Appendix III DNA Extraction Protocol

This time, we will all use Powersoil® DNA Isolation Kit to extract DNA from activated sludge.

Materials

(1) Powersoil® DNA Isolation Kit (MOBIO Catalog# 12888-50 or 12888-100)

http://www.mobio.com/soil-dna-isolation/powersoil-dna-isolation-kit.html

A manufacture protocol will be provided in the Kit

(2) Vortex Genie® 2 Vortex (MOBIO Catalog# 13111-V or 13111-V-220)

http://www.mobio.com/vortex-and-vortex-adapters/vortex-genie-2-vortex.html

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

http://www.mobio.com/vortex-and-vortex-adapters/vortex-adapters-for-vortex-genie-2.html

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

Prepare by yourself or purchase a qualified solution (e.g. 100x TE from Sigma, Catalog# T9285-100ML, http://www.sigmaaldrich.com/catalog/product/sigma/t9285?lang=en&region=US)
You need to dilute the 100X TE to 1X TE with DNase free water. It may be unbelievable, but some people really used the 100X TE directly instead of 1X TE by mistake.

Notes in addition to manufacture protocol:

(1) For each sample, use a pellet from 3 mL mixed liquor to extract DNA by one prep of the kit.

(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.

Fig. 1 Solutions in Powersoil kit. The tubes pointed out by in red circles are 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.

Fig. 2 The positions of bead tubes on the Vortex.

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

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

20. Add 100 µl of **Solution C6** to the center of the white filter membrane. Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica Spin Filter membrane at this step (MO BIO Catalog# 17000-10).

Fig. 3 The step 20 in manufacture protocol. Please use 1X TE buffer instead of solution 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 the top of each tube with Parafilm® or put each tube into a secondary container (e.g. sealed plastic sample bag), to prevent spillage or cross contamination in future shipping.

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