

# Protocol for PCR with EasyPfu DNA Polymerase

## Preparation for Reaction Mixture

Reagent	Final concentration	Volume
10X <i>Taq</i> buffer	1X	2 $\mu$ l
2.5 mM dNTP mix	0.25 mM	2 $\mu$ l
Forward Primer	0.2-0.4 $\mu$ M	1 $\mu$ l
Reverse Primer	0.2-0.4 $\mu$ M	1 $\mu$ l
<i>EasyPfu</i> DNA Polymerase	25 units	1 $\mu$ l
Template DNA	As required	<0.5 $\mu$ g
ddH <sub>2</sub> O to final volume		

## Cycling Conditions

Step	Temperature / $^{\circ}$ C	Time/min
Initial Denaturation	94	5
Denaturation	94	0.5
Primer Annealing	As required	As required
Extending	72	As required(0.5kb/min)
Final Extending	72	10
Number of Cycles	32-35	
Cooling Down	4	10