PCR AMPLIFICATION FROM PLASMIDS

This requires very little plasmid as template - very often at least a 1/100 dilution is necessary from miniprep. Alternatively, can use overnight culture directly or 50% glycerol stock. Pick primers that will enable verification of the insert, and if necessary, primers that will reveal the orientation of the insert within the plasmid.

1. Prepare PCR mix:

1 μl	plasmid DNA (<1ng), overnight culture or 50% glycerol stock
2 μl	10 x PCR buffer
1.2 μl	25 mM MgCl
0.4 μl	10 mM dNTP's
0.5 μl	Primer A (0.1 $\mu g \mu l^{-1}$)
0.5 μl	Primer B (0.1 $\mu g \mu l^{-1}$)
0.2μl	Taq DNA polymerase
14.2ml	H_2O

2. Set up cycles as follows:

	Cycles
94°C (2')	x 1
94°C (30 s), 58°C(30 s), 72°C (30 s)	x 24
72°C (5')	x 1
cooling to 4°C	

3. Run on agarose gel to visualise insert size.

Notes.

- The annealing temperature depends on the melting temperature (Tm) of the primers. Usually it is best to use an annealing temperature 1°C lower than the lowest Tm between the primers used.
- The time for extension should be altered depending on the size of insert to be amplified - the basic rule is 1kb = 1 minute.