Large Volume Marine Cyanophage Phage Purification

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Abstract

Last optimized January 2006, updated by Matt on March 16 2009

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Guidelines

This protocol comes from a group of other protocols.This protocol is (1) of (4):
1. 'Large Volume Marine Cyanophage Phage Protocols'
2. 'DNA Extraction Protocol'
3. 'DNA Precipitation Protocol'
4. 'Checking DNA Concentration with Agarose Gel'

Needed:

- 2L Pro99
- Cyanophage stock
- SYBR titering
- 10 mL chloroform / L phage lysate
- Incubator
- Centrifuge Beckman Ja-14 @ 12,500 rpm
- Kim Wipes
- Polyethylene Glycol (PEG)
- Orbital shaker @ 150 rpm
- CsCl step gradient (1.3, 1.4, 1.5, 1.65 bands)
- Ultracentrifuge tubes
- Concentrated phage samples
- SW28 swinging bucket rotor @ 28,000 rpm
- Slide-A-Lyzer dialysis cassettes
- 3M and 1.8M NaCo
- NaCl buffer
- Tris HCl
- MgCl2

RNase 100ng/ul = 100ug/ml ==> 1:10 = 10ug/ml ==> use 1 ml/L or use UD at 100 ul/L

Protocol

Grow up cells

Step 1.
Grow up cells in large volumes (1-2L) to yield 10^{11} - 10^{12} cells using 'microbubbling' technique.

PROTOCOL

. Microbubbling technique

CONTACT: VERVE Team

NOTES
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Use ‘microbubbling’ technique: acid-washed glass bubblers, along with an air source that has been pre-bubbled through water and pre-filtered before entering the culture

Step 1.1.
Inoculate 2L Pro99 with exponentially growing cells (20-50 ml of dense cells, 10^8 cells ml^{-1})

Step 1.2.
Let cells grow to about mid 10^7 cells ml^{-1}

Step 1.3.
Add as much cyanophage stock as available (up to an infective MOI = 3)

NOTES
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NOTE: Average cyanophage lysates are ~10^8 - 10^9 SYBR ml^-1; only ~0.1-10% of that is usually infective (as assayed with MPNs)

Step 1.4.
Adsorb for about 60 minutes without bubbling

DURATION
01:00:00

Step 1.5.
Start bubbling again

Step 1.6.
Add 1xPro99 amounts of N, P every 3 days until see 'lysis' (by eye, or more rigorously by a decrease in cell concentration)

Step 1.7.
Confirm phage production by SYBR titering

Phage purification (optimized from Sambrook et al. 1989)

Step 2.
Add Rnase A (10ug ml-1 = for 2L, 250µl of Qiagen's 100mg/ml stock) and DNase1 (0.25 SU ml-1 =2L, add 50µl of stock).

NOTES
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This is necessary to minimize phage loss; chews up HMW DNA/RNA in lysate.

Phage purification (optimized from Sambrook et al. 1989)

Step 3.
Incubate for 1 hour at room temperature

DURATION
01:00:00

Phage purification (optimized from Sambrook et al. 1989)

Step 4.
Add 116g NaCl per Liter of lysate (thus 2M addition + seawater yields final concentration 2.6M).

NOTES
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This has been optimized, high salt promotes phage dissociation from particles.

Phage purification (optimized from Sambrook et al. 1989)

Step 5.
Incubate on ice 30 minutes.

DURATION
00:30:00

Phage purification (optimized from Sambrook et al. 1989)

Step 6.
Centrifuge 12,500 rpm (23,975xg) in Beckman Ja-14 for 15 minutes at 4°C

DURATION
00:15:00

NOTES
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This removes cell debris

Phage purification (optimized from Sambrook et al. 1989)

Step 7.
Filter the supernatant through a Kim Wipe tissue paper.

NOTES
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This removes residual cell parts / membranes that float into supernatant

Phage purification (optimized from Sambrook et al. 1989)

Step 8.
Mix in polyethylene glycol (PEG 8,000; 100g L^{-1}) by gentle stirring.

Phage purification (optimized from Sambrook et al. 1989)

Step 9.
Incubate overnight at 4°C.

DURATION
15:00:00

NOTES
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This precipitates phage and any other remaining proteins
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Store here if needed.

Phage purification (optimized from Sambrook et al. 1989)

Step 10.
Collect PEG-phage precipitate by centrifuge (6 tubes at a time for the 2L volumes) at 12,500rpm (23,975xg) in Beckman Ja-14 for 15 minutes at 4°C.

DURATION

00:15:00

NOTES

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Haven't checked, but could possibly up this up to 20-30 minutes instead of 15 minutes

Phage purification (optimized from Sambrook et al. 1989)

Step 11.

Discard supernatant.

NOTES

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If large volumes, one can re-use centrifuge tubes by adding more lysate and spinning again; position the tube such that the phage will pellet in the same place

Phage purification (optimized from Sambrook et al. 1989)

Step 12.

Gently resuspend pellet into 1/150 volume Pro99 (1-2 ml).

AMOUNT

1 ml Additional info:

Phage purification (optimized from Sambrook et al. 1989)

Step 13.

Add in resuspension media -place the tube on its side in syrofoam cut-outs so that it rests at an angle.

NOTES

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May need to tape things down to make sure that the pellet side will be downward facing.

Phage purification (optimized from Sambrook et al. 1989)

Step 14.

Put on orbital shaker (150 rpm) in cold room for overnight re-suspension.

DURATION

15:00:00

Phage purification (optimized from Sambrook et al. 1989)

Step 15.

Combine resuspended PEG concentrated phage into a single tube (now ready for layering onto a CsCl gradient).

Phage purification (optimized from Sambrook et al. 1989)

Step 16.

Prepare a CsCl step gradient (1.3, 1.4, 1.5, 1.65 bands) in ultracentrifuge tubes

NOTES

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See Cesium Chloride Gradients for more details

Phage purification (optimized from Sambrook et al. 1989)

Step 17.

Add concentrated phage sample at top.

Phage purification (optimized from Sambrook et al. 1989)

Step 18.

Spin 28,000 rpm for 4 hours at 4°C in SW28 swinging bucket rotor.

DURATION

04:00:00

Phage purification (optimized from Sambrook et al. 1989)

Step 19.

Remove purified phage band (interface of ρ = 1.4 and 1.5 bands on step gradient)

Phage purification (optimized from Sambrook et al. 1989)

Step 20.

Step dialyze in Slide-A-Lyzer dialysis cassettes (Pierce #66425; 10K MWCO, 0.5-3.0 ml volume) - 30 minutes against 5xNaCl (3M), 3xNaCl (1.8M), two changes of 1xNaCl buffer [MTM100 = 600 mM NaCl, 100 mM TrisHCL (pH = 7.5), 100 mM $MgCl_2$]

DURATION

00:30:00

PROTOCOL

. NaCl Sodium Chloride Buffer

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Step 20.1.

Mix MTM100 (= 600mM NaCl) with 100 mM TrisHCl (pH = 7.5)

Step 20.2.

Mix with 100 mM $MgCl_2$

Phage purification (optimized from Sambrook et al. 1989)

Step 21.

Store dialyzed particles at 4°C until needed