PCR

1. Reaction recipe

Mix the following components (**make sure the stocks you use are at the same concentrations as those in table**):

1	38 ul of deionized sterile water	Used to bring up to 50 uL
2	5 ul of Thermo Pol Buffer 10 X New England Biolabs	
3	1 ul of dNTP mix (25 mM of each)	
4	1 ul of Upstream primer	0.1 mM stock, 100 pmol final
5	1 ul of Downstream primer	0.1 mM stock, 100 pmol final
6	1 ul of DNA template (15 ng/uL)	15 ng final recommended
7	1 ul of Taq DNA polymerase (New England Biolabs, 5units/ul)	

2. Program setting:

Step	Temp. C	Time (min)	Description
1	95	5	Initial denaturation
2	95	1	Denature
3	Tm*	1	Anneal
4	72	1	Elongate (generally, 1kb/min)

5	Goto step 2	29 cycles	Cycle (25-35 only, otherwise enzyme decay causes artifacts)
6	72	10	Final elongation
7	4	hold	
8	End		

***the calculation of Tm**

Tm=4*(G + C) + 2*(A + T) - 5

(usually between 50-65 C)