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Giant Mimiviridae CsCl Purification Protocol

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[Abstract] While different giant viruses' purification protocols are available (La Scola *et al.*, 2003; Byrne *et al.*, 2009; Arslan *et al.*, 2011; Philippe *et al.*, 2013; Campos *et al.*, 2014; Andrade *et al.*, 2017), they are not fully described and they use sucrose gradient that does not reach an equilibrium. Here, we report a protocol for the purification of members of the *Mimiviridae* family virions resulting from *Acanthamoeba castellanii* infections. Viruses are harvested after cell lysis and purified through a high density CsCl gradient to optimize the isolation of the virus from the cell debris or other potential contaminants. Due to the large size of the virion capsids, reaching half a micrometer diameter, the quality of the process can be monitored by light microscopy. The resulting purified particles can then be used to perform new infections, DNA extraction, structural studies, sugar composition analyses, sub-compartment characterization or proteomic experiments.

Keywords: Giant viruses, Mimivirus, CsCl density gradient, Virion purification, Ultracentrifugation

[Background] The discovery of Mimivirus, the first virus visible under a light microscope, overlapping in size and genome complexity with unicellular organisms, initiated a new research area in virology (La Scola *et al.*, 2003; Raoult *et al.*, 2004). Over the past 15 years, many additional members of the *Mimiviridae* family have been isolated from various environments and several protocols have been published to purify the virions. Different approaches were developed, mostly involving sucrose cushion (Campos *et al.*, 2014; Andrade *et al.*, 2017), or sucrose discontinuous gradients (Arslan *et al.*, 2011). However, these protocols are not optimal since the density of the virus is higher than the maximum density of a sucrose solution (1.36 g/cm³ and 1.3 g/cm³, respectively - ICTV 9th report, 2011), meaning the equilibrium cannot be reached. Thus, a long time or high speed centrifugation results in a viral pellet instead of a ring or both depending on the conditions used. We recently reported (Jeudy *et al.*, 2019) an optimized version of our previously published protocol using a CsCl discontinuous gradient (Byrne *et al.*, 2009). Here, we provide the detailed protocol for the purification of *Mimiviridae* particles.

Materiel and reagents

General use

1. Standard pipette filter tips (StarLab, models: TipOne filter tips, catalog number: S1120-3810,

- 40 S1120-1810, S1120-8810 and S1112-1720 or equivalent)
- 41 2. Pipettes (Sarstedt, model: 10 mL serological pipette, catalog number: 86.1254.001)
- 42 3. Ultrapure water
- 43
- 44 **Cell culture**
- 45 1. *Acanthamoeba castellanii* (Douglas) Page (ATCC, catalog number: 30010)
- 46 2. 175 cm² flasks (Greiner Bio-One, catalog number: 660175, or equivalent)
- 47 3. D-(+)-glucose (Sigma, catalog number: G8270)
- 48 4. Sodium citrate tribasic trihydrate (Sigma, catalogue number: C7254)
- 49 5. Proteose Peptone Yeast Extract medium (PPYG; see recipes)
- 50 6. Proteose-Peptone (Sigma, catalog number: 82450)
- 51 7. Yeast extract (Fisher BioReagent, catalog number: BP1422)
- 52 8. Ampicillin Sodium Salt, Cell Culture/Molecular Biology Grade (Euromedex, catalog number:
- 53 EU0400)
- 54 9. Kanamycin Sulfate, Cell Culture Grade (Euromedex, catalog number: UK0010)
- 55 10. Filtropur S 0.2 (Sarstedt, catalog number: 83.1826.001)
- 56 11. Stericup Quick Release Express Plus 0.22 µm PES (Merck Millipore, catalog number:
- 57 S2GPU11RE)
- 58 12. 36% Glucose solution (Sigma, catalog number: G8270; see Recipes)
- 59 13. Ampicillin stock solution (100 mg/mL) (see Recipes)
- 60 14. Kanamycin stock solution (25 mg/mL) (see Recipes)

61

62 **Gradient preparation and recovery**

- 63 1. Plastic Syringes (Terumo, model: 5 mL syringe, catalog number: SS*05SE1)
- 64 2. 21G x 1^{1/2}" needle (Terumo, catalog number: AN*2138R1 or equivalent)
- 65 3. Sterile 50 mL conical tubes (Sarstedt, catalog number: 62.547.254, or equivalent)
- 66 4. Polyallomer Tubes, 38.5 mL (Beckman, catalog number: 326823)
- 67 5. UltraPure Cesium chloride Optical Grade (Invitrogen, catalog number: 15507-023)
- 68 6. Cesium chloride solutions (1.2 g/cm³, 1.3 g/cm³, 1.4 g/cm³ and 1.5 g/cm³; See Recipes)
- 69 7. K36 buffer (see Recipes)

70

71 **Purification quality control**

- 72 1. Glass microscope slide (Biosigma, catalog number: VBS653/A)
- 73 2. Coverslip (Biosigma, catalog number: VBS636)
- 74 3. UVette 220-1600 nm (Eppendorf, catalog number: 952010051)
- 75 4. Formvar and carbon coated 200 mesh copper/rhodium grids (Electron Microscopy Sciences,
- 76 catalog number: FCF200-Cu)
- 77 5. Uranyl acetate 1% in water

78 *Note: Uranyl acetate is a radioactive chemical. It is provided by electron microscopy facilities*

79 *as we are not allowed to use it in the lab.*

80

81 **Equipment**

82

- 83 1. Microbiological safety hood (ADS laminaire, model: Optimal 12, or equivalent)
- 84 2. Incubator Bio Performance (Froilabo, catalog number: BP240 or equivalent)
- 85 3. Centrifuge (Beckman Coulter, model: JXN-30)
- 86 4. Swinging-Bucket Rotor (Beckman Coulter, model: JS24-38)
- 87 5. Spectrophotometer (Eppendorf, model: Biophotometer)
- 88 6. Inverted microscope (Zeiss, model: Axio Observer.Z1)
- 89 7. Transmission Electron Microscope (FEI, model: Tecnai G2)
- 90 8. Autoclave (SHP Steriltechnik SG, model: Laboklav 135)
- 91 9. Peristaltic pump (KNF Lab, model: Laboport)

92

93 **Procedure**

94

- 95 1. Seed 20 175 cm² flasks with 1,000 *Acanthamoeba castellanii* cells per cm² in 20 mL of PPYG
96 medium. Incubate at 32 °C for two days.

97 *Note: You can add ampicillin and kanamycin at final concentrations of 0.1 mg/mL and 0.025*
98 *mg/mL, respectively, in each flask to avoid bacterial contamination.*

- 99 2. After two days, the cell confluence should be between 130,000 and 180,000 cells/cm². Infect
100 the cells with a Mimivirus stock at a multiplicity of infection of 0.2.

101 Incubate at 32 °C for two days or until cell lysis is complete.

102 *Note: There is no need to change the medium before infection or to remove the virus from the*
103 *flasks.*

- 104 3. Harvest the cell debris-containing supernatants from the flasks and pool them into 50 mL
105 conical tubes.

106 *Note: For 20 175 cm² flasks containing 20 mL of medium you will need 7 tubes.*

- 107 4. Centrifuge at 500 x g for 10 min at room temperature (~25 °C, RT) to remove the cell debris.

- 108 5. Decant the supernatant into new 50 conical tubes.

- 109 6. Centrifuge at 10,000 x g for 25 min at RT to pellet the virus.

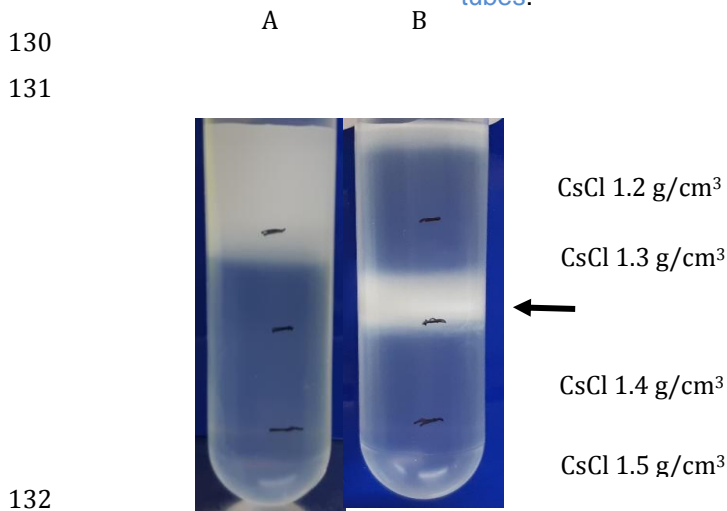
- 110 7. Discard the supernatant.

- 111 8. Resuspend the resulting virus pellets in 10mL K36 buffer by pipeting and pool in two new 50
112 mL conical tubes (35 mL per tube). Complete the volume to 50 mL with K36 buffer.

- 113 9. Centrifuge at 10,000 x g for 25 min at RT to pellet the virus.

- 114 10. During the centrifugation, prepare four CsCl gradients in Beckman polyallomer tubes. Add 7.5
115 mL of CsCl solution density 1.5 g/cm³ at the bottom of each tube, then carefully overlay with 9
116 mL of 1.4 g/cm³ CsCl solution and 9 mL of 1.3 g/cm³ CsCl solution, dropwise. Hold for the final
117 virus-containing 1.2 g/cm³ density layer.

- 118 11. Discard the supernatant from the centrifugation in Step 9.
 119 12. Resuspend the **two viral pellets** with 16 mL of 1.2 g/cm³ CsCl solution **for each pellet by**
 120 **pipeting.**
 121 13. Overlay 8 mL of resuspended virus pellets on top of each **of the four gradients**, dropwise
 122 **(Figure1A).**
 123 14. Centrifuge overnight (between 16 and 18h) at 100,000 x g at 20 °C in a Beckman Coulter
 124 JS24-38 rotor.
 125 15. After centrifugation, harvest the white ring corresponding to the virus fraction (Figure 1B) by
 126 carefully pipetting through the gradient or by puncturing the polyallomer tube and aspirating
 127 with a needle and a syringe **(Figure 2)**. Transfer the virus fraction to two **new** 50 mL conical
 tubes, add K36 buffer up to 50 mL **in each tube** and mix **by inverting the**
tubes.



132 **Figure 1: centrifuge tubes before (A) and after (B) separation of Mimivirus particles on**
 133 **CsCl gradient.** The white ring indicated by the black arrow corresponds to Mimivirus particles.
 134
 135

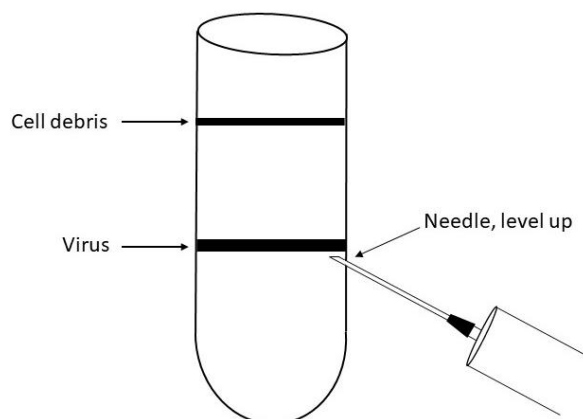


Figure 2: Collection of the viral fraction by side puncture

- 16. Centrifuge at 10,000 x g for 25 min at RT to pellet the virus.
- 17. Resuspend the **two** pellets with 10 mL of K36 buffer each, **complete the volume to 50 mL with K36 and** centrifuge **them** at 10,000 x g for 25 min at RT to wash the virus.
- 18. Repeat Step 16 **and 17** twice. For the last wash, pool the resuspended virus in a single 50 mL conical tube.
- 19. Resuspend the final pellet in 10 mL of K36 buffer.
- 20. The virus is ready to be titrated or stored at -80 °C until further use.

Note: Typically, when purifying Acanthamoeba polyphaga Mimivirus using that protocol, we recover 10 mL of viral solution containing 1 x 10¹⁰ to 4 x 10¹⁰ particles/mL. The purified particles are infectious and can be used to performed new infections or any other experiments.

Data analysis

- 1. Light microscopy
 - Drop 4.5 µl of the purified virus suspension on a microscope slide and overlay with a coverslip. Invert for a few hours in order to let the virus settle onto the coverslip for easier focusing. Observe with a light microscope using the 63x objective to confirm the purified virus does not contain any obvious contaminants (**Figure 3**).

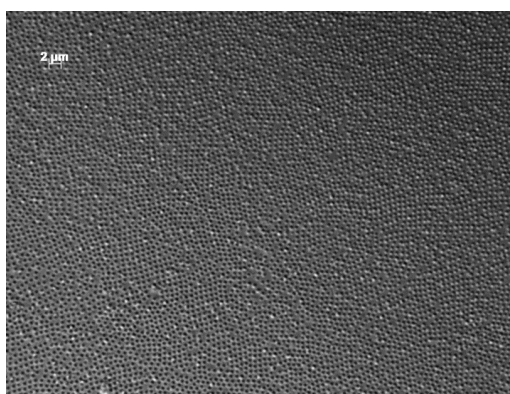
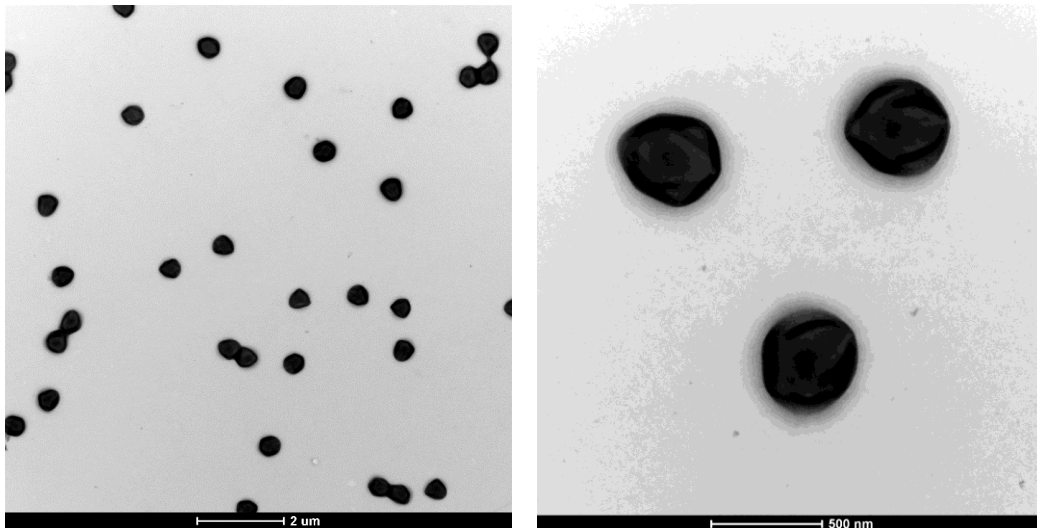


Figure 3. Observation of Mimivirus purified particles with a light microscope (63X objective, 1.6X optovar).

- 2. Transmission Electron Microscopy (TEM)
 - For TEM, dilute the purified Mimivirus sample 1:2 (v/v) in K36 buffer and incubate a 10 µl droplet on a formvar- and carbon-coated 200 mesh copper/rhodium grid for 1 min at RT. Wash the grid with three successive 1% uranyl acetate droplets. Leave the residual uranyl acetate on

168 the grid for 1 min and remove by gently touching the edge of the grid with a filter paper. After
169 drying, examine the grid using an electron microscope (Figure 4).
170



172 **Figure 4. Observations of Mimivirus purified particles by negative staining.**
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174

175 **Recipes**
176

177 1. Glucose solution

178 Dissolve 36 g of glucose and 2 g of sodium citrate in 100 mL of slightly warm water.

179 When the solution is clear, use it to prepare the complete PPYG medium.

180 2. Proteose Peptone Yeast Extract (PPYG) medium

181 **Base medium:**

182 Autoclave 40 g of proteose peptone and 2 g of yeast extract diluted in 1.8 L of ultrapure water
183 in a 2 L glass bottle.

184 Let it cool at room temperature.

185 **Complete medium:**

186 a. Add the following to the basal medium

187 20 mL of $MgSO_4$ 400 mM

188 16 mL of $CaCl_2$ 50 mM

189 20 mL of $Fe(NH_4)_2(SO_4)_2$ 5 mM

190 20 mL of Na_2HPO_4 250 mM

191 20 mL of KH_2PO_4 250 mM

192 and 100 mL of glucose solution.

193 b. Filter through 0.22 μm Stericups using a peristaltic pump and store at room temperature.

194 3. Ampicillin stock solution (100 mg/mL)

195 a. Dissolve 1 g of ampicillin in 10 mL of ultrapure water

- 196 b. Filter through a 0.22 µm filter plugged on a syringe
 197 c. Aliquot in 1.5 mL tubes and store at -20 °C
 198 4. Kanamycin stock solution (25 mg/mL)
 199 a. Dissolve 250 mg of kanamycin in 10 mL of ultrapure water
 200 b. Filter through a 0.22 µm filter plugged on a syringe
 201 c. Aliquot in 1.5 mL tubes and store at -20 °C
 202 5. K36 buffer
 203 a. Dissolve 2.25 g of KH₂PO₄, 5.8 g of K₂HPO₄·3H₂O, 7.4 g of KCl and 0.9 g of NaCl in 1 L of
 204 ultrapure water.
 205 b. Filter through a 1 L 0.22 µm Stericup linked to a peristaltic pump and store at room
 206 temperature.
 207 6. Cesium chloride solutions
 208 Density 1.5 g/cm³: dissolve 22.7 g of CsCl in 27.3 mL of ultra pure water.
 209 Density 1.4 g/cm³: dissolve 19.4 g of CsCl in 30.6 mL of ultra pure water.
 210 Density 1.3 g/cm³: dissolve 15.62 g of CsCl in 34.38 mL of ultra pure water.
 211 Density 1.2 g/cm³: dissolve 11.2 g of CsCl in 38.8 mL of ultra pure water.
 212 Once the solutions appear clear, filter through a 0.22 µm filter plugged on a syringe.
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