

PLASMID DNA MINIPREP

NOTE: Set aside 500 μ l of an O/N culture for storage. Add to this 500 μ l of 30% glycerol/LB in an Eppendorf and store at -70°C. The final concentration of glycerol is thus 15%.

NOTE: When doing more than five minipreps, inoculate 5 ml of LB and grow O/N. This will result in halving all volumes below, and will mean that the supernatant from step 7 will only have to be transferred to one new tube rather than two.

1. Inoculate 10 ml (3 ml) of LB and grow O/N (12-16 hr) in a 50 ml blue-cap tube (2059 tube).
2. Place cells on ice and spin down for 5 min. at setting "7" in our tabletop centrifuge (spin down for 2 min in 1.5ml eppendorf tube at microfuge).
3. Resuspend in 230 μ l (100 μ l) of cooled solution 1 and transfer to an Eppendorf tube. Vortex or use a pipetman.
4. Add 460 μ l (200 μ l) solution 2. Invert tube gently 4 or 5 times. Place on ice 5 min.
5. Add 350 μ l (150 μ l) ice cold solution 3. Invert tube several times. Place on ice 5 min.
6. Spin 15 min. at 4°C in microfuge.
7. Transfer the supernatant to two new Eppendorf tubes (500 μ l) in each).
8. (optional). Extract 1X with 1/2 volume of phenol and 1/2 volume of chloroform and extract. Finally, add one volume of chloroform and extract.
9. Transfer top aqueous layer to a new tube. Add one volume of isopropanol.
10. Precipitate DNA for 5 min. at RT.
11. Centrifuge 8 min. in microfuge. Pour off supernatant.
12. Add 1 ml cold 70% ethanol. Mix to wash pellets of salt and proteins. Spin 2 min. and pour off supernatant.
13. Dry pellet under vacuum until or air dry just dry (about 1 min). Do not overdry pellet or it will be very difficult to resuspend DNA. Alternatively, remove all traces of ethanol from step 12 above with a pipet tip and air dry pellet for 10 min.
14. Resuspend the pellets in 50 μ l (20 μ l) of TE. Expect a yield of 30-50 μ g (10-20 ug)of DNA.

15. Add 4 μ l 20mg/ml RNAase. Inc. 10 min at 37°C.
16. Take 1 μ l to cut with enzyme, or take @ 5ul to sequence.