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RAPID ISOLATION OF PLASMID DNA – MIDI PREP

This protocol was obtained from Drs. Paul Kreig and Doug Melton, Harvard University.

1. Pick a single colony from a plate and inoculate 250 ml of LB broth containing 100 µg ml ampicillin. Grow the cells overnight in a shaker at 37ºC.

2. Centrifuge the cells at 5,000 g, 4ºC, 15 minutes.

3. Resuspend the cell pellet in 6ml of freshly prepared buffer:

	Stock	25 ml	50ml	100 ml
25 mM Tris-HCl, pH 8.0	1M	.625	1.25	2.5 ml
10 mM EDTA	.5 M	.5 ml	1	2 ml
15% sucrose		3.75	7.5	15 g
2 mg/ml lysozyme		50 mg	100 mg	200 mg

4. Add 12 ml freshly prepared:

	100 ml	stock
0.2 M NaOH	20 ml	1 M NaOH
1% SDS	10 ml	10% SDS
	70 ml H_2O	

   Carefully mix by inversion and incubate in ice water for 10 minutes. **DO NOT VORTEX.**

5. Add 7.5 ml of 3 M sodium acetate, pH 4.6. Carefully mix by inversion and incubate in ice water for 20 minutes. **DO NOT VORTEX.**

6. Centrifuge at 33,000 g for 15 minutes. Transfer supernatant to another tube (disposable—30 ml Sarstedt) and discard the precipitate. Use a pipette.

7. Add 50 µl of RNase A (1mg/ml) to the supernatant. Incubate for 20 minutes at 37ºC. 5K 5' discard pellet.

8. Extract twice with an equal volume of phenol:chloroform (1:1), add two volumes of ethanol and place on ice 30 minutes. Centrifuge at 9,500 g for 20 minutes. Dry pellet.

9. Dissolve DNA pellet in 1.6 ml of H_2O. Add 0.4 ml of 4 M NaCl and mix. Add 2 ml of 13% PEG (polyethylene glycol, MW 8,000) and mix. Incubate on ice water 60 minutes.

10. Centrifuge at 15,000 g for 10 minutes. Remove supernatant and wash the pellet with 5 ml 70% ethanol. Centrifuge at 15,000 g for 5 minutes.

11. Dry pellet under vacuum and dissolve DNA in an appropriate volume of TE (100 µl-500 µl). (TE = 10 mM Tris-HCl, pH 7.5-8.0, 0.1 mM EDTA.).
    Measure concentration by measuring absorbance at 260 and 280 nm.