Concentrators (100kDa MWCO). Try to use the size that fits most of your sample in one or two spins; spin at 1000g for 5 min at 10°C and check volume. If you need to add more volume to the retenate, use the flow through to do this.

Bonnie Poulos 19 Jun 2015

You can use up to 1ml sample but more than that will significantly decrease yield of DNA.

Step 4.
Attach minicolumn to bottom of 3ml or 5ml sterile syringe that has had plunger removed.

REAGENTS

Wizard Minicolumns A7211 by Promega

Step 5.
Add resin with sample to the syringe and push through the solution

NOTES

Bonnie Poulos 24 Jun 2015

You can save flow-thru just in case you think you overloaded the resin.

Step 6.
Remove minicolumn from the syringe and pull out plunger.

Step 7.
Reattach minicolumn to the syringe and 2ml of 80% isopropanol to the syringe.

Step 8.
Using the plunger push through the isopropanol to wash the resin.

Step 9.
Remove minicolumn from syringe and place in a sterile 1.5ml centrifuge tube.

Step 10.
Centrifuge 10,000 *g* for 2min to remove any residual liquid.

DURATION
00:02:00

Step 11.
Place minicolumn in new sterile 1.5ml centrifuge tube.

Step 12.
Add 100µl 80°C TE buffer to top of minicolumn.

AMOUNT
100 µl Additional info:

Step 13.
Place tube lid over top of column and vortex gently for 10 seconds.

DURATION
00:00:10

Step 14.
Wait 1 minute

DURATION
00:01:00

Step 15.
Centrifuge at 10,000 *g* for 30 sec to elute DNA.

DURATION
00:00:30

NOTES
Bonnie Poulos 26 Jun 2015
Can repeat a second time using 50µl warm TE (do not pool the 2 elutions until you quantify so as not to dilute the sample).
Usually can recover an additional 10-20% of DNA with the second elution.

Step 16.
Quantify DNA by Quant-iT PicoGreen dsDNA assay kit.

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