

## 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 <sub>2</sub>	2.5mM	PCR cycle	98°C	10sec	
dNTP	300μM		55°C*	30sec	40 cycles
Primer (sense)	200nM		72°C	1min	
Primer (antisense)	200nM		72°C	2min	
Template	~30ng				
Enzyme of company T	25U/mL				

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

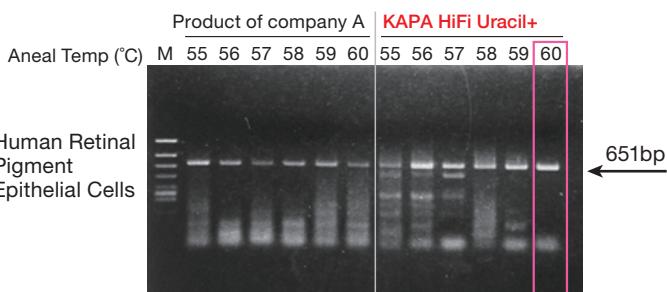
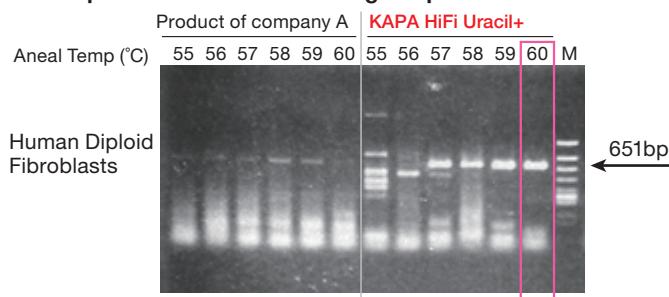
##### KAPA HiFi Uracil+

HiFi Uracil+ Mix	x1	Denature	95°C	5min	
Primer (sense)	300nM	PCR cycle	98°C	20sec	
Primer (antisense)	300nM		60°C*	15sec	40 cycles
Template	~30ng		72°C	1min	

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

### Results

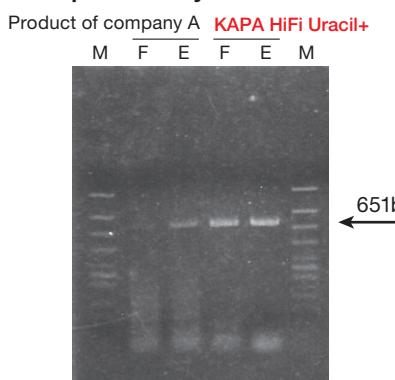
#### 1. Comparison between annealing temperature conditions



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



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.