Fast-Pfu DNA Polymerase

9K-001-0022 (250U) / 9K-001-0023 (500U)

store at -20℃

BIO BASIC INC.

. Fast-Pfu DNA Polymerase (2.5U/μl)
. 10X Fast-Pfu Buffer
. 10 mM dNTP Mix (each 10mM)
. 5X Band Sharpener

Protocol

Fast-Pfu DNA polymerase from Pyrococcus furiosus is a product for long-range and high fidelity PCR as an upgraded version of Pfu DNA polymerase. Processivity and Fidelity of Fast-Pfu are respectively 4 times faster and 3 times higher than regular Pfu DNA Polymerase.

Temperature cycle

Three step PCR cycle		Two step PCR cycle	
95℃ 2 min	x1	95℃ 2 min	x1
95℃ 20 sec	x25~30	95℃ 20 sec	x25~30
AT* 40 sec		68℃ 30 sec/kb	
72℃ 30 sec/kb		68℃ 5 min	x1
72℃ 5 min	x1		

AT*: Annealing Temperature

Adjust to the lower Tm between two primers

AT = Tm-(4~6℃) Tm = 2℃x(A+T)+4℃x(G+C)

Reaction mixture	Volume (for 50μl reaction)	Volume (for 25μl reaction)
Fast-Pfu (2.5U/μl)	0.5 μl	0.25 μl
10X Fast-Pfu Buffer	5.0 μl	2.5 μl
10mM dNTP mix	1.0 μl	0.5 μl
Primer 1 (10pmole/ μl)	2.0 μl	1.0 μl
Primer 2 (10pmole/ μl)	2.0 μl	1.0 μl
Template	X μl	X μl
5X Band Sharpener	0~20μl	0~10μl
D.W. up to	50.0 μl	50.0 μl

*It might be required to optimize PCR conditions depending on target size, Tm of primers, template nature, extension time, annealing temperature, enzyme quantity and cycle(s) numbers.

Technical Information

A. Template (temperature cycle)

. Animal genomic DNA
  50-200 ng (25-35 cycles)
  10-50 ng (30-40 cycles)
. Bacterial genomic DNA
  10-50 ng (20-25 cycles)
  1-5 ng (30-35 cycles)
. Plasmid and lamda DNA
  1-5 ng (20-30 cycles)

B. 5X Band Sharpener *

Band Sharpener is not necessary for regular PCR conditions. In case fragment include high GC region or hard to amplify complex secondary structures, please add Band Sharpener to a final concentration of 0.5x~2x (5-20μl for 50μl reaction) to reaction mixture, as optimization is required (see protocol below).

C. Primer design

- Primer can be designed using a primer design software or manually.
- Avoid repeated sequence at 3' end.
- In case 3'-end is G+C rich, the end have to be A or T.
- In case 3' end is A+T rich, the end have to be G or C.
- It is recommended that Tm of the designed primers is >64℃ and AT >58℃.

D. Extension time

- In general, extension should be performed at 30 sec/kb
- If the amplification is more than 5 kb, extension temperature should be assigned 68℃.

*** Notice
Do not use > 2.5 unit Fast-Pfu DNA Polymerase per reaction
Reduce reaction volume if smears are obtained
Do not use >30sec/Kb (ex: Plasmid, ambda or BAC DNA)

Band Sharpener Optimization protocol

Reaction mixture (Conc. of Band Sharpener)	Mix I (0.0 x)	Mix II (0.5x)	Mix III (1.0x)	Mix IV (1.5x)	Mix V (2.0 x)
10X Fast-Pfu buffer	5.0 μl	5.0 μl	5.0 μl	5.0 μl	5.0 μl
10mM dNTP mix	1.0 μl	1.0 μl	1.0 μl	1.0 μl	1.0 μl
Primer 1 (10pmole/ μl)	3.0 μl	3.0 μl	3.0 μl	3.0 μl	3.0 μl
Primer 2 (10pmole/ μl)	3.0 μl	3.0 μl	3.0 μl	3.0 μl	3.0 μl
Template	X μl	X μl	X μl	X μl	X μl
Band Sharpener	0μl	5.0μl	10.0μl	15.0μl	20.0μl
Fast-Pfu (2.5U/μl)	0.50 μl	0.50 μl	0.50 μl	0.50 μl	0.50 μl
Add D.W. to	50.0 μl	50.0 μl	50.0 μl	50.0 μl	50.0 μl

*** Note:** Band Sharpener included in the Kit is provided for PCR optimization purpose only. If your optimized PCR conditions include Band Sharpener, please order cat#: 9K-001-007.