Appendix III DNA Extraction Protocol

We will all use DNeasy PowerSoil Kit to extract DNA from Anaerobic Digester samples.

Materials

(1) DNeasy PowerSoil Kit (QIAGEN, Catalog# 12888-50 or 12888-100)

https://www.qiagen.com/us/shop/sample-technologies/dna/genomic-dna/dneasy-powersoil-kit/#orderinginformation

A manufacture protocol will be provided in the Kit

(2) Vortex Genie® 2 Vortex (Scientific Industries)

https://www.scientificindustries.com/vortex-genie-2.html

(3) Vortex adapter for 1.5~2 mL tubes (QIAGEN, Catalog# 13000-V1-24)

https://www.qiagen.com/us/shop/automated-solutions/accessories/vortex-adapter/#orderinginformation

(4) 1x TE buffer (10 mM Tris-HCl, 1 mM EDTA.Na_2, pH=8, sterile, DNase free)

Prepare by yourself or prepare from a qualified commercial solution
(e.g. 100x TE from Sigma, Catalog# T9285-100ML,
http://www.sigmaaldrich.com/catalog/product/sigma/t9285?lang=en&region=US)

Notes in addition to manufacture protocol:

(1) For each sample, use a pellet from 1.5 mL sludge to extract DNA by one prep of the kit. Use more pellets if necessary.

(2) Once take the pellet out of freezer, use the bead solution in a PowerBead tube (provided in the kit) to melt, resuspend and transfer the pellet to a PowerBead tube.

(3) Extract 12 samples per round. Always place 12 bead tubes on the Vortex evenly and vortex at maximum speed for 10 min. If you are dealing with less than 12 samples, put some fake bead tubes to ensure 12 bead tubes on the vortex.

Because the number of tubes on the vortex and vortex time can influence lysis efficiency. Let us do it in the same way to minimize the differences between labs.

(3) **DO NOT use solution C6** but use 100 µL 1x TE buffer to elute DNA from the filter at the Step 20 in the manufacture protocol.

* C6 in the kit contains no EDTA. To avoid DNA degradation, we use TE instead of C6.

(4) In the end, use Nanodrop to check the DNA quality and quantity.

260/280 ~1.8, 260/230≥1.7, DNA amount > 3 μg (>5 µg is ideal)

(5) Seal each tube by Parafilm or put each one into a sealed plastic bag.

(6) Store DNA at -80°C (If not available, -20°C may be OK for a short-time storage)**.**