NIPPON Genetics Co.,Ltd.

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

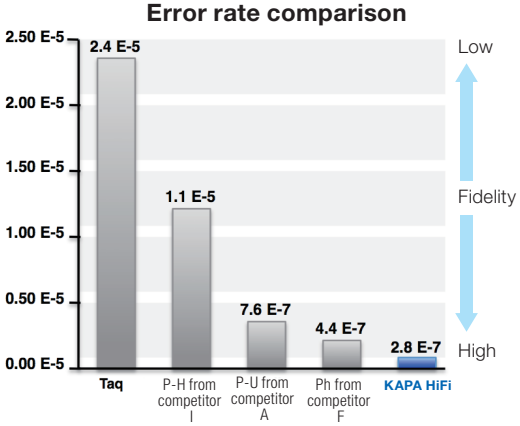
KAPA HiFi DNA polymerase, which was chosen from more than 100 million wild-type DNA polymerase mutants, is a novel PCR enzyme developed and engineered by Kapa Biosystems, exhibiting excellent fidelity and amplification performance.

Next Generation sequencer (Roche FLX) analysis data

	KAPAHiFi™	P-H from competitor I
Type of enzyme	High fidelity engineered single enzyme system	Mixture of high fidelity enzyme and *Taq* polymerase
Total sequence number (base)	17,724,794	7,273,424
Total sequence number / Total error number (base)	3,544,959	89,795
Error rate	2.82 E-07	1.11 E-05

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

Error rate comparison



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 perfrom 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.

1) Markowitz S, Wang J, Myeroff L, et al. Inactivation et al, Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability, Science 1995;268:1336-1338.
2) Yamashita S et al, Methylation Silencing of Transforming Growth Factor-β Receptor Type II in Rat Prostate Cancers, Cancer Res 68:2112-2121, 2008

<PCR>

- **Primer sequence**
  Forward AAGCTCCCCTACCATGACT
  Reverse TGCACTCATCAGAGCTACAG
- **Amplification size**
  118bp
- **PCR reagent**
  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
- **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 fanction (Bior)
- **Cycling program**
  Based on each manufacturer’s recommended conditions
  Gradient PCR was performed to determine the optimum annealing temperature.

<Sequencing>

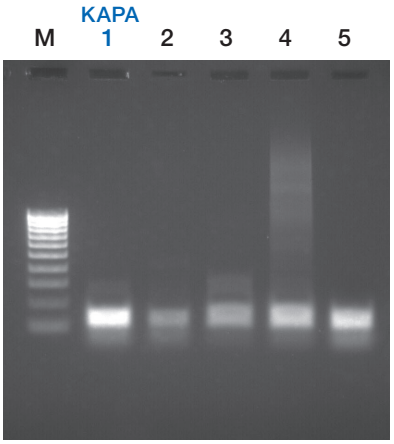
Templates for sequencing were prepared by purifying PCR products with a commercially available kit. We performed “Forward” and “Reverse” sequencing of two templates (22Rv1 and LNCaP) respectively.

- **Primer sequence**
  Forward CCCCTACCATGACTTTATTCT
  Reverse TCATCAGAGCTACAGGAACAC

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)

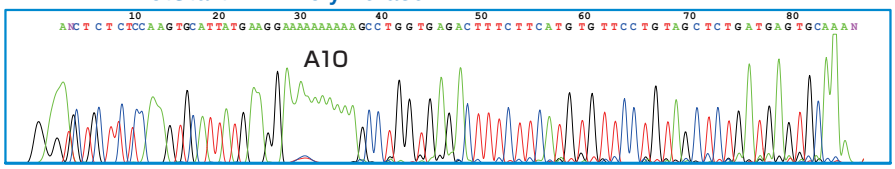
1 KAPA HiFi HotStart DNA Polymerase
2 Ph from competitor F (HotStart)
3 Pr from competitor T (HotStart)
4 Ko from competitor T (HotStart)
5 Wild Type *Taq* DNA Polymerase

Kapa HiFi HotStart marked the best amplification.

<Sequencing>

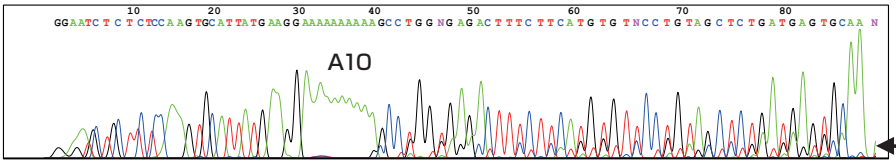
Data of LNCaP (A10)

KAPA HiFi HotStart DNA Polymerase

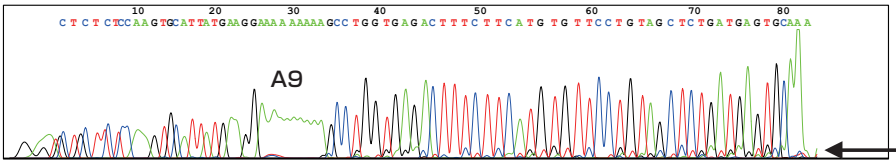


KAPA resulted in low background and very nice single peak.
Ten consecutive “A” s were observed.

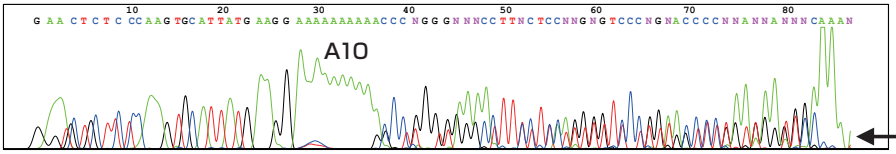
Ph from competitor F (HotStart)



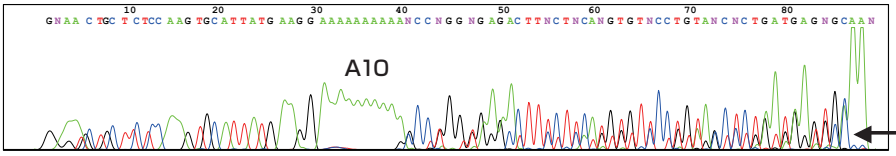
Pr from competitor T (HotStart)



Ko from competitor T (HotStart)



Wild Type *Taq* DNA Polymerase



Slipping (wrong translation) happened at the point of consecutive “A” s, causing high background of sequence data afterwards. The number of consecutive “A” s was only nine for Pr from competitor T.

As a conclusion, Kapa HiFi HotStart resulted in the best PCR amplification.
In addition, on four conditions (two types of templates × double strand) in sequencing, KAPA showed less slipping (wrong translation) and the most accurate sequence data.(The data above is Forward analysis of LNCap)

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.

Nippon Genetics Co.,Ltd http://www.n-genetics.com

Head Office : Hongo Tsuna Bldg., 17-9 Hongo 6-Chome, Bunkyo-ku Tokyo 113-0033 Japan TEL : +81-(0)3-3813-0961 FAX : +81-(0)3-3813-0962
West Sales Office : Raffine Oike 3F, 565, Nishinotoin Oike sagaru, Nakagyo-ku, Kyoto, 604-8277 Japan TEL : +81-(0)75-257-5421 FAX : +81-(0)75-257-5422
Nippon Genetics Europe GmbH : Binsfelder Strasse 77, 52351 Dueren Germany