

Customer feedback on products

Product Name : KAPA HiFi Hotstart Uracil + ReadyMix (KK2801)
Manufacturer : KAPA BIOSYSTEMS
Application : Stable amplification by PCR of bisulfate-treated DNA

The following data were provided by the courtesy of Associate Professor Akira Hattori, Department of System Chemotherapy and Molecular Sciences, Graduate School of Pharmaceutical Sciences, Kyoto University, Japan.

Experimental conditions

It has been difficult to stably amplify bisulfate-treated DNA derived from the following cells using the conventional PCR amplification methods. This experiment was conducted to determine whether bisulfate-treated DNA can be stably amplified using KAPA HiFi Hotstart Uracil + ReadyMix.

●Products compared in the experiment

- (1) Product of company A
(for PCR amplification of bisulfate-treated DNA)
- (2) KAPA HiFi Hotstart Uracil + ReadyMix

●Sample:

Genome DNA which was extracted from the following cell lines and then treated with bisulfate
 Human Diploid Fibroblasts
 Human Retinal Pigment Epithelial Cells

●Amplified DNA size:

651bp
 Target Gene :
 Human C7orf24/γ-glutamyl-cyclotransferase gene

●PCR amplification conditions (reaction volume: 20 μL):

Product of company A

PCR reaction buffer	x1	Denature	95°C	5min
MgCl ₂	2.5mM	PCR cycle	98°C	10sec
dNTP	300μM		55°C*	30sec
Primer (sense)	200nM		72°C	1min
Primer (antisense)	200nM		72°C	2min
Template	~30ng			
Enzyme of company T	25U/mL			

40 cycles

*The range of reaction temperature applied was 55-60°C.
(Recommended temperature: 55°C)

KAPA HiFi Uracil+

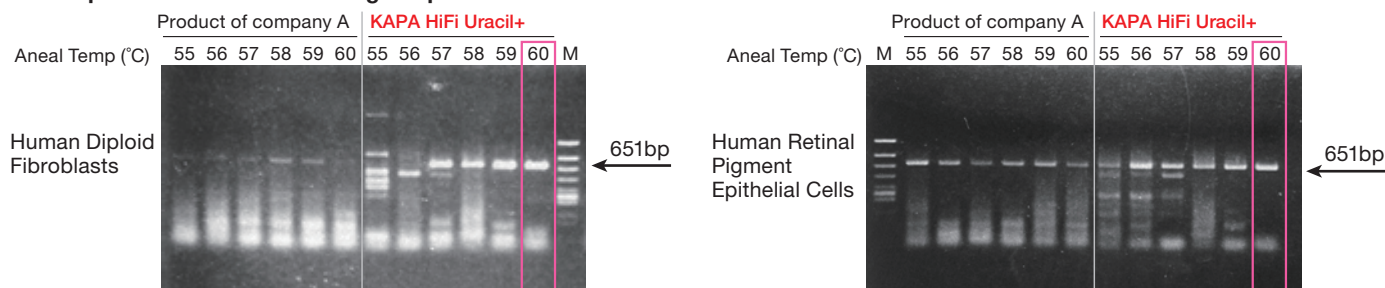
HiFi Uracil+ Mix	x1	Denature	95°C	5min
Primer (sense)	300nM	PCR cycle	98°C	20sec
Primer (antisense)	300nM		60°C*	15sec
Template	~30ng		72°C	1min
			72°C	2min

40 cycles

*The range of reaction temperature applied was 55-60°C.
(Recommended temperature: 60°C)

Results

1. Comparison between annealing temperature conditions

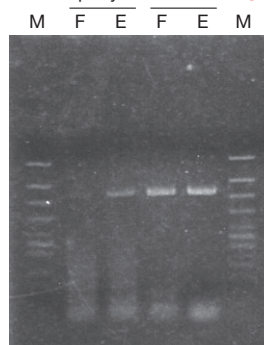


Different annealing temperature conditions were compared.

As a result, when using KAPA HiFi Uracil +, best results were obtained at 60°C as specified in the recommended protocol.

2. Confirmation of the reproducibility at the recommended annealing temperature condition

Product of company A KAPA HiFi Uracil+



M : DNA size Marker (φx174/Hinc II digested)

F : Human Diploid Fibroblasts

E : Human Retinal Pigment Epithelial Cells

PCR amplification reactions using the two products at recommended temperature conditions were compared. As a result, it was confirmed that KAPA HiFi Uracil + has higher specificity and produces higher yields.

Product of company A recommended annealing temperature : 55°C

KAPA HiFi Uracil + recommended annealing temperature : 60°C

<Customer's comments>

When reactions were conducted in accordance with the conditions specified in the protocol, intended bands could be stably amplified with high yields from bisulfate-treated DNA, the amplification of which has been difficult using the conventional methods.