

Protocol for DNA Extraction of samples: (G. Gerdt)

DNA Extraction Protocol modified after Jizhong Zhou et al, Applied & Environmental Microbiology, Vol 62, No.2

- frozen polycarbonate filter(s) (47mm) with biomass were cut into pieces and transferred into 10 ml extraction buffer (100 mM Tris-HCl pH8; 100 mM sodium EDTA pH8; 100 mM sodium phosphate pH8; 1.5 M NaCl; 1% CTAB)
- addition of 37  $\mu$ l Proteinase K (20 mg/ml)
- incubation @ 37°C for 60 minutes
- addition of 1.1 ml SDS 20%
- incubation @ 65°C for 100 minutes
- centrifugation @ 53250 x g for 15 minutes (room temperature)
- extraction of supernatant with equal volume chloroform/isoamylalcohol (24:1 V/V)
- centrifugation @ 3220 x g for 15 minutes (room temperature)
- recovery of aqueous phase and addition of equal volume chloroform/isoamylalcohol (24:1 V/V)
- centrifugation @ 3220 x g for 15 minutes (room temperature)
- recovery of aqueous phase and precipitation with 0.6 volume of isopropanol @ room temperature over night
- centrifugation @ 53250 x g for 20 minutes (room temperature)
- removal of supernatant and washing of the pellet with 2 ml Ethanol 80%
- the pellets were resuspended in 200  $\mu$ l TE buffer (1 mM Tris-HCl pH8, 0.1 mM EDTA pH8) for 2 hours @ room temperature and stored @ 4°C until shipment