

## PCR (using Q5 Polymerase)

Reagent	Final Concentration	Final Volume
H <sub>2</sub> O	to 25 ul	to 25 ul
Template	1 pg-1 ng plasmid DNA (1-1000 ng high complexity gDNA)	1ng
5xQ5 buffer	1X (1:5)	5
Ethylene glycol	1.075 M (1:16.7)	1.5
25mM dNTP	0.2 mM (1:125)	0.2
10uM F primer	0.5 uM (1:20)	1.25
10uM R primer	0.5 uM (1:20)	1.25
Q5 Polymerase	0.02 U/ul (1:100)	0.25

### Cycling Conditions

Cycle step	Temp.	Time	Cycles
Initial denaturation	98°C	30 s	1
Denaturation	98°C	5-10 s	} 35
Annealing	T <sub>m</sub> °C	10-30 s	
Extension	72°C	20-30 s /1 kb (40-50 s/1kb for long products)	
Final extension	72°C	2 min	1
	4°C	hold	