Polymerase Chain Reaction(PCR)

1. Combine the following on ice:

Reagent	1x(volume in ul)	[final]
10x Thermo Pol Buffer	5	1x
10mM dNTPs	1	0.2mM
50mM $MgCl_2$	2	2mM
10 uM F Primer	2	0.4mM
10 uM R Primer	2	0.4uM
H_2O	Vol req for 50 ul total	-
DNA	Vol req for 30 ng <= mass <= 500ng	-
Taq polymerase	0.5	2 U
Total	**50 ul**	-

2. Thermocycle

Segment	Temperature(C)	Length
1. Initial Denaturation	94	3 minutes
2. Denaturation	94	30 seconds
3. Annealing	~55	30 seconds
4. Extension	68	1 min/kb amplified
5. Final Extension	68	5 minutes
6. Final Hold	12	Forever

3. Repeat 25-35 cycles of segments 2-4