

Polymerase Chain Reaction

Overview :

This protocol is generously provided by the lab.

Component	20 μ L reaction	50 μ L reaction	Final cocentration
ddH ₂ O	To 20 μ L	To 50 μ L	
5X Phusion GC Buffer	4 μ L	10 μ L	1X
10 mM dNTPs	0,4 μ L	1 μ L	200 μ M
10 μ M Forward Primer	1 μ L	2,5 μ L	0,5 μ M
10 μ M Reverse Primer	1 μ L	2,5 μ L	0,5 μ M
PhUision DNAPol	0,2 μ L	0,5 μ L	1.0 units/50 μ L PCR
Template DNA	<ul style="list-style-type: none"> - Use 1pg–1ng of plasmid templates - Use 1ng–1μg of genomic templates 		

Program :

Step	Temperature	Time	Cycles
Initial denaturation	94°C	5 min	x 1
Denaturation	94°C	30 sec	x 35
Annealing	X°C	30 sec	
Elongation	72°C	Y:YY	
Final extention	72°C	10 min	x 1
Final hold	15°C	∞	x 1

X°C – Annealing temperature - 5°C

Y:YY – Extension time (1 min/kb)