2X Pfu mix

Cat No	Pack size	conc
PFM0500	5 ml	2x
PFM2500	5x5ml	2x

Description :***Pfu*** DNA polymerase is a thermostable enzyme isolated from *Pyrococcus furiousus.* The enzyme replicates DNA at 75˚C, catalyzing the polymerization of nucleotides into duplex DNA in the 5'-3' direction. ***Pfu*** DNA polymerase possesses 3'-5' exonuclease (proofreading) activity. Base misinsertions that may occur during polymerization are rapidly excised by the proofreading activity of the polymerase. ***Pfu*** DNA polymerase is recommended for use in PCR and primer extension reactions that require high-fidelity synthesis. ***Pfu*** DNA polymerase-generated PCR fragments are blunt-ended.

2X Pfu mix is optimized mixture contain of Pfu polymerase, reaction buffer, dNTP And enhancer as 2-fold concentration. 2x Pfu mix is designed to allow the user for quick ,easy preparation of reaction mixture. The 2x Pfu mix can be amplification PCR products up to 3-5 kb.

storage conditions: long time at -20˚C
short time at 4 ˚C

Template
2 x Pfu mix is suitable for amplifying targets up to 3 kb from the following templates:

Genomic DNA: 10–200 ng
Plasmid DNA : 1–5 ng
cDNA : ~100 ng starting total RNA

Primers
Use 0.3 μM per primer as a general starting point. For larger amounts of template (e.g., 200 ng genomic DNA), increasing the concentration up to 0.5 μM per primer may improve yield.

Annealing Temperature
The annealing temperature is slightly higher than with typical PCR. The optimal annealing temperature should be ~2˚C lower than the Tm of the primers used. A range of 58–68˚C is recommended.

Extension Time: As little as 60 seconds per kb is suitable for most targets. Use up to 120 seconds per kb for maximum yield.

PCR Protocol:
1.Thaw the 2x Pfu mix at room temperature. Vortex the 2x Pfu mix and then spin it briefly in a micro centrifuge to collect the material in the bottom of the tube.
2. Prepare one of the following reaction mixes on ice:

Component	Volume
2x Pfu mix	12.5 ul
Primer1 (20 pmol)	1-2 ul
Primer2 (20 pmol)	1-2 ul
template	1-10 ul
ddH_2O	Up to 25 ul
Total	25 ul

3. If necessary you can scale up your volume

1. Program the thermal cycler as follows:

Step	Temperature	Time	Cycle
Initial denaturation	94-96°C	0.5-2mins	1
Denaturation	94-96°C	0.2-2mins	15-30
Annealing	50-68	0.2-2mins	
Extension	68-75	2min/1kb	
Final extension	68-75	1-10mins	1

Step
After cycling, maintain the reaction at 4˚C. Samples can be stored at –20˚C until use.

Analyze products using standard agarose gel electrophoresis.

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