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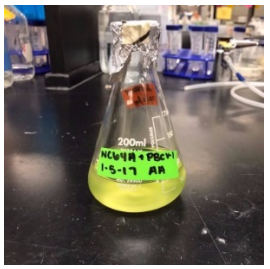
Long term Cryopreservation of Chloroviruses by Infection of Chlorella V.3



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The Aquatic Microbial E...

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

Contact Dr. Steven Wilhelm (wilhelm@utk.edu) or Samantha Coy (srose16@vols.utk.edu) for additional information regarding this protocol.

Pre-cryopreservation

1 Confirm that the lysate (PBCV-1 in this case) does not contain anything other than the virus that is being used. This can be completed with PCR and gel electrophoresis of known gene markers.

2 Perform a plaque assay to determine the plaque forming units (PFU/mL) of the virus to determine the volume required for the desired MOI at which the *Chlorella* (NC64A) cells will be infected pre-cryopreservation.

3 Grow a stock culture of NC64A for 48 hr under continuous light at 25ºC to ~$2*10^7$ cells/mL at the desired volume.

Cryopreservation

4 Always use the '1/3 rule'. That is, infect enough host so that 1/3 of the sample volume (PBCV-1 + NC64A) is left after all of the samples have been collected and aliquoted with CPAs.

Note

The 1/3 rule is used to have extra biomass leftover incase you need extra for sampling errors, and to prevent bottle effects in your final samples.

5 Make 30% stocks of DMSO, Ethylene glycol, and L-proline diluted in Milli-Q. Combine all three for a final concentration of 10% each in a clean flask. Aliquot desired volume to each cryovial, set on ice.

*You want to have a 50:50 ratio of virus to cryoprotectants (CPAs). I use 1 mL PBCV-1+NC64A and 1 mL CPAs in cryovials. You can determine the amount of CPAs needed based on the desired amount of cryopreserved host infected virus samples.

Note

Sterilize cryoprotectants by filtering through a 0.45 um filter.

6 Infect NC64A with *Chlorovirus* at a multiplicty of infection (MOI) = ~5.

7 Collect samples at the desired time points during the infection cycle in duplicates and add the desired volume of host infected virus to CPAs in cryovials.

Note

In our hands, collection of a sample at 240 min post-infection shows the greatest recovery of viable cells (and thus viruses) for PBCV-1 post-crypreservation

8 Transfer cryovials directly to a slow-freeze container, such as Mr. Frosty, filled with isopropanol at -80ºC overnight.

9 Transfer samples into a -150ºC freezer until the desired treatment period is over.

Post-cryopreservation

10 Transfer vials to ice and let thaw slowly.

Note

Be aware that the infection cycle will continue once thawed, and if it completes, the number of virus in the sample will dramatically increase. This will make it impossible to determine % viability.

11 Pellet infected cells at 4300 G, 5 min.

12 Resuspend pelleted materials in appropriate buffer, such as PBS buffer, to the same volume that you started with pre-cryopreservation (volume of host infected virus+CPAs).

13 Perform a plaque assay to determine PFU/mL and % viability post-cryopreservation. Calculate the number of viable cells by assuming 100% viability based on the number of cells that were originally infected.

Viablity calculations:
(NC64A initial concentration)*(total volume of *Chlorovirus* infected host)*(MOI) =
(Total NC64A)/(*Chlorovirus* initial concentration) =
total mL *Chlorovirus* to infect *Chlorella*

(volume of *Chlorovirus* used)*(*Chlorovirus* initial concentration) = total *Chlorovirus* PFU
(volume of *Chlorella* used)*(*Chlorella* initial concentration) = total *Chlorella* cells
Ratio: (total *Chlorovirus*)/(total *Chlorella*)

(total *Chlorovirus*)/(total volume of *Chlorovirus* infected host) = (Amount of *Chlorovirus* (PFU/mL) in the total volume)/(2) = (PFU/mL)/(Ratio) = PFU/mL for 100% viability

Note

I typically see a ~80% viability at the timepoint 240 min post-infection and ~20% viability at the timepoint 180 min post-infection.