



Fast-Taq DNA Polymerase

9K-001-0003 (500U) / 9K-001-0004 (2500U)

store at -20°C

- . Fast-Taq DNA Polymerase (5U/μL)
- . 10X Fast-Taq 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 Fast-Taq DNA polymerase, extension time, annealing temperature, and cycle number according to the product size, primer T_m value, and template type.

Reaction mixture (for 50 μl reaction)		Temperature cycle	
10X Fast-Taq buffer	5.0 μl	95°C 5 min	x1
10mM dNTP mix	1.0 μl	95°C 20 sec	} x10~40
primer 1(10pmole/μl)	2.0 μl	AT* 40 sec	
primer 2(10pmole/μl)	2.0 μl	72°C 1 min/kb	
template	X μl	72°C 5 min	x1
Band Sharpener	0~20 μl		
Fast-Taq (5U/μl)	0.25 μl		
D.W. to	50.0 μl		

AT*: Annealing Temperature

Adjust to the lower T_m between two primers

$$AT = T_m - (4 \sim 6^\circ\text{C}) \quad T_m = 2^\circ\text{C} \times (A+T) + 4^\circ\text{C} \times (G+C)$$

*It might be required to optimize PCR conditions depending on target size, T_m 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. Fast-Taq DNA Polymerase

Please use 1.25~5 unit in case target size is >3kb.
In case the target is >3kb, we recommend LR DNA Polymerase.

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 2.5x~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 T_m of the designed primers is >64°C and AT >58°C.

E. Extension time

- Normally, extension time is 1 min/kb. In case target is >3kb, the extension time should be increased to 1.0-2.0 min/kb.

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-Taq 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)	2.0 μl	2.0 μl	2.0 μl	2.0 μl	2.0 μl
Primer 2 (10pmole/μl)	2.0 μl	2.0 μl	2.0 μl	2.0 μl	2.0 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
Fast-Taq (5U/μl)	0.25 μl	0.25 μl	0.25 μl	0.25 μl	0.25 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.