Modified Protocol for Sterivex Filter DNA Extraction

1. Prepare the Powersoil PRO Bead tube:
   Spin briefly to ensure that the beads have settled at the bottom, then add **800µl** of Solution **CD1**.
2. Push out the RNALater from the Sterivex & rinse:
   Fill a new syringe with air, connect it to the sterivex, and push the RNALater out while keeping the outlet facing upwards.
   Fill a new syringe with **20ml** of **PBS**, connect it to the sterivex, and rinse the filter inside using the PBS, rotating the filter to ensure that all sides have been rinsed.
   Fill the syringe with air and push the PBS out of the sterivex.
3. Break open the Sterivex filter outer casing: Place pliers at the broader edge & crack open. Change parafilm & disinfect with bleach for every sterivex.
4. Cut the filter into strips using sterile razor blades. Use tweezers to place strips into the PowerSoil kit bead tube. Make sure that the tweezers are also disinfected between samples.
5. Vortex briefly, then incubate at 70°C at 500rpm for 10 minutes.
6. Repeat step 5.
7. In the fumehood, add up to **600µl** of Phenol-Chloroform-Isoamyl alcohol.
8. Vortex at maximum speed for 10 minutes.
9. Centrifuge at 15,000g for 1 minute.
10. Transfer **800µl** of the aqueous supernatant to a clean 2ml collection tube.
11. Add **200µl** of Solution **CD2** and vortex briefly.
12. Centrifuge at 15,000g for 1 minute
13. Avoiding the pellet, transfer up to **800µl** supernatant to a clean 2ml collection tube
14. Add **700µl** of Solution **CD3** (Shake before use). Vortex briefly.
15. Load **600µl** of mixture into the spin filter, then centrifuge at 15,000g for 1min.
    Take the filter out, discard flow-through, put the filter back in.
    Repeat this step until all of the sample has been processed.
16. Add **500µl** of Solution **EA**, centrifuge at 15,000g for 1 min. Discard flow through.
17. Add **500µl** of Solution **C5**, centrifuge at 15,000g for 1 min. Discard flow through.
18. Centrifuge again at 15,000g for 2min to remove the remaining C5.
19. Carefully place the spin filter in a clean 2ml collection tube.
20. Add **60µl** of **Nuclease-free water** to the centre of the white filter membrane to elute DNA. Incubate at room temperature for at least 1 minute.
21. Centrifuge at 15,000g for 1 min.
22. Optional: Repeat steps 19-21 to ensure that the DNA has been completely eluted.
23. Discard spin filter. DNA Extraction completed.

16S PCR amplification

Recipe:

- DNA template (5ng) 2.5uL
- Primer 926F (10uM) 0.5uL
- Primer 1392R (10uM) 0.5uL
- KAPA Hotstart (2x) 12.5uL
- Holy 9uL

Total: 25uL

Setting:

- 95°C 3min
- **98°C 30sec**
- **55°C 30sec**
- **72°C 30sec**

in bold: repeat 25 cycles

- 72°C 5min
- 4°C ∞

PCR cleanup with SPRI Beads

Always prepare fresh ethanol to wash the beads.

1. Add PCR product : Beads in **1:0.8** ratio
2. Incubate at room temperature for 5min
3. Place on magnetic stand and incubate for 2min until it is clear and colourless
4. Remove supernatant. Add 200uL 70% ethanol
5. Discard ethanol. Add 200uL 70% ethanol
6. Discard ethanol thoroughly
7. Open lid and allow to dry at room temperature for 5min
   (Do NOT over dry bead, this will significantly decrease elution efficiency)
8. Remove from magnetic stand, add 42.5uL nuclease free water and mix well with the bead by pipetting in and out
9. Incubate at room temperature for 2min
10. Place the tube back to the magnetic stand until supernatant has cleared (about 2 minutes)
11. Transfer 40ul supernatant to a clean new tube.

The supernatant is the purified PCR product.

Qubit

1. Prepare Qubit solution as below:
   - Buffer - 199 x (number of samples + 2 standard + 1)
   - Dye - 1 x (number of samples + 2 standard + 1)
2. For standards: Add 190uL Qubit solution + 10uL standard
3. For samples: Add 198uL Qubit solution + 2uL DNA
4. Briefly vortex and let rest for 1min
5. Use a kimwipe to clean the tube before taking the measurement
6. Record the measured DNA concentration