PCR protocol

1.Pipet the following into a microfuge tube：

reagent	50μ l reaction system	final concentration
2×EsTaq MasterMix	25μ l	1×
Forward Primer，10μ M	2μ l	0.4μ M
Reverse Primer,10μ M	2μ l	0.4μ M
Template DNA	<1μ g	<1μ g/reaction
RNase-Free Water	up to 50μ l	

The table below provides standard reaction conditions for PCR.

Mg2+	**KCl**	**dNTPs**	**Primers**	**DNA polymerase**	**Template DNA**
1.5 mM	50 mM	200 μM	1 μM	1-5 units	1 pg to 1 μg

2. cycling condition：

procedure	temperature	time
initial denaturation	94℃	2min
denaturation	94℃	30s
primer annealling	55-65℃	30s
extending	72℃	1min/Kb
final extending	72℃	2min
cooling down	4℃	10min