Can Crusher-Culley Protocol

Alexander Culley, Grieg Steward

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).

Citation: Alexander Culley, Grieg Steward Can Crusher-Culley Protocol. **protocols.io** dx.doi.org/10.17504/protocols.io.c3jykm
Published: 28 Jul 2015

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

Protocol

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

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

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

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

Step 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**.

NOTES
Bonnie Poulos 21 Jul 2015

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.

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

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

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

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

NOTES

Bonnie Poulos 21 Jul 2015

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

Step 10.
Remove the Anotop from the syringe.

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

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

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

Step 14.
Wrap the parafilmed Anotop in the labeled aluminum foil.

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

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

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

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

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