CsCl Step Gradient to Purify Phage

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Abstract

April 2000 chp

Phage are best purified by a CsCl gradient. This is separated by density not sedimentation as in a sucrose gradient.

This is a protocol for P22 like phage not T4 with delicate tail fibers.

Citation: Jonathan King Lab CsCl Step Gradient to Purify Phage. **protocols.io**
dx.doi.org/10.17504/protocols.io.c4zyx5
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Guidelines

This protocol is part of a larger collection of Cesium-Chloride related protocols. This is number (2) 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:

- Phage
- Beckman Ti45
- 10% PEG
- 0.5M NaCl
- Centrifuge 8K GSA
- CsCl
- Buffer
- Tris
- MgCl2
- SW 28.1 Beckman Ultra Clear Tube
- Needle
- Syringe
- Guage Needle
- Stop cork grease
- Plunger
- Pierce Dialysizer (optional)
- 18 guage needle (necessary if using pierce dialysizer)
- Tube
- Mg buffer

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.

Grams of CsCl to be added to Buffer

p	M	25 mls	50 mls	75 mls	100 mls	200 mls
1.3	2.4	10.10	20.20	30.31	40.41	80.82
1.4	3.2	13.47	26.94	44.16	53.86	107.76
1.45	3.6		30.24		60.48	
1.5	4	16.87	33.74	50.61	67.48	
1.55	4.4	18.48	36.96		73.92	
1.6	4.8	20.2	40.40			
1.65	5.2	21.89	43.78		87.55	
1.7	5.6	23.57	47.15	70.7	94.29	

(Yamamoto 1970 Virology 40, 734-744)

Materials

- Beckman Type 45 Ti Rotor (45,000rpm 6x94ml) 339160 by Contributed by users
- Fisher Scientific Marathon Model 8K Centrifuge Discontinued by Contributed by users
- SW 28.1 Beckman 342214 by Contributed by users

Protocol

Make your phage

Step 1.
Make your phage: author used to being at about 10^9-10^{10} /ml.

NOTES
VERVE Team 10 Aug 2015
Now it needs to be concentrated.

Make your phage

Step 2.
To put on the top of the gradient we use two methods:

a. In the Beckman Ti45 1 hr 35K
b. PEG ppt using 10% PEG (6K) 0.5 M NaCl (Yamamoto 1970 Virology 40, 734-744)

REAGENTS
- Beckman Type 45 Ti Rotor (45,000rpm 6x94ml) 339160 by Contributed by users

DURATION
01:00:00

NOTES

VERVE Team 19 Jun 2015

These methods were handled in two ways: Adding the phage dry, or adding it as an autoclaved 50% weight/volume.

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1) Cammie adds it dry

2) Patricia adds it as an autoclaved 50% weight/ volume.

Make your phage

Step 3.

Mix in the cold and allow to sit at least 1 hour; recommended overnight

DURATION

01:00:00

Collection

Step 4.

The phage is collected by cfg, 8K GSA 20 min a waxy pellet resuspend in a small volume.

DURATION

00:20:00

NOTES

VERVE Team 19 Jun 2015

Keep in mind you will need to load on to a gradient if using a SW 28.1 about 3-5 mls/tube.
If SW50.1 about 0.5-1 mls.

Preparation of CsCl Step Gradient [Cammie's recipe unknown source]

Step 5.

Prepare CsCl step gradient. [Cammie's recipe unknown source]

PROTOCOL

. CsCl Step Gradient Buffer

CONTACT: VERVE Team

Step 5.1.

Weigh out the CsCl
M = 8 (p25-1) MW = 168.37

Grams of CsCl to be added to Buffer

p	M	25 mls	50 mls	75 mls	100 mls	200 mls
1.3	2.4	10.10	20.20	30.31	40.41	80.82
1.4	3.2	13.47	26.94	44.16	53.86	107.76
1.45	3.6		30.24		60.48	
1.5	4	16.87	33.74	50.61	67.48	
1.55	4.4	18.48	36.96		73.92	
1.6	4.8	20.2	40.40			
1.65	5.2	21.89	43.78		87.55	
1.7	5.6	23.57	47.15	70.7	94.29	

Step 5.2.
Add a little Mg2+ (high in Mg2+) buffer with stir bar.

Step 5.3.
Add CsCl slowly until all the CsCl is dissolved

Step 5.4.
Bring up the volume, rinse the stir bar.

Step 5.5.
Add 50 mM Tris pH 7.6

Step 5.6.
Add 100 mM $MgCl_2$

Preparation of CsCl Step Gradient [Cammie's recipe unknown source]

Step 6.
Make gradient in a SW 28.1 Beckman ultra clear tube holding 17 mls

REAGENTS
SW 28.1 Beckman 342214 by Contributed by users

NOTES
VERVE Team 19 Jun 2015
The polyallomar are easier to puncture but you can't see through them with the SW 28.1 Beckman ultra clear tube

Preparation of CsCl Step Gradient [Cammie's recipe unknown source]

Step 7.
Layer the lowest density first

Preparation of CsCl Step Gradient [Cammie's recipe unknown source]

Step 8.
Displace it with the next heavier using a long canular needle

Preparation of CsCl Step Gradient [Cammie's recipe unknown source]

Step 9.
Displace with the next heavier, and so on.

Preparation of CsCl Step Gradient [Cammie's recipe unknown source]

Step 10.
Slow layer sample on top.

NOTES
VERVE Team 19 Jun 2015
Try not letting the sample volume be more than half the gradient. Try to keep it less than a third.
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These were the volumes used from the author's experiment:
1.3 is 4 mls, 1.4 is 4 mls 1.5 is 3 mls, and 1.7 is 2 mls.

Preparation of CsCl Step Gradient [Cammie's recipe unknown source]

Step 11.
Centrifuge for 2.5 hours 24K in the SW 28.1

REAGENTS
SW 28.1 Beckman 342214 by Contributed by users

DURATION
02:30:00

NOTES

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If run longer the bands will stay at the same position since it is an equilibrium density gradient

Drawing

Step 12.

Draw a sketch of the layers in the tube.

Sealing

Step 13.

The band between 1.4.-1.5 can be pulled out with a syringe and 20 or above gauge needle.

NOTES

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This will act as your sealant.

Phage are a bluish whitish band is between the 1.4 and 1.5. The higher bands are empty heads and the bottom stuff are ribosomes.

Sealing

Step 14.

Put a bit of stop cork grease on the tube and the middle of the needle.

NOTES

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This will act as your sealant.

Sealing

Step 15.

Plunge the syringe a few times.

NOTES

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If you don't, it is tough to start pulling the band.

Sealing

Step 16.

Puncture by slowly twisting and pushing the needle, beveled side up, a few mm below the bluish white band.

NOTES

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Once through, the resistance decreases and you might go through the other side so slowly. Even pressure is the key.

Sealing

Step 17.

Now through ensure the hole around the needle is sealed with grease. Collect the band by slowly moving the needle back and forth under the band

Dialyze

Step 18.

Change needle to a 18 gauge if using in a Pierce Dialysizer, if not, take needle off and put in a tube until you can dialyze it.

NOTES

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Change needle and syringe before going to the next tube even if it is the same sample.

Repuncturing the tube dulls the needle enough where it can cause problems on the next collection.

Dialyze

Step 19.

Dialyze the band against a high Mg buffer.

NOTES

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T4 needs to have step dialysis 1.1 because the heads will pop if put directly into a buffer without CsCl.

Warnings

This is a protocol for P22 like phage, not T4 with delicate tail fibers.