Viruses Purification of Perkinsus spp. Version 3

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Abstract

The purpose of this protocol is to isolate and characterize viruses that infect *P.olseni, P.marinus.*

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Guidelines

Refs :

Fichorova RN, Lee Y, Yamamoto HS, Takagi Y, Hayes GR, Goodman RP, et al. (2012) Endobiont Viruses Sensed by the Human Host – Beyond Conventional Antiparasitic Therapy. PLoS ONE 7(11): e48418. doi:10.1371/journal.pone.0048418

Bessarab IN, Liu H-W, Ip C-F, Tai J-H (2000) The complete cDNA sequence of a Type II Trichomonas vaginalis virus. Virology 267: 350–359.

Hafiz Ahmed, José A. Fernández-Robledo, and Gerardo R. Vasta (unpublished work) Glycosidases from Perkinsus marinus : Purification and Characterization of ß-D-glucosidase

Before start

What you need before you start:

- UV and sterilize the hood with reagent alcohol. Work with gloves to prevent contaminating the hood.
- Your *Perkinsus* spp. grown to exponential phase

Materials

Bottle Assembly, Polypropylene, 500 mL, 69 x 160 mm (qty. 6) 355607 by Beckman Coulter

Avanti J-30I 363118 by Beckman Coulter

Centrifuges 5810 R View by Eppendorf Centrifuge

0.5mm diameter glass beads SI-BG05 by Scientific Industries, Inc.

Disruptor Genie SI-D237 by Scientific Industries, Inc.

PBS - Phosphate-Buffered Saline (10X) pH 7.4 AM9625 by Thermo Fisher Scientific

Phenylmethanesulfonyl fluoride P7626 SIGMA by Sigma Aldrich

Protocol

Step 1.
First, Fill centrifuge bottles with 450mL *Perkinsus* Culture in the logarithmic growth phase.

Step 2.
Centrifuge bottles at 1500 g; 15 min at room temperature.

Step 3.
Transfer supernatant to a clean tube, and store at 4°C for further analyses.

Step 4.
Resuspend pellet in 10% initial culture volume supernatant in a 50mL Falcon Tube.

Step 5.
Centrifuge tubes at 1500 g; 15 min at room temperature.

NOTES
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During this centrifugation, prepare 2.5X PBS buffer (2.5mL of 10X PBS Buffer in 10mL mQ water QS) with protease inhibitor cocktail (1mM Phenylmethanesulfonyl fluoride = 1.74 mg for 10mL of solution). This solution must be freshly prepared (the half-life of PMSF is short = 55min at pH = 7.5)

Step 6.
Transfer supernatant to a clean tube, and store at 4°C for further analyses.

Step 7.
Resuspend pellet in 4mL PBS/0.35 M NaCl) containing protease inhibitor cocktail.

Step 8.
Transfer the pellet resuspended in 2mL Eppendorf Tubes (1.5mL/tube).

Step 9.
Add 100mL Glass beads.

NOTES
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We tried Sonication at differents times (15sec-->10min) with or without ice --> NOT worked

Step 10.
Disruptor Genie for 15sec**.**

Step 11.
Verify cell disruption : place a 20mL under light microscope.

NOTES
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No cell disruption was observed after 15sec Disruptor Genie.

Step 12.
Disruptor Genie for 1min**.**

Step 13.
Verify cell disruption : place a 20mL under light microscope.

NOTES
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No cell disruption was observed after 1min Disruptor Genie.

Step 14.
Disruptor Genie for 3min**.**

Step 15.
Verify cell disruption : place a 20mL under light microscope.

NOTES
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Cell disruption was observed after 3min Disruptor Genie.

Step 16.
Centrifugation at 10,000 g; 30 min, 4°C

Step 17.
Removed the supernatant to clean 1.5mL Eppendorf tube, discard the pellet (cell debris)

Step 18.
Centrifugation again at 10,000 g; 30 min, 4°C

NOTES
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To be sure to discard the cells debris well.

Step 19.
Removed the supernatant to clean 1.5mL Eppendorf tube, discard the pellet (cell debris)

Step 20.
Conserve the tubes at 4°C

Warnings

- Wear gloves and lab coat at all times during this procedure.
- Before high speed centrifuging, make sure that all tubes are perfectly balanced with a scale before loading them into the rotor.
- 2.5X PBS buffer with protease inhibitor cocktail (1mM Phenylmethanesulfonyl fluoride) must be freshly prepared (the half-life of PMSF is short = 55min at pH = 7.5)