LARGE SCALE PLASMID PREP FOR 100 ML SUPERBROTH CULTURES

1. Spin down the bacteria from a 100 ml culture in two 65 ml centrifuge bottles at 5000 rpm for 5 min. in the Hermle.
2. Discard the supernatant. Resuspend pellets in 4 ml each (8 ml total) by vortexing. Pool the solutions into one tube.
3. Add 16 ml of Solution II, mix gently by rotating the centrifuge tube slowly. The bacteria should lyse and the solution become translucent.
4. Add 12 ml of Solution III, shake hard to mix. A flocculent white precipitate should form. Spin 5000 rpm for 10 min. to pellet the precipitate.
5. Decant supernatant to fresh centrifuge bottle (65 ml) through gauze to trap any floating bits of precipitate. Add 0.6 volumes (25 ml) of isopropanol, mix and spin 8000 rpm for 10 minutes.
6. Discard supernatant, remove as much as possible by aspiration. Dissolve pellet in 3 ml TE and transfer to a 12 ml centrifuge tube (Nalgene PSF tubes or disposable 14ml Falcon tubes). Add 3 ml cold (-20 degrees C) 5M LiCl. Mix well and spin 5000 rpm for 10 minutes.
7. Transfer supernatant to fresh 12 ml centrifuge tube, add an equal volume of isopropanol (7 ml or fill tube), mix well and spin 5000 rpm for 10 minutes.
8. Aspirate the supernatant and leave to air dry.
9. Dissolve the pellet in 300 ul of TE + 20 ug/ml RNase A; transfer to a microfuge tube, incubate at room temperature for 15 minutes.
10. Add an equal volume of 26% PEG in 1.6M NaCl, put on ice for 5 min, mix well and spin 15 minutes at 4ø in microfuge to pellet DNA. Wash the pellet with 70% ethanol, speed vac dry before resuspending in TE. This will remove a lot of the excess PEG, making the phenol extractions much more bearable!
11. Dissolve pellet in 400 ul of TE. (Be sure that the pellet is completely dissolved before proceeding or your DNA yield may be considerably reduced.) Extract once with an equal volume of chloroform, twice with an equal volume of buffered phenol, and once more with an equal volume of chloroform. Add ammonium acetate to 0.5M plus two volumes of 100% ethanol. Mix by gently inverting the tube several times; you should be able to see the DNA coming out of solution. Let stand for 10 minutes at room temperature, spin 5 minutes in microfuge to pellet the plasmid DNA.

PHENOL IS AN ORGANIC ACID. CONTACT WITH SKIN WILL RESULT IN SEVERE BURNS. WEAR GLOVES, LAB COAT, EYE PROTECTION, AND WORK IN THE FUMEHOOD.

CHLOROFORM IS A POISON, WEAR GLOVES, LAB COAT AND EYE PROTECTION. READ MSDS SHEETS FOR THESE MATERIALS BEFORE HANDLING.

ETHANOL IS HIGHLY FLAMMABLE; EXERCISE CAUTION WHEN USING NEAR AN OPEN FLAME

12. Aspirate supernatant, wash pellet with 200 ul of 70% ethanol, vortex and spin to repellet DNA. Aspirate supernatant and dry pellet under vacuum.
13. Dissolve DNA pellet in 50 to 100 ul of TE, depending on the size of the pellet. Read OD260 of a 1:100 dilution to quantitate (1 OD unit = 50 ug/ml of dsDNA). Add TE to adjust the DNA concentration to 1 mg/ml. Store at -20 degrees C.

    Note: when using the spectrophotometer, be sure to allow the UV lamp to warm up for at least 15 min before quanitifying your DNA. Failure to do so will result in incorrect DNA concentration.
14. Use 0.5 - 1 ug of DNA for a restriction digest to map plasmid.

Solution I (Plasmid Prep)	**Glucose Buffer**	
	100mL	500mL
0.05M glucose (m.w. 180.16)	0.9g	4.504g
0.025M Tris (pH 8.0)	1.25mL	6.25mL of (2M stock) 0.01M EDTA
	2 mL	10mL of (0.5M stock)

pH 8.0 (should be) adjust if not

SOLUTION II (MAKE FRESH) (PLASMID PREP)

1% SDS	5mL of 20% SDS stock
0.2N NaOH	5mL of 4N NaOH stock
	make up to 100mL with dH2O

Note: put some water in container first as NaOH + SDS will ppt!

SOLUTION III (PLASMID PREP)

	1000mL	**500mL**	**250mL**

3.0M KoAC	294g	147g	73.5g
2.0M HoAC	115mL	57.5mL	28.75mL
Make up to required volume with dH2O			

pH 4.8

Keep at -20 C to deter bugs from growing in it!

PEG IN NaCl

- 13% PEG 8000 13 g
- 1.6 M NaCl 40 mL 4 M NaCl

Make up to 100 ml with d H2O