Isolation of Viral Like Particles (VLP) from Tissues of Molluscs Version 2

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Abstract

This is a protocol especielly for the isolation of Viral Like Particles (VLP) from the tissues of Molluscs, such as abalone, oyster, clam, scallop and so on. This maybe also applicable for other animal tissues, such as shrimp and fish, based on the principle of the protocol. But we havn't verified this idea yet.

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Guidelines

Protocol

Sampling

Step 1.

Tissue samples are sliced off and freezed in liquid Nitrogen instantly. Then transfer to -80℃ freezer after return to laboratory.

NOTES

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Visceral mass and gills are the best target tissues for isolation, as viruses are easily concentrated in those organs.

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We have tested with from minimum 20 mg of oyster spat samples to maximum 20 g of abalone samples for VLP isolation. And we all acquired certain amount of Viral reads after seqenced by Illunima platform.

Releasing VLP from Tissues

Step 2.

Add about 1 to 1.5 times volume of SB (Stabilizing Buffer, 0.2 M NaCl, 50 mM Tris-HCl, 5 mM $CaCl_2$, and 5 mM $MgCl_2$, pH 7.5) and grind with grinding machine at 4℃.

TEMPERATURE

4 °C Additional info:

NOTES

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For example, adding 1-1.5 mL SB buffer to homogenize 1g tissues

Releasing VLP from Tissues

Step 3.

Add extra SB to fix a final volume at 3-5 times of weightened tissue, and make it fully homogenized.

Releasing VLP from Tissues

Step 4.

Put above homogenate into three freezing and thawing cycles

NOTES

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Use liquid nitrogen and 50℃ water bath to proceed the cycle. But it needs to be removed **immediately** after melting in the water bath.

Releasing VLP from Tissues

Step 5.

Add extra SB to fix a final volume at 5-10 times of weightened tissue, if you want to maximize the dissolution of VLP from cell debris.

Separation of VLP from Cell Debris

Step 6.

Conduct differential centrifugation at 4 °C, sequentially from 1,000 3,000 5,000 8,000 10,000 g, each time for 5 min.

NOTES

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Cell debris will precipitate to the bottom of tubes, and VLP will be keeped in supernatant.

Separation of VLP from Cell Debris

Step 7.

Supernatants then filtrated with Millex-HV 0.45 µm and 0.22 µm filters (Merck Millipore, Billerica, MA, USA) sequentially

NOTES

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0.22 µm filters will effectively remove most of bacterial cells. Please NOTICE that 0.22 µm not suitable for enriching viruses larger than 200 nm in diameter.

Concentrating VLP via Ultracentrifuging

Step 8.

Sucrose cushion (sucrose-SB solution, 28% w/w) was added to the bottom of the ultracentrifuge tube

first.

Concentrating VLP via Ultracentrifuging

Step 9.

Transfer supernatants (step 7) into ultracentrifuge tubes, softly laying onto 28% (w/w) sucrose cushion.

NOTES

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Operation with carefulness, as 28% sucrose solution is easily disturbed

Concentrating VLP via Ultracentrifuging

Step 10.

Centrifuging at 300,000 g for 2 h, then remove supernatant. VLPs are just in the precipitation

NOTES

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You can pause at this step, and put VLP pellet into -80℃ for reservation.

Wiping out Free DNA Fragments

Step 11.

Add the appropriate amount of DNase solution （820 µl ddH_2O, 90 µl 10× DNase I Buffer, 90 µl DNase I (1 U/µl)） to the tube, and use pipette tip to fully suspend precipitation.

Wiping out Free DNA Fragments

Step 12.

Incubating at 37 °C for 60 min in shaker at 150 rpm.

Wiping out Free DNA Fragments

Step 13.

Centrifuging at 10000 g for 5 min and then transferr supernatant to a new tube.