DNA Extraction from Sterivex Filters for Metagenomics Sequencing

Modified 2015 by the Brazelton Lab from protocols by Rika Anderson, Colleen Kellogg, Julie Huber, and Byron Crump. Incorporated some recommendations from Lever et al. (2015) Frontiers in Microbiology doi: 10.3389/fmicb.2015.00476.

Do ahead of time:

Notes:

- TE is good for DNA storage, but EDTA inhibits PCR. So this low EDTA TE buffer is a good compromise for storing DNA for later PCR amplification. You can also just use EB (10 mM Tris-HCl, pH 8 or 8.5).
- Will need 1.4 mL DEB and at least 200 uL low EDTA-TE, 2.8 mL 100% ethanol, and 1 mL 70% ethanol per sample.

Steps:

1. Heat water bath or oven to 65ºC.
2. Prepare sodium acetate: 3 M sodium acetate, pH 5.2
3. Prepare 70% Ethanol:

   Requires for 50 mL:

100% Ethanol	35 mL
Ultrapure Water	15 uL

   a. Add UltraPure water to 50 mL falcon tube.
   b. Add 100% Ethanol to the water. The ethanol should be added after the water to reduce the risk of evaporation, which will alter the concentration.
   c. Store at -20 ºC.
4. Prepare DNA Extraction Buffer (DEB):

   Requires for 45 mL:

0.1M Tris-HCl (pH 8)	4.5 mL of 1.0 M
0.1M Na-EDTA (pH 8)	9 mL of 0.5M
0.1M KH_2PO_4 (pH 8)	0.54 g
1.5M NaCl	13.5 ml of 5M
0.8M Guanidine HCl	3.44 g
0.5% Triton-X 100	0.225 mL (225 µL) of 100%

   a. Add above ingredients to 50 mL tube.
   b. Add milli-Q water to ~40 mL.
   c. Add NaOH to pH 10 (several drops at a time).
   d. Add milli-Q water to 45 mL.
   e. Filter-sterilize to remove possible spores with 0.22 µm syringe filter.
   f. Autoclave (~30 minutes). Slightly loosen lid so that it is not air-tight. Recover from autoclave soon after the autoclave cycle is completed to prevent loss from evaporation, which will alter the concentration of the solution.
   g. Pour autoclaved solution into fresh 50 mL tube.
   h. Aliquot into 1.5 or 2.0 mL microtubes.
5. Prepare low EDTA-TE:

   Requires for 50 mL:

10 mM Tris-HCl
0.1 mM EDTA
milliQ H_2O

a. Combine 500 µl autoclaved 1 M Tris-HCl (pH 8.0) and 10 µl autoclaved 0.5 M EDTA (pH 8.0) with 50 mL milliQ H_2O.
b. Filter-sterilize to remove possible spores with 0.22 µm syringe filter.

Hot Lysis:

Steps:

1. Add 1.4 mL of DEB to each Sterivex. Position the pipette tip to just below the mouth of the Sterivex so that it does not come back out the top. Do not fill to the top – stop before solution covers the white filter.
2. Place sterivex caps on either end of the filter if missing.

Possible Stopping Point. Store at -20ºC

3. Place sterivex filter in 50 mL tube (with holes).
4. Incubate at 65ºC for 30 minutes either on a Genemate spinning machine or manually shaking every 5-10 minutes.
5. Vortex each filter inside a Falcon tube (without holes) for 30 seconds.

Bead Beating:

Preparation:

1. Label two 1.7 mL microtubes and one 0.1 mm bead tube per sample.

Steps:

1. Using a syringe, withdraw fluid from each Sterivex and eject into bead tube (glass 0.1 mm for bacteria).
2. Bead beat for 40 seconds.
3. Centrifuge for 2 minutes at 5000 g (1 rcf = 1000 g).
4. Without disturbing the beads, transfer supernatant into a fresh tube. Add no more than 900 µL in each tube (or no more than 750 µL if using 1.5 mL tubes).

Phenol / chloroform extraction:

Preparation:

1. Label two 1.7 mL microtubes for each tube from the previous section.
2. Prepare fume wings or fume hood to work in.

Steps:

1. Add equal volume phenol / chloroform / isoamyl alcohol (25:24:1, bought pre-mixed with alkaline buffer) to each tube. Avoid pipetting the overlying phenol.
2. Gently shake a few times and then centrifuge at 14,000g for 1 minute.
3. Transfer supernatant to fresh tube, carefully avoiding the bottom layer.
4. Add equal volume of chloroform / isoamyl alcohol (24:1) to each tube.
5. Gently shake a few times and centrifuge at 14,000g for 1 minute.
6. Transfer supernatant to fresh tube, carefully avoiding the bottom organic layer.

Ethanol precipitation:

Preparation:

1. Label one 1.7 mL eppendorf tubes for each tube from the previous section.

Steps:

1. Redistribute aqueous phase among 3 tubes so that each 2.0 mL tube has 550 µL or less and each 1.7 mL tube has 450 µL or less.
2. Add 0.1x volume 3M, pH 5.2 sodium acetate (*e.g*. add 55 µL to 550 µL).
3. Add 2 volumes 100% ethanol (*e.g.* add 1210 µL to 605 µL).
4. Invert several times to mix.
5. **Incubate at -20ºC for at least 1 hr or overnight.** Incubation on ice might work just as well and yield a cleaner pellet.
6. Centrifuge for 40 minutes at 16,000g (Optional: used cooled centrifuge at 0ºC).
7. Pour out supernatant. Do not completely invert tube; keep at a gentle angle to minimize the chance of the pellet falling out.
8. Add 500 µL cold 70% ethanol to each tube.
9. Invert the tube to mix. Make sure the pellet is dislodged from the bottom so that it is properly washed.
10. Centrifuge at 16,000g for 10 minutes.
11. Discard liquid again with pipette, being careful to avoid pellet.
12. Place tubes with open lids in the Vacufuge. Spin for 7 minutes at 30ºC on the V-AL setting. If you can see ethanol in the tube, spin for another 2-5 minutes. If the pellets become powdery, they are too dry.
13. Resuspend in 100 µL of low EDTA TE.
14. Heat to 55ºC for 10 or more minutes to dissolve pellet and store at 4ºC. Gently finger-flick to mix, if necessary. For long-term storage, place at -20 or -80ºC, but avoid repeated freezing and thawing of the DNA. One strategy is to keep half at 4ºC for the working sample and store the other half at -80ºC as the archive sample.