Polymerase Chain Reaction

For normal PCR, we have found that the following conditions appear to work well.

Dilutions:

- Primers diluted to 25pmol/uL (1:60 of 300pmol ul-1 stock)
- Template plasmid DNA diluted to 100pg/uL (2 serial dilutions of 1/100 of stock)
- dNTPs prepared at 5 mM stock

Set up the following reactions:

	100uL Reaction
template	2
primer 1	1
primer 2	1
10X buffer	10
dNTPs	4
H2O	81
Vent	1

Sample PCR conditions

1. First, 1 cycle for 1 min at 94 degree C
2. Then, 25 cycles:

- 1 min in 94 degree C
- 1 min in melting temperature
- 1 min/kbase at 72 degree C
- 72degree 2 min
- hold 4 degree C

For DNA stretches of up to 1kbp, then 30" for each step in the cycle is usually enough.

For complete (3-4kbp) plasmids, increase the extension time to 1'30"

Calculating Primer Concentration

C= A x10(6) /E

Where:

- C= amount of DNA primer in pmoles
- A= absorbance at 260nm
- E= extinction coefficients of each nucleotide

(11.7x #G's) + (7.3x #C's) + (15.4x #A's) + (8.8x #T's)

Volume to resuspend primer in to get 25 pmol/uL = c/25