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I. Introduction.

The *AccuPower® Pfu* PCR PreMix contains Pfu DNA polymerase, dNTPs, and reaction buffer in a premixed format that is freeze-dried into individual tubes. Patented chemical stabilizer maintains the activity of the premixture for over a month even when stored at room temperature(25°C) and over 2 years in the freezer.

II. Advantages.

- High fidelity.** *AccuPower® Pfu* PCR PreMix has a high accuracy activity.(error rate=1.9x10⁻⁶)
- High purity.** *AccuPower® Pfu* PCR PreMix a high purity using polymerase, which passed E.coli genomic DNA contamination test. (template free PCR achievement done by E.coli genome detection primer.)
- Stability.** *AccuPower® Pfu* PCR PreMix contains a stabilizer (Patented in US and Korea), which can maintain the stability of the polymerase up to 2 years at -20°C, for over a month at room temperature, and for 20 minutes at 94°C. Also, pre-denaturation during PCR has no effects on the activity of the DNA polymerase.
- Reproducibility.** *AccuPower® Pfu* PCR PreMix has been batch produced using strict Q.C. procedures. Errors that often occur during mass production have been completely eliminated and our current batch processing system allows the most accurate and reproducible end-products.
- Simplicity.** The fewer manual steps allow reduction in potential errors. Each tube contains tracking dye and precipitant for agarose gel electrophoresis, eliminating the needs for a separate loading buffer.

III. Application.

AccuPower® Pfu PCR PreMix is recommended for use in Polymerase Chain Reaction(PCR), primer extension reactions, Gene Synthesis, site-directed mutagenesis and other applications that demands high fidelity.

IV. Contents.

Reaction size component	20 ul reaction	50 ul reaction
Pfu DNA polymerase	20 ul reaction	2.5 U
Each:dNTP(dATP, dCTP, dGTP, dTTP)	250 uM	250 uM
10X reaction buffer	2 ul	5 ul
Stabilizer and tracking dye		

V. Experimental Protocol.

- Add template DNA and primers to *AccuPower® Pfu* PCR PreMix tube.
- Concentration of template DNA and primer.

Reaction size	20 ul reaction	50 ul reaction
Template DNA	1 ~ 100 ng	1 ~100 ng
Primer	5 ~20 pmole	10 ~ 50 pmole
- Add distilled water to *AccuPower® Pfu* PCR PreMix tubes to a total volume of 20 ul or 50 ul.
- Dissolve the lyophilized blue pellet by vortexing, and briefly spin down.
- Perform PCR of samples.
- Load samples on agarose gel without adding a loading-dye mixture, and perform electrophoresis.

VI. PCR Cycling condition.

- Typical PCR amplification.

Step	Temperature	Time	Number of cycles
Initial Denaturation	94°C	2~5 min	1 cycle
Denaturation	94°C	0.5~1 min	25~35 cycle
Annealing	42~65°C	0.5~1 min	
Extension	72°C	1~2 min	
Final Extension	72°C	5min	1 cycle

- The extension time of Pfu PCR PreMix is needed approximately 1~2 minutes for every 1kb to be amplified.
- In case of long PCR, it is recommended to use two-step PCR methods of denaturation step(94°C for 30 sec) and annealing/extension step (68°C for 1~2 min / 1 kb).

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VII. Quality test.

1. Template Range & Sensitivity test.

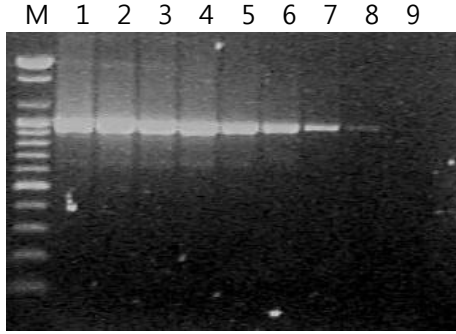


Fig 1. Test of working range & sensitivity of *Pfu* PCR PreMix for Lambda DNA template.

M : 100bp DNA Ladder Line 1 : 100ng
Line 2 : 10ng Line 3 : 1ng
Line 4 : 100pg Line 5 : 10pg
Line 6 : 1pg Line 7 : 100fg
Line 8 : 10fg Line 9 : Template negative

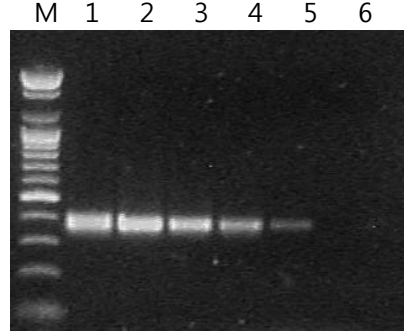


Fig 2. Test of working range & sensitivity of *Pfu* PCR PreMix for Human DNA template.

M : 100bp DNA Ladder Line 1 : 100ng
Line 2 : 10ng Line 3 : 1ng
Line 4 : 100pg Line 5 : 10pg
Line 6 : Template negative

2. Long kb test.

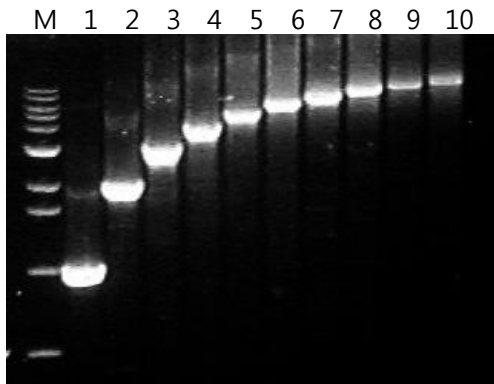


Fig 3. Amplification of Lambda DNA of 1 kb to 10kb with *Pfu* PCR PreMix

M : 1kb DNA Ladder
Line 1 : 1kb fragment Line 2 : 2kb fragment
Line 3 : 3kb fragment Line 4 : 4kb fragment
Line 5 : 5kb fragment Line 6 : 6kb fragment
Line 7 : 7kb fragment Line 8 : 8kb fragment
Line 9 : 9kb fragment Line 10 : 10kb fragment

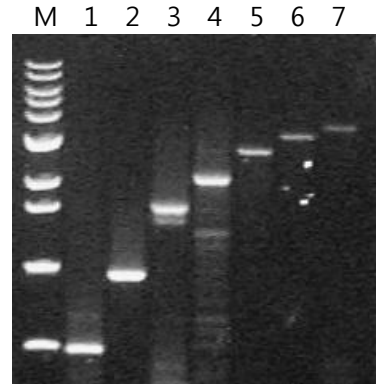


Fig 4. Amplification of Human DNA of 500bp to 3.5kb with *Pfu* PCR PreMix.

M : 1kb DNA Ladder
Line 1 : 0.5kb fragment Line 2 : 1kb fragment
Line 3 : 1.5kb fragment Line 4 : 2kb fragment
Line 5 : 2.5kb fragment Line 6 : 3kb fragment
Line 7 : 3.5kb fragment

VIII. Ordering Information

Tube	20ul	K-2022	0.2 ml thin-wall 8-strip tubes with attached cap / 96 tubes
		K-2024	0.2 ml thin-wall 8-strip tubes with attached cap / 480 tubes
	50ul	K-2023	0.2 ml thin-wall 8-strip tubes with attached cap / 96 tubes
		K-2025	0.2 ml thin-wall 8-strip tubes with attached cap / 480 tubes
		K-2027	0.5 ml thin-wall tubes with attached cap / 100 tubes
MasterMix	—	K-2026	1 ml of 2 X MasterMix solution