Pfu DNA Polymerase

9K-001-0009 (250U) / 9K-001-0010 (500U)

store at -20℃

. Pfu DNA Polymerase (2.5U/µl)
. 10x Pfu reaction Buffer
. 10 mM dNTP Mix (each 10mM)
. Band Sharpener

Protocol

The recommended protocol for reaction mixture and temperature cycle in PCR is as below. You may modify the amount of template and Pfu DNA polymerase, extension time, annealing temperature, and cycle number according to the product size, primer Tm value, and template type.

Reaction mixture (for 50µl reaction)		**Temperature cycle**		
10X Pfu Taq buffer	5.0 µl	95℃ 2 min		x1
10mM dNTP mix	1.0 µl	95℃ 20 sec	┐	
primer 1(10pmole/µl)	3.0 µl	AT* 40 sec	┘	x10~40
primer 2(10pmole/µl)	3.0 µl	72℃ 1 min/kb		
template	X µl	72℃ 5 min		x1
Band Sharpener	0~20 µl			
Pfu (2.5U/µl)	0.50 µl			
D.W. to	50.0 µl			

AT*: Annealing Temperature

Adjust to the lower Tm between two primers

AT = Tm-(4~6℃) Tm = 2℃x(A+T)+4℃x(G+C)

*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. Pfu DNA Polymerase

- For amplification >3 kb from animal genomic DNA, the amount of Pfu should be increased to 2 or 2.5U.
- LR DNA Polymerase is recommended for long-range PCR reaction.

C. 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).

D. 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℃.

E. Extension time

- Generally, extension should be performed at 2 min/kb.
- For amplification >5 kb, extension temperature should be assigned to 68℃.

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 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	0 µl	5.0 µl	10.0 µl	15.0 µl	20.0 µl
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.