**Application Note**

**Product Name**: KAPA HiFi HotStart DNA polymerase  
**Manufacturer**: KAPA BIOSYSTEMS  
**Application**: Fidelity verification of KAPA HiFi HotStart DNA polymerase by Human TGFBR2 mutation analysis

**Introduction**

KAPA HiFi DNA polymerase, which was chosen from more than 100 million wild-type DNA polymerase mutants, is a novel PCR enzyme developed and engineered by Kapa Biosystems, exhibiting excellent fidelity and amplification performance.

**Next Generation sequencer (Roche FLX) analysis data**

<table>
<thead>
<tr>
<th></th>
<th>KAPA HiFi™</th>
<th>P-H from competitor I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of enzyme</td>
<td>High fidelity engineered single enzyme system</td>
<td>Mixture of high fidelity enzyme and Taq polymerase</td>
</tr>
<tr>
<td>Total sequence number (base)</td>
<td>17,724,794</td>
<td>7,273,424</td>
</tr>
<tr>
<td>Total sequence number / Total error number (base)</td>
<td>3,544,859</td>
<td>89,795</td>
</tr>
<tr>
<td>Error rate</td>
<td>2.82 E-07</td>
<td>1.11 E-05</td>
</tr>
</tbody>
</table>

*Data courtesy of Dr. Phillip Buckhaults, University of South Carolina*

A huge amount of time and effort is required in order to quantify error rates of high fidelity PCR enzymes. Common methods are “to carry out a great number of sequencing” or “to quantify error rates through blue/white colony selection by cloning E-coli”, both of which are tiring. We performed PCR/sequence analysis of a sequence of about 100bp which is difficult to correctly amplify by PCR, comparing the fidelity of KAPA HiFi DNA Polymerase with competitors’ products in a manner users actually do.

In this Human TGFBR2 mutation analysis, you need to analyze Wild Type with ten consecutive “A” s in a sequence and Mutant with nine consecutive “A” s in a sequence. However, the existence of these consecutive “A” s makes it difficult to perform accurate PCR amplification. In this case, a sequencing using PCR products will not lead to good sequencing results.

In order to verify which PCR reagent brought about the most accurate amplification, we amplified the sequence with a variety of commercially available PCR reagents including KAPA HiFi HotStart DNA Polymerase, and performed sequence analysis.

**Procedure**

Human TGFBR2 mutation analysis was performed in accordance with the following reference literature.


**<Primer>**

<table>
<thead>
<tr>
<th>Primer sequence</th>
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</table>
| Forward | AAGCTCCCCCTACCATGACT  
| Reverse | TSGACTCACAGAGCTCAGAG  

**Amplification size**

118bp

**<PCR reagent>**

1. KAPA HiFi HotStart DNA Polymerase  
2. Ph from competitor F (HotStart)  
3. Ph from competitor T (HotStart)  
4. Ko from competitor T (HotStart)  
5. Wild Type Taq DNA Polymerase

**Template DNA**

We used the following two types of genomic DNA templates purified from human prostate cancer-derived cell line, whose sequence information is clear.

**Reaction setup**

Based on each manufacturer’s recommended conditions

**Thermal cycler**

LifePro with gradient function (Bior)

**Cycling program**

Based on each manufacturer’s recommended conditions

Gradient PCR was performed to determine the optimum annealing temperature.

**<Sequencing>**

Templates for sequencing were prepared by purifying PCR products with a commercially available kit. We performed “Forward” and “Reverse” sequencing of two templates (22Rv1 and LNCaP) respectively.

<table>
<thead>
<tr>
<th>Primer sequence</th>
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| Forward | CCCCCTACCATGACTTTATTCTC  
| Reverse | TCATCAGAGCTCAGAGACAC  

<table>
<thead>
<tr>
<th>Error rate comparison</th>
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</table>

 Error rate comparison

Next Generation sequencer (Roche FLX) analysis data
Result

<PCR>
Gradient PCR was performed for each PCR reagent in order to determine the optimum annealing temperature where we can observe the most efficient amplification and no non-specific amplification. As a result, we obtained nice PCR results depending on optimum conditions for each PCR reagent. We used PCR products, as templates for sequencing, obtained at the optimum conditions for each reagent.

Data of LNCaP (A10)
1. KAPA HiFi HotStart DNA Polymerase
2. Ph from competitor F (HotStart)
3. Pr from competitor T (HotStart)
4. Ko from competitor T (HotStart)
5. Wild Type Taq DNA Polymerase

KAPA HiFi HotStart marked the best amplification.

<Sequencing>
KAPA HiFi HotStart DNA Polymerase

Data of LNCaP (A10)

As a conclusion, KAPA HiFi HotStart resulted in the best PCR amplification.

Acknowledgement
In this verification experiment of Human TGFBR2 Mutation Analysis, we received a lot of guidance and advice from Mr. Satoshi Yamashita at Division of Epigenomics, National Cancer Center Research Institute. We truly appreciate his support.