

Genomic DNA Extraction from Activated Sludge

This protocol is based on Purkhold U. et al. (2000). Appl Environ Microbiol 66:5368-82,
and modified by Suzan Yilmaz

1. Centrifuge activated sludge samples in 50-mL falcon tubes at 5,000 x g for 5 min.
2. Resuspend 0.25 g (wet weight) (approx. 20 ml) pellets in 625 µl DNA extraction buffer in 2 mL screw cap tubes.
3. Add 50 µl enzyme mixture I, and mix gently by inversion. Incubate at 37°C for 30 min.
4. Add 50 µl enzyme mixture II, and mix gently by inversion. Incubate at 37°C for 30 min. again
5. Add 75 µl 20% SDS, and incubate @ 65°C for 2 hrs.
6. Add 600 µl phenol:chloroform:isoamyl alcohol (25:24:1), mix by inversion, and incubate @ 65°C for 20 min.
7. Vortex, and centrifuge at 10,000 x g for 10 min at RT. Transfer the aqueous phase into a fresh tube.
8. Add 100 µg/ml RnaseA to 10 µg/ml final conc., and incubate at 37°C for 30 min.
9. Extract samples with 1 volume chlorofom:isoamyl alcohol (24:1), mix by inversion, and centrifuge at 10,000x g for 10 min at RT. Transfer the aqueous phase into a fresh tube.
10. Precipitate nucleic acids by adding 0.6 vol of isopropanol and incubating at room RT for 1 hr. Centrifuge at 10,000 x g at 4°C for 20 min. Discard the supernatant.
11. Wash pellets with 2.5 vol cold 70% ethanol. Invert tubes, and air-dry pellets.
12. Resuspend pellets in TE, and store high molecular weight DNA in the fridge.

DNA Extraction Buffer

Stock Solution	Volume	Final Concentration
1 M Tris-HCl (pH 8)	10 ml	100 mM
0.5 M EDTA (pH 8)	20 ml	100 mM
1 M NaPhosphate (pH 8)	10 ml	100 mM
NaCl	8.36 g	1.5 M
10% CTAB/0.7 M NaCl	10 ml	1 % CTAB
H ₂ O	to 100 ml	

Enzyme Mixture I

Enzyme	mg/50 µl	Final Concentration
Lysozyme	0.71	14.2 mg/ml
Lipase	1.13	22.6 mg/ml
Pectinase	0.84	16.8 mg/ml
b-Glucuronidase	0.53	10.6 mg/ml

Enzyme Mixture II

Enzyme	Stock Solution	Volume	Final Concentration
Proteinase K	20 mg/ml	25 µl	10 mg/ml
Protease	100 mg/ml	5 µl	10 mg/ml
Pronase*	100 mg/ml	20 µl	40 mg/ml

* Needs to be self-digested. Dissolve powder in 10 mM Tris-Cl (pH 7.5) and 10 mM NaCl, and digest at 37°C for 1 hr