

Isolation of total DNA (Cao et al. (1990) Cell 61, 1089-1101)

- take 40 ODU cells (e.g. 40 ml of OD₆₀₀=1), optional: fix in 50 % ethanol at -20°C
- wash 1x in TE
- resuspend in 0.5 ml 1 M sorbitol, 10 mM NaPO₄ pH 7.2 (or KPO₄), 50 mM EDTA, 3 µl β-mercaptoethanol, 5 µl of 1 mg/ml zymolyase T100
- 15 min 37°C
- 15 sec. microfuge, resuspend in 0.5 ml 50 mM EDTA, 0.3 % SDS, 5 µl of 20 mg/ml Prot.K and incubate 30 min at 65°C
- cool on ice and add 200 µl 5 M KOAc and keep 20 min on ice
- 10 min microfuge at 4°C
- decant SN into new tube and extract 3x with PCIA (49:49:2), extract 1x with ether
- add 2 vol ethanol and 0.1 vol NaOAc, keep 15 min in dry ice/EtOH bath
- wash pellet with 70 % ethanol and let pellet air dry
- resuspend pellet in 30 µl TE
- use 6 µl for restriction digest in Southern analysis include RNase