PLASMID DNA MINIPREP

1. Use 1 ml of an overnight liquid culture or a colony scrape off a plate.

2. If using a liquid culture, pellet cells in a microfuge.

3. Add 100 µl **Solution I**. Stand at room temperature for 5'.

4. Add 200 µl **Solution II**, shake gently and place on ice for 5 - 10'.

5. Add 150 µl **Solution III**, vortex and place on ice for 10'.

6. Spin down and remove supernate to fresh tube.

7. Add 750 µl ethanol to the supernate, incubate at room temperature for 5' and then pellet.

8. Resuspend in TE, add 1 µl 10 $mgml^{-1}$ RNAase A, incubate for 10' at 37°C.

9. Phenol/chloroform extract, ethanol precipitate and resuspend at concentration required for use.

Solution I (100ml)

10 mM Tris pH 8	500 µl 2 M
1 mM EDTA	200 µl 0.5 M

Solution II (50 ml) (Make fresh each time)

0.2 M NaOH	10 ml 1 M
1% SDS	5 ml 10%

Solution III (100 ml)

3 M K Ac pH4.8	29.4 g

(dissolve in as little water as possible. Needs about 12 ml acetic acid to pH)