

Customer feedback on products

Product Name	: KAPA HiFi HotStart ReadyMix with Dye (KK 2601)
Manufacturer	: KAPA BIOSYSTEMS
Application	: Cloning of target gene from cDNA samples derived from human cancer cells

The following data were provided by the courtesy of Dr. Masashi Okada of Department of Molecular Cancer Science of Yamagata University Faculty of Medicine, Japan.

Experimental conditions

cDNA prepared from total RNA derived from human cancer cells was used as a template. The target gene was amplified using KAPA HiFi Hot Start Ready Mix and subsequently cloned.

1) PCR Reaction mixture composition

2.5 pmol/μl primer Fw	:	6 μl
2.5 pmol/μl primer Rv	:	6 μl
HiFi Hot Start Ready Mix	:	25 μl
DMSO	:	2.5 μl
cDNA mix	:	2 μl
DDW	:	8.5 μl

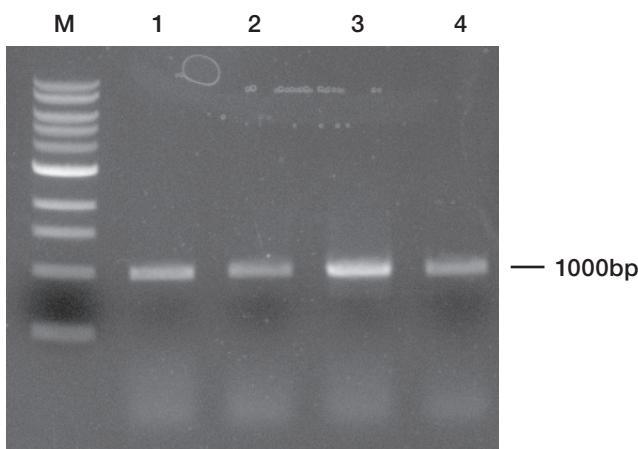
total volume of 50 μl

Thermal cycle program

95°C	3 min x1
98°C	20 sec
60°C	15 sec
72°C	80 sec
72°C	5 min x1
4°C	o/n

Thermal cycler : Takara Thermal cycler Dice

Results



Amplification of the target genes was confirmed in all samples (lanes 1 through 4)

<Customer's comments>

The overall GC content of the amplified gene (1,000 bp) is about 65%, but there is a GC-rich region of about 250 bp with a GC content of approximately 90% (87%).

This GC-rich region had been an obstacle in amplifying this gene with other enzymes, and the region was sometimes left unsequenced or prone to sequence errors.

The KAPA HiFi HotStart ReadyMix could amplify the gene in about 30 cycles to an extent comparable to that achieved using other enzymes.

We used the amplified product for cloning, and no error was found in the DNA sequence.

The readymix is reliable and easy-to-use.

This experiment could be easily conducted by a student who had just joined our lab.



Nippon Genetics Co.,Ltd <http://www.n-genetics.com>