NIPPON Genetics Co.,Ltd.

Customer feedback on products

Product Name : KAPA HiFi HotStart ReadyMix (KK2601)
Manufacturer : KAPA BIOSYSTEMS
Application : 16S rRNA gene PCR using wild animal feces extract as a template

The following application data were provided by the courtesy of Dr. Sayaka Tsuchida, Laboratory of Animal Science, Kyoto Prefectural University, Japan.

Method

In order to conduct an exhaustive analysis of intestinal bacterial flora of wild animals, DNA was extracted* from feces of wild animals and 16S rRNA genes were amplified using the solution of the crude DNA as a template. When commercially available Taq polymerase was used, either no amplification or very weak amplification was shown. Thus, we tried PCR amplification using KAPA HiFi HotStarty ReadyMix.

* After bead-beating treatment and protease treatment, DNA was recovered with ethanol precipitation.

①PCR reaction mixture

〈**KAPA**〉

2×KAPA HiFi HotStart Ready Mix	12.5µl
Forward primer (10uM)	0.75µl
Reverse primer (10uM)	0.75µl
Templete DNA (<25ng)	1µl
dH2O	10µl
	25µl

〈**Product from another manufacturer**〉

2×enzyme Ready Mix	12.5µl
Forward primer (10uM)	1µl
Reverse primer (10uM)	1µl
Templete DNA (<25ng)	1µl
dH2O	9.5µl
	25µl

②PCR condition

〈**KAPA**〉

95℃	3min	
98℃	20sec	×30cycle
55℃	15sec	
72℃	15sec	
72℃	3min	
4℃	∞	

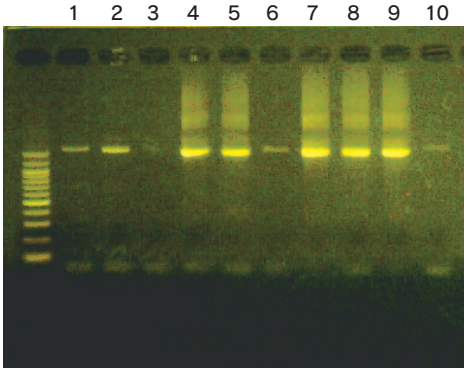
〈**Product from another manufacturer**〉

94℃	3min	
94℃	30sec	×30cycle
55℃	30sec	
72℃	90sec	
72℃	3min	
4℃	∞	

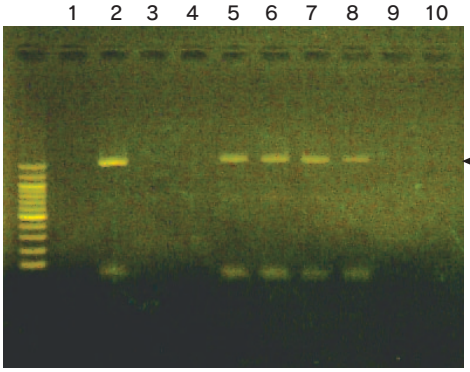
- **Thermal Cycler: BIO-RAD iCycler**

Results

KAPA HiFi HotStart ReadyMix



Product from another manufacturer



When KAPA HiFi HotStart ReadyMix was used, bands were detected in all of the 10 specimens (lanes 1 to 10).

When a product from another manufacturer was used, band was not detected for 5 specimens (lanes 1, 3, 4, 9 and 10).

Gel: 1.2% TAE agarose
Volume applied: 3 µl of PCR product/lane

In the templates, all the bacteria-derived DNA in the feces were mixed. Therefore, also in the target range, bacterial species with high GC contents and those with low GC contents were mixed.
In a previous study, we conducted PCR with annealing temperatures of approximately 50°C using a product from another manufacturer, and we were not able to detect any species with high GC contents in cloning or metagenomics.
To learn the distribution of bacterial species with high GC contents, we decided to use KAPA HiFi HotStart ReadyMix in the last experiment. We successfully detected the amplification, and the product allowed us to do further analyses.

<Customer's comments>

Crude DNA solutions derived from wild animal feces often contain plant secondary metabolites (e.g., phenols), which inhibit the PCR amplification. As a result, we were not able to obtain enough volumes of amplification products in many cases or in some cases no amplification occurred. To cope with these problems, we tried to lower the annealing temperature, but the temperature cannot be lowered too much in amplification of GC-rich sequences. When we tried KAPA HiFi HotStart ReadyMix, we were able to obtain the amplification at the optimal annealing temperature even with the specimens containing a PCR-inhibiting substance.

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