

Customer feedback on products

Product Name : KAPA HiFi HotStart ReadyMix (KK 2601)
Manufacturer : KAPA BIOSYSTEMS
Application : Target gene cloning from a cDNA sample derived from human smooth muscle cells

The following data were provided by the courtesy of Dr. Takuya Azechi of the Department of Clinical Chemistry, Hoshi University, Japan.

Experimental conditions

1) PCR reaction mixture composition used

A : Manufacturer T's high-fidelity enzyme

Enzyme	1 μ l
dNTPs	4 μ l
5xBuffer	10 μ l
Primer F (10 μ M)	1 μ l
Primer R (10 μ M)	1 μ l
Nuclease free water (N.W.)	28 μ l
Template	5 μ l
Total	50 μl

B : KAPA HiFi

2xKAPA HiFi HotStart Ready Mix	25 μ l
Primer F (10 μ M)	1.5 μ l
Primer R (10 μ M)	1.5 μ l
N.W.	17 μ l
Template	5 μ l
* 7.5 μ L of template was used in the second experiment	
Total	50 μl

2) Thermal cycler, PCR program, amplified size

Thermal cycler : GeneAmp PCR System 9700

Amplified size : 3892 bp

PCR program

A : Manufacturer T's high-fidelity enzyme

98°C, 1min
 98°C, 10sec
 60°C, 15sec
 68°C, 4min
 68°C, 5min

x35 cycle

B : KAPA HiFi (1st experiment)

95°C, 5min
 98°C, 20sec
 60°C, 15sec
 72°C, 2.5min
 72°C, 5min

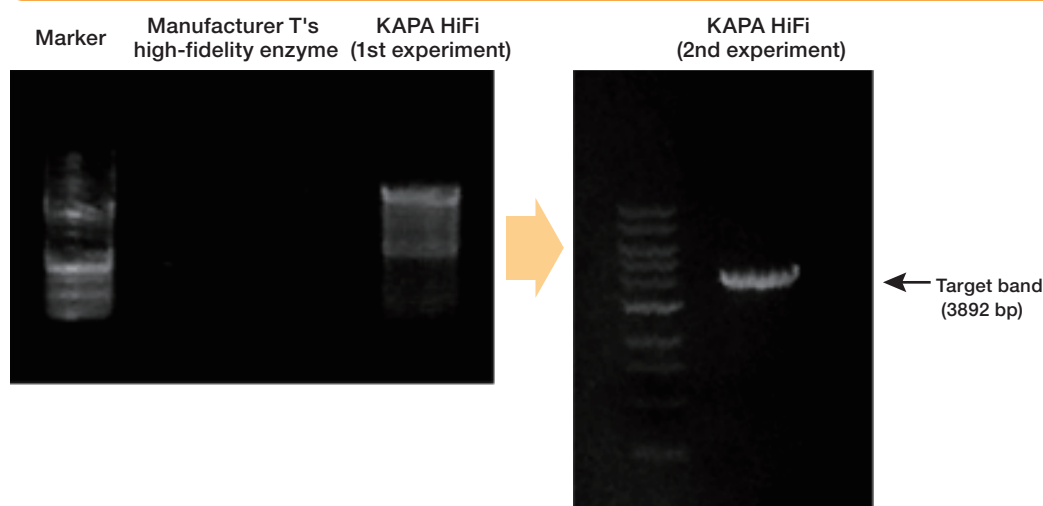
x35 cycle

C : KAPA HiFi (2nd experiment)

95°C, 5min
 98°C, 20sec
 63°C, 15sec
 72°C, 2.5min
 72°C, 5min



Results



PCR using Manufacturer T's high-fidelity enzyme produced no band. We also tried different annealing temperatures, but no amplification could be detected (data not shown).
 PCR using KAPA HiFi produced a ladder of bands, so we raised the annealing temperature from 60°C to 63°C in the second PCR reaction and could clearly detect the target band.

<Customer's comments>

We have mainly used Manufacturer T's high-fidelity enzyme in our past cloning experiments. This time, we used KAPA HiFi, which enabled faster amplification compared to the Manufacturer T's high-fidelity enzyme and also enabled cloning of a long fragment (3.9 kb). High fidelity of KAPA HiFi could be confirmed by sequence analysis, so the enzyme should be suited for cloning applications.