CALeDNA Water samples DNA extraction (0.22 or 0.45 micron Sterivex Columns)
by Jen Quick-Cleveland 4/26/2022
Based on David Halfmaerten protocol Research Institute for Nature and Forest from 3/15/2017

DAY 1

Materials:
10% bleach in spray bottle (for cleaning)
20% bleach in 50 mL falcon tube
80% ethanol in 50mL falcon tube
100% ethanol in 50 mL falcon tube
Tweezers
Pipe cutter
Microbead sterilizer
2 ml eppendorfs
ATL buffer (Qiagen lysis buffer)
Proteinase K solution from Qiagen (20mg/mL)

1. Sterilize your work area by spraying working surfaces with 10% bleach, followed by 70% ethanol. Spray pipettes with RNAse-Away which will kill any DNA on surfaces *(RNAse-Away = alkali hydroxide plus some detergent)*

2. Wipe the outside of the Sterivex column with 10% bleach, let this dry and then wipe with 70% ethanol.

3. Sterilize the tweezers by letting them soak in 20% bleach solution in a 50 mL falcon tube for 1 minute. Rinse them for ~20 seconds in 70% ethanol and then in 100% ethanol and place them in the micro-bead sterilizer. After they stop sizzling (ethanol evaporating off) take them out and set them aside too cool.

4. Hold the filter columns with the notched side up and with the pipe cutters gently pry off the lid of the column. The column should be gently removed. The buffer can go to the waste OR you can save it in an eppendorf tube at -20°C for future extractions (*recommended*). Note: some clients may expect half of the filter to be saved, too. We fold these into foil and put them in a whirlpak bag.

5. With your sterile and cool tweezers peel the filter off of the column in small pieces (this improves the extraction). Place the small pieces into a 2 mL tube

6. Add 900 ul of ATL Buffer to the 2 mL tube to lyse the sample

7. Add 100 ul Proteinase K solution (20mg/ml per Qiagen website) to the 2 mL tube

8. Shut the tubes securely (parafilm closed if you like - I haven't found this to be necessary with 2 mL tubes) and incubate at 56^0C for 24 hours in a rotating incubator. *Meyer lab note:* Set rotator at speed 10.

DAY 2

You will either continue either with the DNeasy Blood and Tissue Kit (Qiagen: product number 69504). OR you will use DNeasy PowerSoil Pro Kit (Qiagen: product number 47016) depending on what your research questions are. If you are asking questions that deal with mammals and larger eukaryotes, you will get better results using DNeasy Blood and Tissue Kit. If your questions focus more on prokaryotes, use the DNeasy Power Soil Pro Kit from the humic acid precipitation step and on - as written.

DNeasy Blood and Tissue Kit protocol - with mods from David Halfmaerten

Set-up: Bring 100ul/sample of TE buffer to 70^{0}C in a heatblock
Preheat thermomixer or heat controlled rotator to 60-70^{0}C

1. Pipette 650ul of your lysed sample into a labeled DNeasy Mini Spin column sitting in a 2 mL collection tube
2. Centrifuges at ≥6000xg for 1 minute
3. Discard the flowthrough into a liquid waste - reuse the 2mL collection tube - tap on a kimwipe to remove all remaining liquid
4. Repeat the first 3 steps until all your sample has been filtered through the column *note: *some samples may have a lot of gunk that clogs the column - you can go up to 20,000xg according to the manufacturer so try spinning at a higher rcf!)*
5. Add 500 ul of AW1 to the column and centrifuge at ≥6000xg for 1 minute. Discard the flow though and reuse collection tube
6. Add 500ul of AW2 and centrifuge at 14,000xg for 3 minutes. Discard the flow through along with the collection tube
7. Place the column into a new collection tube (either 2 mL or 1.5 mL tube with caps removed).
8. Centrifuge again for 1 minute at 14,000xg to remove any little bit of remaining AW2 that might still be in the column
9. Transfer the column to new collection tubes again
10. Elution of the DNA: Add 100ul of 70^{0}C TE buffer to the filter - incubated at RT for 10 minutes
11. Centrifuge for 1 minute at ≥6000xg
12. Do a second round of elution by retrieving the flow-through (*this is your DNA)* and applying this to the column again
13. Incubate at RT for 10 minutes
14. Centrifuge for 1 minute at ≥6000xg
15. Discard the column and retrieve the flow-through, transfer to a new and appropriately labeled 1.5 mL low-bind eppendorf
16. Run 5 ul on a 2% agarose gel if you wanted to check size (we don't usually do this)
17. Store extracted DNA at -20^{0}C