Bacteriophage Precipitation with Polyethylene Glycol (PEG)

Fabian Kunisch
Faculty of Medicine, Westfälische Wilhelms-Universität Münster, Münster, Germany
Jeroen Wagemans
Department of Biosystems, KU Leuven, Leuven, Belgium
Mercedes Gonzalez Moreno (mercedes.gonzalez-moreno@charite.de)
Center for Musculoskeletal Surgery, Charité-Universitätsmedizin Berlin, Berlin, Germany
https://orcid.org/0000-0003-3586-6544

Method Article

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Abstract

This protocol can be employed to both purify and concentrate bacteriophages (phages) after their production. As a hygroscopic substance, polyethylene glycol in combination with its co-precipitant sodium chloride binds water molecules leading to the precipitation of the phages. After centrifugation, those can then be collected and resuspended in a buffer solution. While the purification with polyethylene glycol is a cost effective, fast (approx. 4:50 h) and easy to use method, it also has weaknesses related to the quality of purification, as both PEG and NaCl, as well as bacterial components, can contaminate the centrifuged phage precipitate.

Introduction

For the purification and concentration of phages, needed for many downstream analysis and applications, it is important to choose the protocol that best fits to ones needs regarding purity, costs, and protocol duration. To that end, alternative protocols to PEG/NaCl precipitation are the caesium chloride (CsCl) density gradient ultracentrifugation, precipitation at the phage's isoelectric point or with spermidine, and chromatographic approaches.

Reagents

1. Double-distilled water (ddH_2O)
2. Hydrochloric acid (HCl 37%; Carl Roth GmbH, Karlsruhe, Germany; cat. no.: 7476.2)
3. Ice
4. Magnesium sulfate heptahydrate ($MgSO_4.7H_2O$; Sigma-Aldrich, St. Louis, USA; cat. no.: M2773)
5. Polyethylene glycol 8000 ($HO(C_2H_4O)_nH$; BioChemica, AppliChem, Munich, Germany cat. no.: A2204)
6. Sodium chloride (NaCl; Molekula GmbH, Munich, Germany; cat. no.: 41272436)
7. TRIS ($C_4H_{11}NO_3$; Carl Roth GmbH; cat. no.: 2449.1)

8. Saline magnesium buffer (SM-buffer): Prepare 1 M Tris-HCl buffer (pH 7.5) in a 100 ml bottle by adding 6.06 g of TRIS to 50 ml ddH_2O and adjusting the pH to 7.5 with hydrochloric acid. Then, add in an autoclaved 1 l bottle 5.8 g of sodium chloride, 2 g of magnesium sulfate and 50 ml of the prepared 1 M Tris-HCl (pH 7.5), and adjust to 1 l with ddH_2O.

9. 20%-PEG/NaCl solution: Dissolve 100 g polyethylene glycol 8000 and 75 g sodium chloride in 400 ml ddH_2O under stirring at room temperature. Transfer the solution to an autoclaved bottle and bring it to

a final volume of 500 ml. Filter the solution with a 0.22 µm filter and store it at room temperature.

Equipment

1. Centrifuge (e.g., Eppendorf 5810 R; Eppendorf SE, Hamburg, Germany; cat. no.: 5811000015)
2. Centrifuge tube (50 ml and 15 ml; Falcon® Corning Inc., Corning, USA; cat. no.: 352070 and 352096)
3. Ice bucket
4. Permanent marker
5. Pipette controller and serological pipettes (5 ml and 10 ml)
6. Pipettes and pipette tips (5 ml)
7. Refrigerator
8. 0.22 µm Syringe filter (Merck Millipore, Massachusetts, USA; cat. no.: SLGSM33SS)
9. 50 ml Syringe (BD, New Jersey, USA; cat. no.: 300865)

Procedure

1. Transfer the phage lysate solution into a 50 ml centrifuge tube and add the filtered 20%-PEG/NaCl solution to a final concentration of 8 % (e.g., 15 ml phage solution and 10 ml 20%-PEG/NaCl).
2. Seal the centrifuge tube with the lid and mix the solution by inverting the tube 10 times.
3. Cool down the solution for 1.5 h on ice to allow the phage particles to precipitate. Refresh the ice each half an hour and ensure the centrifuge tube is fully covered with ice.
4. Using a permanent marker, draw a vertical line on the 50 ml centrifuge tube.
5. Place the centrifuge tube in the centrifuge with the vertical line facing up (this way, the line will indicate the location of the phage pellet). Centrifuge the tube for 40 min at 4 °C and 13,000 g to precipitate the phages.
6. Without disturbing the phage pellet (pellet might not be visible), carefully collect the supernatant in a new sterile centrifuge tube and keep it in case something went wrong.
7. Invert the centrifuge tube containing the precipitated phage for 15 min to allow the remaining fluid to drain away from the pellet (pellet might not be visible). Carefully remove any residual fluid with a pipette. There will always be residual PEG on the wall of the centrifuge tube.

8. Resuspend the phage pellet in 3 ml of SM-buffer without moving around the centrifuge tube to avoid collecting any residual PEG on the tube wall. Leave the tube at room temperature for 1 h so that the SM-buffer covers (tilted position) and soaks the pellet.

9. To resuspend the phage pellet, carefully pipette up and down the SM-buffer solution.

10. Let the phage pellet soak for another 45 min.

11. Pipette the solution up and down again and then transfer it with a 5 ml serological pipette into a sterile 15 ml centrifuge tube.

12. Store the solution in a refrigerator at 4 °C until further use.

Troubleshooting

Step: 1

Problem: Phage concentration failed (no titer increase)

Possible reason: Not enough phage lysate or low initial phage titer

Solution: Increase the initial volume and/or concentration of phage lysate and/or resuspend two or more independently precipitated pellets together in a single buffer solution

Step: 1

Problem: Phage concentration failed

Possible reason: Not enough polyethylene glycol (PEG/NaCl)

Solution: Increase the final concentration of PEG/NaCl to 10 % or higher

Step: 3

Problem: Phage concentration failed

Possible reason: Precipitation of phage particles

Solution: Cool down the solution overnight at 4 °C and/or under agitation (e.g., 150 rpm)

Step: 8

Problem: Phage concentration failed

Possible reason: Breaking phage particles during resuspension

Solution: Let the pellet soak in the SM buffer for several hours or overnight at 4 °C

Step: 8

Problem: Low phage concentration

Possible reason: Resuspension of phages in 3 ml of SM-buffer

Solution: Reduce resuspension volume (e.g., 1 ml)

Step: 9 & 11

Problem: Contamination risk of pipettes

Possible reason: Due to an increased viscosity of the resuspended solution

Solution: Use of a 5 ml pipette (slowly) or filter pipette tips

To reduce the contamination of the final phage precipitate by bacterial DNA and other bacterial structures (e.g., ribosomes), adding 1 µg/ml of DNase I and RNase A to the phage lysate at the onset of the protocol should reduce their precipitability. When the phages are not sensitive to chloroform, chloroform can be added to the resuspended phage solution to remove any remaining PEG. Use an equal volume of chloroform and centrifuge the mixture for 5 min at 4 °C and 4,000 g. Phages are present in the aqueous phase on top.

Time Taken

The protocol takes approx. 4:50 h, including 4:20 h of waiting time.

Anticipated Results

By the precipitation with polyethylene glycol, the phage solution can be considered both purified (no adverse effects observed after injection into *Galleria mellonella* larvae) and having an unchanged or increased concentration compared to the initial phage lysate titer.

References

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