Plasmid DNA Miniprep -- Alkaline Lysis Method

Samples:

1. Inoculate 3 ml of LB and grow o/n (12-16 hr) in a 2059 tube.
2. Place cells on ice and spin down for 2 minutes in 1.5 ml eppendorf tubes.
3. Resuspend in 100 µl of cooled solution I using a pipetman.
4. Add 200 µl solution II. Invert tube gently 5 times. Place on ice 5 minutes.
5. Add 150 µl cooled solution III. Invert tube several times. Place on ice 5 minutes.
6. Spin 15 minutes at 4°C in microfuge.
7. Transfer the supernatant to new eppendorf tube.
8. Add one volume of isopropanol; vortex to ensure mixing.
9. Precipitate DNA for 5 minutes at RT.
10. Centrifuge 8 minutes in microfuge. Remove supernatant.
11. Add 1 ml cold 70% ethanol. Vortex and spin 2 minutes. Remove supernatant.
12. Air-dry pellet for 10 minutes.
13. Resuspend the pellets in 20µl of TE + µl 10mg/ml RNAase. Incubate 10 minutes at 37°C.
15. Use 1-2 µl to cut with enzyme, or use 5 µl to sequence.