DNA Extraction from Sterivex Filters

Modified 2015 by the Brazelton Lab from Brazelton et al. (2010), influenced by protocols from Rika Anderson, Colleen Kellogg, Julie Huber, and Byron Crump, and incorporating some recommendations from Lever et al. (2015). Clean-up with magnetic beads follows Rohland & Reich (2012).

References

Brazelton W.J., K.A. Ludwig, M.L. Sogin, E.N. Andreishcheva, D.S. Kelley, C-C. Shen, R. L. Edwards, J.A. Baross (2010) Archaea and bacteria with surprising microdiversity show shifts in dominance over 1000-year time scales in hydrothermal chimneys. *Proceedings of the National Academy of Sciences USA*. 107: 1612-1617. doi:10.1073/pnas.0905369107.

Lever M.A., Torti A., Eickenbusch P., Michaud A.B., Šantl-Temkiv T., Jørgensen B.B. (2015) A modular method for the extraction of DNA and RNA, and the separation of DNA pools from diverse environmental sample types. Frontiers in Microbiology doi:10.3389/fmicb.2015.00476.

Rohland N, Reich D. (2012) Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. Genome Research. doi:10.1101/gr.128124.111.

Protocol

Do ahead of time:

Prepare solutions:

Amended DNA Extraction Buffer (DEB):	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
0.8M guanidium HCl	3.44 g
0.5% Triton-X 100	0.225 mL (225 µL) of 100%

Add above ingredients to 50 mL tube.
Add milli-Q water to ~40 mL
Add NaOH to pH 10
Add milli-Q water to 45 mL
Filter-sterilize to remove possible spores
Autoclave. Slightly loosen lid so that it is not air-tight. Recover from autoclave very soon after the autoclave cycle is completed.
Pour autoclaved solution into fresh 50 mL tube.
Aliquot into 1.5 mL tubes.

Recipe for low EDTA TE:

10 mM Tris-HCl
0.1 mM EDTA

For 50 ml:
500 µl 1 M Tris-HCl (pH 8.0) autoclaved
10 µl 0.5 M EDTA (pH 8.0) autoclaved
→ to 50 ml with milliQ H_2O
→ filter sterilize with 0.22 µm syringe filter

BSF Sample Prep:

1. Flame ceramic mortar and pestle
2. Wipe down mortar and pestle with dichloromethane (treat DCM as you would phenol)
3. Crush and homogenize sample
4. Divide sample into two 50mL falcon tubes-- half for chemistry and half for biology
5. Store homogenized sample at -80˚C

Hot Lysis:

1. Measure 0.25g of sample
2. In a 2mL tube, add 1.4 mL of DEB to 0.25g of sample
3. Freeze sample (Possible stopping point, store sample in -20˚C freezer)
4. Incubate at 65ºC for 30 mins in the ThermoMixer at 1500rpm

Bead Beating:

5. Using a pipette, withdraw fluid and any undissolved sediment and eject into bead tube (glass 0.1 mm for bacteria).
6. Bead beat for 40 s.
7. Centrifuge for 2 min at 5000 g.
8. Transfer fluid - avoiding beads - into fresh Eppendorf 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:

9. Add equal volume of phenol / chloroform / isoamyl alcohol (25:24:1, bought pre-mixed with alkaline buffer) to each tube.
10. Gently shake a few times and then centrifuge at 14,000g for 1 minute.
11. Remove supernatant to fresh tube.
12. Add equal volume of chloroform / isoamyl alcohol (24:1) to each tube.
13. Gently shake a few times and centrifuge.
14. Remove supernatant to fresh tube, carefully avoiding the bottom organic layer.

Ethanol precipitation:

15. Redistribute aqueous phase among 3 tubes so that each 2.0 mL tube has 550 µL or less and each 1.6 mL tube has 450 µL or less. Add 2 volumes 100% ethanol. (*e.g.* add 1210 µL to 605 µL.)
16. Invert a few times to mix.
17. **Incubate at -20ºC overnight.**
18. Centrifuge for 40 minutes at 16,000g.
19. Pipette out supernatant without disturbing the pellet.
20. Add 500 µL of -20˚C 70% ethanol to each tube.
21. Invert the tube to mix. Make sure the pellet is dislodged from the bottom so that it is properly washed.
22. Centrifuge at 16,000g for 10 minutes.
23. Remove liquid again with pipettor. Be careful to avoid pellet.
24. Place tubes with open lids in the Vacufuge. Spin for 5 minutes at 30ºC on the V-AL setting. If you can see ethanol in the tube, spin for another 2-5 minutes. If pellets become powdery, they are too dry.
25. Resuspend in ~100 µL of low EDTA TE. Heat to 55ºC for 10 or more minutes to dissolve pellet and store at 4ºC. For long-term storage, place at -20 or -80ºC, but avoid repeated freezing and thawing of the DNA.

Amicon Clean-Up

1. Make sure to qubit, volume, and nanodrop the sample before cleanup. Record values before proceeding.
2. Bring sample up to 500ul volume with TE
3. Load diluted sample into Amicon 30K Ultra Centrifugal Filter Unit and centrifuge for 4 min at 14000g. You should have ~100-75uL of sample in amicon column
4. Add ~400uL of TE (1mM EDTA 10mM Tris) at 65˚C to column -- final volume should be 500uL
5. Centrifuge column for 4min at 14000g.
6. Repeat steps 4-5
7. Flip column over into clean tube.
8. Centrifuge 2 min at 1000g to collect sample
9. Qubit and nanodrop

Final Bead Cleanup

1. Bring speed beads to room temperature.
2. Vortex speed beads to mix.
3. Add twice the sample volume of speed beads to sample (e.g. for 50uL of sample add 100uL of speed beads), pipette to mix.
4. Incubate 5 mins at room temperature.
5. Spin down tube.
6. Pellet on magnetic rack for 3 mins or until supernatant is clear.
7. Leaving the sample of the magnetic stand, discard supernatant without disturbing beads (DNA is bound to beads).
8. Remove the tube from the magnetic stand.
9. Add 200 ul of 80% ethanol to the tube.
10. Pipette to remove beads from side of tube.
11. Place back on the magnetic stand.
12. Wait 3 minutes or until supernatant is clear, and discard the supernatant.
13. Repeat wash steps (steps 8-12).
14. Leave tube open and let beads air dry from 3-5min. Careful not to overdry ("cracked" beads are overdry).
15. Resuspend beads in 50uL low EDTA-TE.
16. Incubate 3 mins at room temperature.
17. Spin down tube.
18. Pellet on magnetic stand for 3 mins or until supernatant is clear.
19. Transfer supernatant to a clean eppendorf tube.
20. Measure final concentration with qubit, record nanodrop values, and you’re done!