

Protocol for Plasmid DNA Miniprep

http://csmbio.csm.jmu.edu/biology/courses/bio480_580/mblab/miniprep.html

Birnboim and Doly (Nucleic Acids Research 7:1513, 1979)

P1: 50 mM Tris-HCl, 10 mM EDTA, 100 ug/mL RNase A, pH 8.0, keep at 4°C

P2: 1% SDS, 0.2 M NaOH

P3: 3.0 M Potassium Acetate, pH 5.5, keep at 4°C

1st day

Prepare mini-culture and incubate at 37°C with shaking overnight

2nd day

1. Collect cell pellet into 1.5mL- Eppendorf tubes
2. Resuspend the cell pellet in 150 uL of P1
3. Add 150 uL of P2 and invert 5 times. Let tubes stand for 3 min
4. Add 200 uL of P3 and invert 5 times. Let tubes stand for 3 min
5. Centrifuge for 5min at highest speed by Table centrifuge
6. Collect the supernatant, add 400uL of **Isopropanol** and Invert 5 times
7. Apply the mixture into Spin column. Centrifuge 1min at highest speed by Table centrifuge
8. Add 700ul of **70% ethanol**. Centrifuge 1min at highest speed by Table centrifuge
9. Spin for another 5min to rid of ethanol
10. Dissolve the pellet in 40ul ddH₂O

§ (1) The amount of solutions mentioned above is for 2.5mL of start culture. If culture volume is increased, the amount of solution (P1-P3) should be increased as well.

(2) To precipitate DNA, Isopropanol should be add from 0.7-1 volumes of sample (the supernatant in Step 6).

(3) The amount of 70% ethanol (for washing) does not need to be adjusted.