

Alkaline Lysis/PEG Precipitation DNA Prep

This method is especially useful for obtaining large amounts of very clean low-copy plasmid.

1. Inoculate 500 mL 2xYT or LB/0.1% w/v glucose from 1-5 mL overnight
2. Grow to $OD_{600}=0.5-1$ and add 170 $\mu\text{g/mL}$ Cm for low copy plasmids. If the plasmid confers Cm resistance, use 25 $\mu\text{g/mL}$ spectinomycin.
3. Grow 12-16 more hours.
4. Harvest 7k rpm 4°C GS3 10 min.
5. Resuspend cells in 4 mL glucose/Tris/EDTA and transfer to SS34 tube.
6. Add 50-200 μg RNase A (DNase-free), and 25 mg HEW lysozyme in 1 mL GTE. RT 10 min.
7. Add 10 mL freshly-prepared 0.2M NaOH/1% SDS (200 μL 10M NaOH, 1 mL 10% SDS). Stir gently until homogenous and clear. Ice 10 min.
8. Add 7.5 mL KOAc solution and stir to precipitate. Ice 10 min.
9. Spin 13k rpm 4°C SS34 10 min.
10. Transfer S/N to two fresh SS34 tubes and add 0.6 vol isopropanol. Invert to mix. RT 10 min.
11. Spin 11.5k rpm 4°C SS34 10 min. (RT okay here)
12. Wash pellet with 70% ethanol, spin briefly, aspirate ethanol and dry pellet.
13. Resuspend pellet in 1 mL GTE. Add 20 μg RNase A and incubate 37°C 20 min.
14. Add 2 mL freshly-prepared 0.2M NaOH/1% SDS. Invert to mix. RT 10 min.
15. Add 1.5 mL KOAc solution. Invert to mix. RT 10 min.
16. Spin 11k rpm 4°C SS34 10 min.
17. Transfer S/N to 15 mL conical vials. Extract with phenol/chloroform and then with chloroform.
18. Transfer aqueous layer to SS34 tube.
19. Add $\frac{1}{4}$ vol of 10M ammonium acetate and mix. Add 2 vol 100% ethanol. Dry ice 10 min.
20. Spin 8k rpm 4°C SS34 10 min.
21. Wash pellet with 70% ethanol, spin, dry.
22. Resuspend pellet in 2 mL TE. Transfer to two eppendorfs. Add 0.4 mL PEG/NaCl solution per 1 mL resuspended pellet. Mix 4°C 12-16 h
23. Spin 30 min top speed benchtop centrifuge.
24. Resuspend pellet in 0.4 mL TE per tube. Ethanol precipitate with 0.1 vol 3M NaOAc (5) and 2.5 vol ethanol.
25. Dry and resuspend in water or 1-10 mM Tris (8).

Glucose/Tris/EDTA (GTE) [500 mL]

50 mM glucose [4.5 g]
25 mM Tris•HCl (8) [6.25 mL 2M]
10 mM EDTA [10 mL 0.5M]
SF or autoclave

PEG/NaCl solution

30% w/v PEG 8000
1.6M NaCl

KOAc solution (~5.5) [500 mL]

147g KOAc (3M)
55 mL glacial HOAc