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Can Crusher-Culley Protocol

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Abstract

Culley Protocol (from Culley & Steward (2007), **New genera of RNA viruses in subtropical seawater, inferred from polymerase gene sequences**, Applied & Environmental Microbiology 73(18):5937-5944).

Guidelines

Supplies needed (per 100 mL sample):

Sample (0.2 µm filtered seawater)One 60 mL syringe
One Anotop (0.02 µm filter)
One piece of parafilm
One piece of aluminum foilOne Ziploc freezer bag
Sharpie (ultra-fine)
Can crusher

1 Using sterile technique, remove the plunger from the 60mL syringe.

2 Attach the 0.02 µm filter (Anotop) to the end of the syringe.

3 Pour 50 mL of 0.2 µm filtrate into the 60-mL syringe.

4 Replace plunger and mount the syringe and filter into the can crusher.

5 Push the 0.2 µm filtrate **very slowly** through the Anotop, using the can crusher and applying **gentle, steady pressure**. Watch carefully for the last drop of water to go into the Anotop, and then **stop pushing**.

Note

a) Do not let any air push through the Anotop—that may cause it to burst.
b) Do not suck up any water into the outlet of the Anotop.

6 Using sterile technique, remove the Anotop from the syringe. Do not touch the filter inlet or outlet.

7 Suck up the remaining 50mL of 0.2 µm filtrate for this depth into the 60-mL syringe.

8 Re-attach the Anotop to the end of the syringe.

9 Repeat Step 5: Push the 0.2 µm filtrate **slowly** through the Anotop, using the can crusher.

Note

Be careful not to push air through the Anotop or suck any water back up into it.

10 Remove the Anotop from the syringe.

11 Write on the Anotop itself using a waterproof Sharpie: date, depth, location (or a shorthand for location if key is explained elsewhere)

12 Wrap the Anotop in clean parafilm, covering both the inlet and outlet.

13 Label a piece of aluminum foil with date, depth, and location (using waterproof Sharpie).

14 Wrap the parafilmed Anotop in the labeled aluminum foil.

15 Label a Ziploc bag with date, depths, and location.

16 Collect several Anotops to put into one labeled Ziploc: one location/sample site per bag.

17 Put the foil-wrapped Anotops into the labeled Ziploc bag.

18 Freeze the Ziploc in -80°C freezer (if no -80°C freezer is available, use a -20°C freezer).

19 Discard the syringe and filtrate (unless you need some sterile water).