Purifying Viruses Using Sucrose Cushion

Shannon Williamson

Abstract

This protocol describes the use of a sucrose cushion to purify viruses. It is the Matthew Sullivan Lab adaptation of the Shannon Williamson protocol.

DNase I treatment, CsCl purification, and sucrose purification methods were compared using replicated viral metagenomics in Hurwitz et al. 2012.

Citation: Hurwitz, B. L., Deng, L., Poulos, B. T., & Sullivan, M. B. (2012). Evaluation of methods to concentrate and purify ocean virus communities through comparative, replicated metagenomics. Environmental Microbiology. doi:10.1111/j.1462-2920.2012.02836.x

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Guidelines

Shannon Williamson's Protocol

1.) DNase I Treatment (Can also be done post sucrose cushion)

1.1 Dilute the sample to approximately 10--20% glycerol with MilliQ water. Most samples will be in 50% glycerol solutions. This will generally be a volume of ~20ml. *Only applies if glycerol was used as a cryo--protectant.*

1.2 Add 1 unit of DNase I (RNase free) for every milliliter of sample.

1.3 Mix sample and incubate at room temperature for 2 hours.

1.4 Stop reaction by adding EDTA and EGTA to a final concentration of 100mM.

1.5 Store at 4°C

2.) Pelleting Viral Particles Through a Sucrose Cushion

2.1 Rinse one ultracentrifuge tube per sample with sterile water.

2.2 Load sample (no more than ~25 to 30ml) into ultracentrifuge tube.

2.3 Add up to 10ml of 38% Sucrose in SM buffer to the bottom of ultracentrifuge tube to form a sucrose cushion. The tube should be filled to the very top and balanced to within +/- 0.1g to avoid

being crushed by the ultracentrifuge's vacuum.

2.4 Centrifuge for 3 hours, 23°C at 32,000 RPM in a SW32.1Ti rotor. See **Note** below.

2.5 Gently pipette off the supernatant and save at 4°C.

2.6 Allow pellet to dry for 5 - 10 minutes, and resuspend in 500ul of TE.

2.7 Add EGTA and EDTA to a final concentration of 100mM.

Note: Email correspondence with author indicated that the ultracentrifugation is at 32,000 RPM as opposed to 32,000 x g. The difference is very large between these two: in this rotor 32,000 x g would be about 13,000 RPM whereas 32,000 RPM = 180,000 x g. See email correspondence below. BTP

Emails concerning protocol details:

From: Matthew Sullivan
Sent: Monday, October 19, 2009 3:32 PM
To: Williamson, Shannon
Subject: few quick Qs

Hey Shannon,
We're setting up to implement your sucrose protocol. A few questions came up ...

1) Should the EDTA and EGTA both be at 100mM final concentration or is this a combined molarity of both?

2) Why are EDTA and EGTA also added to the resuspended pellet? (is DNase still potentially present)

3) Your protocol says to use a SW32.1Ti rotor and just prior says to use no more than 25-30ml of sample with 10ml sucrose but the tubes for this rotor only holds 17ml (whereas the SW32Ti rotor uses 38ml tubes). Can you clarify the amounts and rotor? (just want to confirm g force for the centrifugation since 32K rpm in 32.1Ti = 187,000g whereas 32K RPM in 32Ti = 175,000g)

From: "Williamson, Shannon"
Date: October 21, 2009 9:33:34 AM GMT--07:00
To: "Matthew Sullivan"
Subject: RE: few quick Qs

Hi Matt, Here are some answers for you...

1) 100mM is the final concentration of each. So 100mM EGTA and 100mM EDTA.

2) The EDTA/EGTA is added here just as a precaution to remove Mg and Ca in the case that we have any enzymes after we do the DNA extraction. The possibility of having bacterial cells here is very slim but we do not take chances especially with viral DNA yields being what they are. Also, it is possible to do the DNAse reaction after the sucrose cushion, which is recommended if you want to work in

smaller volumes and save money and reagents.

3) For the sucrose cushion you want to make sure you have about 1/8-1/6 of the total ultra centrifuge tube volume to be your sucrose cushion with the remainder being your concentrated VC. As long as you can get up to 30,000g or 32,000g it doesn't matter which rotor or tubes you use. It really comes down to how comfortable you are concentrating your VC to small enough volumes to fit the tubes. We have done the sucrose cushion in the 5ml, 15ml, and 38ml ultra tubes, and all have worked well.

Please let me know if you have any additional questions.

Best wishes,
Shannon

From: Matthew Sullivan
Sent: Thursday, October 22, 2009 9:31 AM
To: Williamson, Shannon
Subject: Re: few quick Qs

Hey Shannon,

Thanks so much for the response ... do you mean 30,000 g or RPM below? (my first time doing a sucrose gradient and I'm told these things are very spin sensitive)

All the best,
Matt

From: "Williamson, Shannon"
Date: October 22, 2009 1:35:45 PM GMTÂÂ□07:00
To: "Matthew Sullivan"
Subject: RE: few quick Qs

It's RPM, sorry about that!

From: Matthew Sullivan
To: Bonnie Poulos
Subject: Fwd: few quick Qs
Date: Thu, 22 Oct 2009 13:37:54 ÂÂ□0700

Hey Bonnie it's RPM ...

Protocol

Step 1.
Prepare sucrose (Molecular Biology Grade or higher) as 38% (weight to volume) in SM buffer (100mM NaCl, 8mM MgSO4, 50mM TrisJHCl, pH 7.5) that has been 0.2µm filtered.

Step 2.
Rinse SW40 tubes with SM buffer or sterile water.

NOTES

Bonnie Poulos 03 Aug 2015

The SW 40 tubes hold 12 ml total volume. The protocol calls for using 2.5ml 38% sucrose as the cushion, which leaves 9.5 ml volume for the sample. If the DNase treated sample (reaction stopped with EDTA/EGTA) is less than this, add SM buffer to bring the sample up to 9.5 ml. Place the 9.5ml sample into the rinsed ultracentrifuge tube.

Step 3.

Place the 9.5ml DNase I-treated sample into the rinsed ultracentrifuge tube.

Step 4.

Using a 3cc syringe and cannula, slowly layer the 2.5 ml of 38% sucrose under the sample trying not to cause any mixing of sample and sucrose.

Step 5.

Centrifuge at 175,000g for 3.25 hr at 18°C.

DURATION

03:15:00

Step 6.

Using a sterile transfer or serological pipet, pull off the 9.5 ml sample layer.

Step 7.

Pull off the interface and 2.5 ml sucrose, being careful not to disturb or suction off the pelleted sample at the bottom.

NOTES

Bonnie Poulos 22 Jul 2015

Pellets will look orange in color due to iron chloride.

Step 8.

Keep this sucrose layer until virus counts from the pellets are completed.

Step 9.

Air dry the pellets in a fume hood for 15-20 min.

DURATION

00:20:00

Step 10.

Add a mixture of TE containing 0.1M each EDTA/EGTA to resuspend the pellets (500 µl is recommended).

Step 11.

Perform virus counts and then extract DNA