Pfu DNA Polymerase

Description:

Pfu DNA Polymerase is a thermostable enzyme with a molecular weight of 90 kDa. It catalyzes the polymerization of nucleotides into duplex DNA in the 5'_3' direction, results in blunt-ended PCR products **without 3'-dA overhangs.**

Pfu DNA Polymerase exhibits 3'_5' exonuclease (proofreading) activity that enables the polymerase to correct the mis-incorporation of nucleotides, and lacks 5'_3' exonuclease activity. It is suitable for PCR and primer extension reaction that requires high fidelity when the PCR fragment is relatively **shorter than 3 kbp.** The enzyme exhibits 3´>5´ proofreading activity, resulting in over 10-fold higher PCR fidelity than possible with Taq DNA Polymerases.

Contents:

Components	500U
Pfu DNA poly. 5 U/µl	500U
$MgCl_2$ Solution 25 mM	1 mL
5X Pfu Buffer $MgCl_2$ free	1 mL

General Reaction Protocol:

1. Thaw 5X reaction buffer, dNTP mixture.
2. Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes or plates.
3. Add templates DNA to the individual PCR tubes or wells containing the master mix.

Component	Volume	Final conc.
5X Reaction Buffer	4 µL	1X
$MgCl_2$ Solution 25 mM	1.6 µL	2 mM
40 mM dNTPs Mix (10 mM each)	0.4 µL	0.2 mM
Forward Primer (10 pmol/ µL)	1 µL	0.5 pmoles/µL
Reverse Primer (10 pmol/ µL)	1 µL	0.5 pmoles/µL
Template DNA	Variable	10 fg to 1 µg
Pfu DNA poly. (5 units/µl)	0.25 µL	
PCR grade water	Up to 20µL final volume	-
Total Volume	**20 µL**	

4. Program the PCR machine according to the program outlined.

Cycle	Time	Temp °C
1	4 min	95
30 - 35	30 sec	94
	30 sec	57
	60 sec	72
1	5 min	72

Notes:

* Longer extension time makes nonspecific bands.
* Extension rate for this enzyme is near 500bp/min.

Agarose Gel Electrophoresis:

Run the total 5-7 µL of PCR products alongside 3 µL DNA marker on a 2% agarose gel containing Green Viewer Dye DNA safe stain.