

Alkaline Lysis Miniprep. Modified by Rahul Patharkar.

Solution 1 = 10 mM EDTA pH 8.0, 1-20 µg/mL RNAase A. Store at 4°C.

Solution 2 = (0.1 M NaOH, 1% SDS). Store at room temperature.

Solution 3 = 250g/L Potassium Acetate, 15% vol/vol Acetic Acid. Store at 4°C.

1. Grow 2 mL bacteria (or 1.7 mL if growing in 2 mL tubes) with vigorous shaking or rolling in LB broth.
2. Transfer the saturated bacterial culture to a 2 mL microcentrifuge tube if culture was not grown in a 2 mL tube. Spin at $\geq 10,000xg$ for 30 seconds. Discard supernatant.
3. Resuspend the pellet in 100 µL Solution 1 by vortexing or pipeting.
4. Add 200 µL Solution 2 and mix by gentle swirling (**it is very important to be very gentle or you will get E. coli genomic DNA contamination in your plasmid prep**).
5. Add 75 µL cold (4°C) Solution 3 and mix by gentle inversion (**it is very important to be very gentle or you will get E. coli genomic DNA contamination in your plasmid prep**).
6. Place tubes in a rack stored at -20°C and store at -20°C for 1 minute.
7. Spin at max speed ($\geq 10,000xg$) for 5 minutes.
8. Transfer up to 375 µL of the supernatant to a clean 1.5 mL tube (**avoid taking any white precipitate**).
9. Add 225 µL 100% isopropanol and mix by vortexing. Spin at max speed ($\geq 10,000xg$) for 5 minutes. Carefully discard the supernatant by pouring it out. Briefly spin down and remove any residual supernatant with a pipet.
10. Add ≥ 1 mL 70% ethanol. Then pour away the ethanol.
11. Spin at $\sim 10,000xg$ for 5 seconds. Pipet away the residual ethanol.
12. Leave on bench horizontally to dry for 2-5 minutes or speedvac for 1 minute.
13. Resuspend the pellet in 75 µL (or 50-100 µL) in one of the following: 2mM Tris pH 8.5, sterile H₂O, TE.

View video protocols here: <https://rahulpatharkar.000webhostapp.com/2018/10/super-cheap-plasmid-miniprep-without-columns>