DNA extraction from sterivex filter
(Staley Lab 2008)
Modified from:
Vetriani et al., (2003) Applied and Environmental Microbiology 69: 6481-6488.

Solutions needed:
Solution 1
50 mM glucose
10 mM EDTA
25 mM Tris-Cl (pH 8)

Lysozyme Solution (Remake daily)
0.8 mg/mL lysozyme in Solution 1

Proteinase K Solution (Remake daily)
10 mg/mL in TlowE

1.) Heat close filter bottom. Parafilm bottom.
2.) Pipet 1 mL of Solution 1 onto filter.
3.) Freeze thaw sample between dry ice/Ethanol slurry and 55ºC water bath 8-10 times. Transfer quickly.
4.) Add 400 µL Solution 1, 200 µL Lysosyme solution, 100 µL 500 mM EDTA, 20 µL proteinase K. Parafilm top.
5.) Turn for 30 minutes at 30ºC.
6.) Take solution out of filter case with syringe. Split between 2 or 3 microcentrifuge tubes.
7.) Add 150 µL of 10% SDS followed quickly by phenol chloroform (pH 7) (1:1 volume with sample). Invert 10 times.
8.) Spin in centrifuge at max speed for 3 minutes.
9.) Remove top phase with pipet. Add to new tube.
10.) Add 1:1 volume of phenol chloroform. Invert 10 times. Spin in centrifuge at max speed for 3 minutes. Remove top phase with pipet. Add to 2 mL tube.
11.) Precipitate DNA: add 50 µL of 3.0 M sodium acetate and 2X volume of 100% ethanol.
12.) Put tubes in -15ºC freezer for at least 3 hours. (Overnight is ok.)
13.) Spin in centrifuge at max speed for 15 minutes.
14.) Wash pellet with 100 µL 70% ethanol. Centrifuge 5 minutes on max.
15.) Dry in speed-vac.
16.) Resuspend in 100 µL TlowE for several hours in the fridge.
17.) Clean DNA with Qiagen Qiaquick PCR purification kit.
18.) Resuspend in 50µL TlowE.