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Resuspension and concentration of iron chloride precipitated viruses from seawater for HMW DNA extraction

Iron Chloride Precipitation of Viruses from Seawater

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This protocol describes an alternative resuspension protocol for iron chloride flocculated viruses from natural water. This method produces much higher molecular weight DNA than the original.

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Citation for original iron flocculation method: John, S.G., Mendez, C.B., Deng, L., Poulos, B., Kauffman, A.K.M., Kern, S., Brum, J., Polz, M.F., Boyle, E.A., & Sullivan, M.B. (2011). A simple and efficient method for concentration of ocean viruses by chemical flocculation. Environ Microbiol Rep. 3(2), 195-202. doi:10.1111/j.1758-2229.2010.00208.x.

Resuspension

1 Prepare 0.2M citrate 0.2M $MgCl_2$ buffer, pH 6-6.5.

1.1 Recipe for 500mL buffer
Add the following to ~400mL milliQ water:
29.41g sodium citrate
5.09g magnesium chloride hexahydrate
pH to 6-6.5 using HCl
fill to 500mL with milliQ water
Autoclave to sterilize and store at 4C.

2 Make sure the precipitate is accessible to the buffer - if filters were folded precipitate side in, this will necessitate unfolding and re-folding the filter. If you do not want to resuspend the whole filter (i.e., the filer has 20L precipitated onto it, but you only need 10L), cut the filter and move the portion you need to a new tube. Sterilize your scissors and forceps with 10% bleach and rinse with milliQ water.

3 Add 1ml buffer for each 1mg Fe, e.g., 20ml of 1x buffer for 20L seawater precipitate.

4 Place tubes on a rotator and rotate at 4°C overnight.
18:00:00

If there is a significant amount of precipitate still on the filters, leave rotator at room temp for a few hours.

5 Using bleached and rinsed forceps pull an edge of the filter onto the tube edge so it is caught in the lid when secured.

6 Centrifuge at low speed (less than 1000 rpm) for 3-5 minutes to remove buffer from the filter.

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7 If the filter still has substantial precipitate on it, add more buffer, incubate again for a couple hours, and collect liquid as stated above.

DNase treatment

8 Follow this protocol to treat the viral resuspension with DNase:
https://dx.doi.org/10.17504/protocols.io.c3myk5
We recommend incubating for 1hr at 37C to aid in more complete dissolving of the viral resuspension.

Concentration

9 Fill the upper reservoir of a 15mL 100kDa Amicon centrifugal device with your sample.

10 Centrifuge at 1000 - 3000g until the level in the upper reservoir is below 250ul. Start with 1000g for 15 min and see how much sample goes through. Continue centrifuging in 15-30 minute increments (tou may need to increase the time). If very little sample is going through in 30 min at 1000g, increase speed to 2000g. If the sample is still not going through, increase to 3000g. As more sample passes through the filter, it will become clogged and may slow down, so expect to start at 1000g and possibly need to increase speed as you go.

This process usually takes 2-6 hours.

10.1 If your sample volume is more than 15mL, empty the bottom reservoir as sample passes through and refill the top until all your sample has been added.

11 Remove the upper reservoir from the Amicon and place the cap on top.

12 Vortex at ~1,500rpm for 20 sec, being sure to tilt the upper reservoir to each side to maximize resuspension.

13 Pipet the viral concentrate out of the upper reservoir into a 2mL tube using a 200ul pipettor.

Add 190ul of resuspension buffer to the upper reservoir and repeat steps 12-13, adding the

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14 recovered viral concentrate into the same 2mL tube.

15 Repeat step 14 (this is a total of 3x rounds of vortexing).

16 Viral concentrate should be stored at 4C. Proceed to DNA extraction for HMW DNA https://dx.doi.org/10.17504/protocols.io.6cbhasn.

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