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):

<table>
<thead>
<tr>
<th>Product of company A</th>
<th>KAPA HiFi Uracil+</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR reaction buffer</td>
<td>x1</td>
</tr>
<tr>
<td>MgCl2</td>
<td>2.5mM</td>
</tr>
<tr>
<td>dNTP</td>
<td>300µM</td>
</tr>
<tr>
<td>Primer (sense)</td>
<td>200nM</td>
</tr>
<tr>
<td>Primer (antisense)</td>
<td>200nM</td>
</tr>
<tr>
<td>Template</td>
<td>~30ng</td>
</tr>
<tr>
<td>Enzyme of company T</td>
<td>25U/mL</td>
</tr>
</tbody>
</table>

Denaturation 95°C 5min
PCR cycle 10 sec
55°C 30 sec
72°C 2 min

Denaturation 95°C 5min
PCR cycle 10 sec
98°C 30 sec
55°C 30 sec
72°C 2 min

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

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.

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.

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