

## PCR (using Phusion Polymerase)

Reagent	Final Concentration
H <sub>2</sub> O	to 25 ul
Template	1 pg-10 ng plasmid DNA or 50-250 ng high complexity gDNA
5xHF buffer	1:5
Ethylene glycol	1:16.7 (1.075 M)
25mM dNTP	1:125 (0.2mM)
10uM F primer	1:20 (0.5uM)
10uM R primer	1:20 (0.5uM)
Phusion Polymerase	1:100 (0.02U/ul)

### 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	15-30 s /1 kb	
Final extension	72°C	5-10 min	1
	4°C	hold	