ALKALINE LYSIS MINI PREP PROCEDURE

(Procedure from Maniatis cloning manual)

1. Inoculate a 5 ml culture of LB/amp (50-100 µg/ml) with a single bacterial colony. Place tube in 37°C shaker overnight.

2. Fill an eppendorf tube with approximately 1.5 ml of the culture and microfuge 1 minute. Remove the supernatant and add another aliquot of culture to the tube. Again, microfuge 1 minute and remove the supernatant. Repeat until the entire 5 ml culture is spun down in one tube.

3. Resuspend pellet in 100 µl of solution I:

Solution I

25 mM Tris pH 8.0	100 ml:	1 M Tris	25.0 ml
10 mM EDTA pH 8.0		0.5 M EDTA	2.0 ml
50 mM Glucose		1 M Glucose	5.0 ml
		dH_2O	68.0 ml

4. Add 20 µl 10 mg/ml lysozyme solution:

   1 ml: 0.01 g lysozyme
   Fill to 1 ml with 0.250 M Tris pH 8.0

   Mix well and let the tube stand at room temp 2 minutes.

5. Add 200 µl of Solution II:

Solution II

1 ml:	50 µl	20% SDS
	20 µl	10 N NaOH
	930 µl	dH_2O

   Mix and ice 5 minutes.

6. Add 150 µl Solution III: (3 M K+, 5 M Acetate)

Solution III

100 ml:	5 M KOAc	60 ml
	glacial acetic acid	11.5 ml
	dH_2O	28.5 ml

5 M Potassium acetate

100 ml: 49.075 g potassium acetate
Fill to 100 ml with dH_2O

7. Vortex gently to form small white clumps. Ice 5 minutes.

8. Microfuge 5 minutes in cold microfuge.

9. Transfer supernatant to new tube. Add 400 µl of phenol:chloroform. Vortex and microfuge 2 minutes.

10. Transfer aqueous (upper) phase to a new tube. Add 1 ml room temp EtOH. Mix well and stand at room temp for 2 minutes.

11. Microfuge 5 minutes in cold microfuge. Pour off EtOH and let pellet dry completely.

12. Resuspend pellet in 50 µl of TE/ RNase (20 µg/ml):

    1 ml: 20 µl 10 mg/ml RNase
    980 µl TE

13. Place tube at 37°C for 30 minutes.

14. Restriction digest of mini-prep DNA:

    10 µl DNA (approximately 1 µg)
    2 µl enzyme buffer
    1 µl enzyme (approx. 10 units)

    Incubate for 2-12 hours at 37°C (for most enzymes--check appropriate temperature before incubation).

15. Prepare a 1% agarose gel with 0.2% EtBr

    100 ml: 1 g agarose
    100 ml 1xTBE
    --boil to dissolve agarose--
    20 µl 10 mg/ml EtBr

    Add 2 µl gel loading dye and microfuge the tube approx. 5 seconds. Load 10 µl sample.