

BOILING METHOD MINI PREP PROCEDURE

This is an alternative method for the preparation of plasmid DNA.

Materials:

STET buffer: 8% sucrose
 0.5% triton X-100
 50 mM EDTA, pH 8
 10 mM Tris, pH 8
Lysozyme Solution: 10 mg/ml in 0.25 M Tris, pH 7.5
RNase (DNase free): 2.5mg/ml solution in 10mM Tris, pH7.5/15mM NaCl
 boil for 15 min. and slowly cool to inactivate any DNase
 activity.

Procedure:

1. One amp^R colony is used to inoculate 5 mls LB + amp (at 50 µg/ml) for each sample; incubate at 37°C with shaking for >16 hours.
2. Spin bacterial cultures in centrifuge tubes at room temperature for 5 min. at 5,000 rpm to pellet cells.
3. Discard supernatant and resuspend pellet in 350 µl STET buffer.
4. Add 25 µl freshly prepared lysozyme solution to each. Transfer to Eppendorf tubes, mixing by pipetting up and down.
5. Place in a boiling water bath for 40 seconds, then place on ice.
6. Spin in Eppendorf microfuge for 15 minutes at 5°C (cold room).
7. Pipet off supernatant into fresh Eppendorf tube, then precipitate with two volumes of -20°C isopropanol.
8. Place at -20°C for 2 hours (or overnight).
9. Centrifuge in Eppendorf microfuge for 15-30 minutes at 4°C.
10. Let pellets drain for 5-10 minutes then resuspend in 25 µl TE each.
11. 5-10 µl of this resuspended DNA can easily be detected on a 0.7-1.0% agarose gel.
12. If restriction digestion is desired, at step (11) resuspend pellet in 200 µl TE.
13. Add 4 µl of 2.5 mg/ml DNase-free RNase stock to each. Final concentration 50 µg/ml. Place at 37°C for one hour.
14. Phenol extract once with equal volume.
15. Phenol: chloroform (1:1) extract once with equal volume.
16. Add 15 µl 3M sodium acetate pH 5.5 to each, then 400 µl -20°C 100% ethanol. Place in dry ice/ethanol bath for 20 minutes.
17. Spin down 15 minutes at 4°C in Eppendorf microfuge; resuspend pellets in 30-40 µl TE each.
18. Use 10 µl of DNA per restriction digest.

Reference: Holmes, D.S. and M. Quigley. 1981. A rapid boiling method for the preparation of bacterial plasmids. *Anal. Biochem.* 114, 193-197.