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

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Abstract

Version 1b

17 October 2012

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

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:
 $\% \text{ wt/wt} = 137.48 - 138.11/p$

where 'p' is the desired density. For example, for $p = 1.7 \text{ g/mL}$, use 56.24g CsCl and 43.76 mL H₂O.

Materials

MATERIALS

 Wizart® PCR Preps DNA Purification Resin **Promega Catalog #A7181**

 Quant-iT dsDNA Pico Green assay kit (Invitrogen) **Life Technologies Catalog #P7589**

 Wizart Minicolumns **Promega Catalog #A7211**

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Troubleshooting



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

2 Shake resin vigorously to resuspend particles

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

 1 mL

Note

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 retentate, use the flow through to do this.

Note

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

 Wizard® PCR Preps DNA Purification Resin **Promega Catalog #A7181**

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

 Wizard Minicolumns **Promega Catalog #A7211**

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

Note

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

6 Remove minicolumn from the syringe and pull out plunger.

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

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



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

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

 00:02:00

11 Place minicolumn in new sterile 1.5ml centrifuge tube.

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

 100 µL

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

 00:00:10

14 Wait 1 minute

 00:01:00

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

 00:00:30

Note

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.

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

 Quant-iT dsDNA Pico Green assay kit (Invitrogen) **Life Technologies Catalog #P7589**