Plasmid Mini-Preps via P1, P2, & P3

This method for plasmid isolation is based on the QIAprep Spin Miniprep kit (Qiagen) except that no column is utilized in our protocol. Instead, the plasmid DNA is precipitated with isopropanol. The isolated DNA is acceptable for restriction digests, ligation, and other subcloning applications. It is not, however, suitable for sequencing.

1. Add 1.5 ml of culture to 1.5 ml eppendorf tube. Spin at 16,000 X *g* for 1 to 2 min to pellet cells. Decant supernatant, and repeat up to two times if desired.
2. Resuspend cells in 250 µl of P1 (250 µl per 5 ml cells). No cell clumps should be visible in suspension.
3. Add 250 µl of P2 (250 µl per 5 ml cells) and gently invert 4-6 times to mix. DO NOT vortex! Solution should become viscous and slightly clear. DO NOT let lysis proceed for more than 5 minutes!
4. Add 350 µl of P3 (350 µl per 5 ml cells) and invert tube immediately, but gently, 4-6 times to mix. Solution should become cloudy.
5. Centrifuge at 16,000 X *g* for 10 minutes to pellet debris.
6. Transfer 700 µl of the supernatant to a tube containing 700 µl of isopropanol and cool on ice for 15 min.
7. Centrifuge at 16,000 X *g* for 30 minutes to pellet plasmid DNA.
8. Add 100—500 µl of chilled 70% ethanol, and centrifuge at 16,000 X *g* for 10 minutes.
9. Remove supernatant and dry pellet via air or speed-vac for 20 minutes.
10. Resuspend pellet to desired volume with TE, EB (elution buffer), or water.

Recipes:

P1: 6.1 g Tris, 3.7 g EDTA-2H2O pH 8.0 w/ HCl/1 liter, add 100 µg/ml RNAse A as needed, usually 10 mg RNase in 100 ml batches, store 4 degrees.

P2: 8.0 g NaOH in 900 ml H2O plus 100 ml of 10 % SDS/1 liter, store R.T.

P3: 294 g KAcetate in 500 ml H2O pH to 5.5 with Acetic Acid (~110 ml), bring to 1 liter, store R.T.

EB: 10 mM Tris, pH 8.5

P1, P2, and P3 recipes were taken from www.fhcrc.org