ALKALINE LYSIS DNA PREP (E. COLI)

(Howard Judelson)

Stock solutions:

BD1- 50 mM glucose, 25 mM Tris pH 8.0, 10 mM EDTA (added from 0.5 M pH 8.9 stock) BD2- 0.2 M NaOH, 1% SDS (made fresh from 2 M and 10% stocks)
BD3- Make 3M solution of KOAc, then add glacial acetic acid to pH 4.8

Procedure:	Miniscreen	Large-scale
1. Start with overnight culture - LB with 50 ug/ml amp - or TB with 50 ug/ml amp (TB gives more cells than LB)	1.5 ml	250 ml
2. Pellet celis	1 min, microfuge (room temp)	Skxg, 5 min (4C)
3. Resuspend pellet in BD1	100 µl	10 ml
4. Add BD2; gently mix by inversion 2X	200 µl	20 ml
5. Add BD3; gently mix by inversion 2-3X	150 µl	15 ml
6. Wait 2 min, then spin	5 min, microfuge (4C or room temp)	5kxg, 10 min (4C)
7. Save supernatant in new tube (filter large volumes through 2 layers of cheesecloth)		
8. Precipitate nucleic acids; add alcohol at room temp, wait 2 min, then spin 10 min at room temp.	2.5 vol EthOH	1X vol IPOH

9a. MINISCREENS ONLY: Add 40 p1 TE containing RNAse (10 µg/ml). Wait 10 minutes at room temp. Then add 2.5X vol ethanol, wait 2 min, then pellet 5 min in microfuge. Resuspend in 40 p1 iof TE.

9b. LARGE SCALE PREPS ONLY: Gently add 20 ml 70% ethanol to pellet, pour off, invert tubes to drain, then dry partially under vacuum. Purify further using either EtBr-CsC1 centrifugation, or PEG procedure.

CsC1 method Completely resuspend pellet in 9.5 ml TE. Add 1 g CsC1 for every ml of DNA solution; mix well. Add 0.8 ml of 10 mg/ml EtBr solution for every 10 ml of DNA-CsC1 solution. Spin at least 18 hr (usually 20-22) in 70.1Ti rotor, 60 K.

Suck off (lower) band using 18 guage needle (less than 3 ml). Extract with equal volume of 20X SSC-saturated isopropanol until solution is no longer pink (3-4 times; DNA stays in bottom phase).

Add 2 volumes of TE. Then add 2 volumes of room temperature ethanol. Wait 5 minutes (don't cool down or the salt will precipitate). Spin 10 x g, 10 minutes. Dry. Resuspend in 0.5 ml 0.3 M NaOAc pH 5.5 (helped usually by heating to 65C). Transfer to microfuge tube, add 2.5 volumes ethanol, spin 5 minutes. Rinse with 0.5 ml 70% ethanol, pour or aspirate off the wash, then dry. Resuspend in TE (0.2 ml) with assistance of 65C incubation.

PEG Method Resuspend DNA in 3 ml TE. Proceed as in the "new" Maniatis.