Cesium Chloride Gradients

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Abstract

The usefulness of cesium chloride (CsCl) step gradients and continuous gradients for the separation of viruses is based on the differing buoyant densities of viruses, bacteria, and extracellular debris. This protocol provides a method for Cesium Chloride and DNA Extraction for Viruses (See guidelines for DNA Extraction).

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Guidelines

This protocol is part of a larger collection of Cesium-Chloride related protocols. This is number (1) 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:

- Cesium chloride
- Seawater that has been 0.02µm or 0.2µm filtered and autoclaved
- Balance
- Sterile pipet tip
- Gradient fractionator
- Centrifuge (SW40 or SW28 Beckman) @ 24,000rpm
- Sterile 0.5ml tubes
- Sterile 1.5ml tubes
- Rack
- Ring stand
- Sterile 20 guage needle
- 4-place balance
- SYBR Gold Nucleic Acid Gel Stain
- Fluorescent Scope
- Wizard DNA Purification Resin
- Wizard Minicolumn
- Syringe
- Plunger
- 1.5ml centrifuge tube
- 80% isopropanol
- TE buffer

DNA extraction
Wizard DNA Purification Resin (Promega #A7181)
Wizard Mini Columns (Promega #A7211)
Phage Buffer to dilute if necessary (150mM NaCl, 40mM Tris-Cl, pH7.4, 10mM MgSO4 in nuclease-free water; fliter sterilized)

Mix 1ml DNA Purification Resin with 0.5 ml CsCl sample (can use up to 1ml sample but more than that will significantly decrease yield of DNA). Attach minicolumn to bottom of 3ml or 5ml sterile syringe that has had plunger removed. Add resin with sample to the syringe and push through the solution (can save flow-thru just in case you think you overloaded the resin). Remove minicolumn from the syringe and pull out plunger. Reattach minicolumn to the syringe and 2ml of 80% isopropanol to the syringe. Using the plunger push through the isopropanol to wash the resin. Remove minicolumn from syringe and place in a sterile 1.5ml centrifuge tube. Centrifuge 10,000 g for 2min to remove any residual liquid. Place minicolumn in new sterile 1.5mil centrifuge tube. Add 100µl 80oC TE buffer to top of minicolumn. Place tube lid over top of column and vortex for 10 seconds. Wait another 30 seconds and then immediately centrifuge at 10,000 g for 30 sec to elute DNA. Can repeat this 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 addition 10-20% of DNA with the second elution.

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.

Protocol

Cesium Chloride gradients
Step 1.
Prepare cesium chloride densities of p1.2, p1.4, p1.5 and p1.65 in seawater (sw) that has been 0.02µm (or 0.2µm) filtered and autoclaved.

Cesium Chloride gradients
Step 2.
Place tube of prepared cesium on balance

Cesium Chloride gradients
Step 3.
Tare balance to 0

Cesium Chloride gradients
Step 4.
Remove 1ml with sterile pipet tip.

p1.2	11.19g per 50ml seawater
p1.4	26.94g per 50ml seawater
p1.5	33.74g per 50ml seawater

p1.65 43.78g per 50ml seawater

AMOUNT
1 ml Additional info:

NOTES
VERVE Team 24 Jun 2015
Difference in weight should be equal to the density of the solution (eg, 1ml of p1.2 should weigh 1.2ml). Adjust as needed.

Cesium Chloride gradients

Step 5.
Layer CsCl from the bottom using a gradient fractionator if you have one or by hand:

SW40 rotor:	2ml p1.65
	3ml p1.5
	3ml p1.4
	1ml p1.2
SW28 rotor:	5ml p1.65
	8ml p1.5
	9ml p1.4
	3ml p1.65

NOTES
VERVE Team 24 Jun 2015
The p1.2 layer is to equilibrate sample before it hits the p1.4 cesium layer
VERVE Team 06 Jul 2015
Use thin-walled SW40 or SW28 tubes!
VERVE Team 06 Jul 2015
This was done with a fraction collector (Labconco) but you can carefully layer these solutions with Pasteur pipets.

Cesium Chloride gradients

Step 6.
Carefully layer on sample (5ml can be layered with SW40 15ml with SW28).

NOTES
VERVE Team 24 Jun 2015
Use thin-walled SW40 or SW28 tubes!
~5ml can be layered with SW40, ~15ml with SW28)
VERVE Team 06 Jul 2015
This was done with a fraction collector (Labconco) but you can carefully layer these solutions with Pasteur pipets.

Cesium Chloride gradients

Step 7.
Weigh and balance tubes.

Cesium Chloride gradients

Step 8.
Centrifuge using SW40 or SW28 Beckman rotor @ 24,000rpm for 4hr at 4°C

DURATION
04:00:00

NOTES
VERVE Team 06 Jul 2015
With a SW-48 swinging-bucket rotor (Beckman), setting 9 was used for deceleration. This slower deceleration should reduce mixing in your gradient.

Cesium Chloride gradients
Step 9.
Have sterile 0.5ml tubes (SW40) or 1.5ml tubes (SW28) labeled 1-24

Cesium Chloride gradients
Step 10.
Open tubes in a rack.

Cesium Chloride gradients
Step 11.
Place tube on ring stand

Cesium Chloride gradients
Step 12.
Puncture about 2mm from the bottom side of the tube using a sterile 20 guage needle, bevel up.

NOTES
VERVE Team 24 Jun 2015
To make things easier, can pull off top sample layer with pipette as it will not contain any viruses after centrifugation; but drops will slow down as you reach the end of the collection.

Cesium Chloride gradients
Step 13.
Collect droplets to fill each tube in order.

NOTES
VERVE Team 24 Jun 2015
Do not need to collect bottom layer as virus will not be in that layer.

Cesium Chloride gradients
Step 14.
Place tube with sample on 4-place balance in holder.

NOTES
VERVE Team 24 Jun 2015
Make one by cutting off top 1" of 15cc tube with cap on.

Cesium Chloride gradients
Step 15.
Tare to 0.

Cesium Chloride gradients
Step 16.
With sterile pipet tip, remove 100µl.

AMOUNT
100 µl Additional info:

Cesium Chloride gradients
Step 17.
Take measurement.

Cesium Chloride gradients

Step 18.

Return sample to tube.

Cesium Chloride gradients

Step 19.

Remove another 100µl, take measurement, return sample to tube.

AMOUNT

100 µl Additional info:

Cesium Chloride gradients

Step 20.

Remove yet another 100µl, take measurement, and return sample to tube.

AMOUNT

100 µl Additional info:

Cesium Chloride gradients

Step 21.

After all tubes have been measured, take average of the 3 values for each tube.

Cesium Chloride gradients

Step 22.

Multiply by 10.

NOTES

VERVE Team 24 Jun 2015

This will equal the density of the CsCl in that tube. For viruses, collect the p1.4-1.5 samples.

Cesium Chloride gradients

Step 23.

Pool and extract DNA or can confirm presence of virus first by staining 10µl with SYBR gold

AMOUNT

10 µl Additional info:

Cesium Chloride gradients

Step 24.

Count under fluorescent scope.

Cesium Chloride gradients

Step 25.

Pool all samples with virus.