Introducing **KAPA**HiFi™ HotStart

World's highest fidelity polymerase for PCR.

KAPABIOSYSTEMS

next generation thinking
in enzyme technology

Overview

KAPAHiFi™ HotStart is the engineered KAPAHiFi™ DNA Polymerase with an antibody-based hot start technology and improved buffer system.

KAPAHiFi™ HotStart exhibits:

- World leading fidelity – confirmed by next-generation DNA sequencing.
- High sensitivity.
- Robust performance across a wide range of difficult templates (GC-rich).
- Long range - amplification of complex targets > 15 kb.
- High speed - reduce reaction times by up to 75%.

Overview

KAPAHiFi™ HotStart Key Product Features:

- Antibody hot start technology increases reaction efficiency and sensitivity by eliminating spurious amplification products resulting from non-specific priming events during reaction setup and initiation.

- KAPAHiFi™ HotStart includes improved Fidelity and GC buffers. These buffer modifications confer dramatic improvements to fidelity and sensitivity.

- KAPAHiFi™ HotStart is capable of amplifying longer, more complex targets at greater sensitivity – greater than 15 kb from genomic targets.

- KAPAHiFi™ HotStart is recommended for all previous KAPAHiFi™ applications and expands the opportunity set for new applications.

Overview

Applications:

- In addition to traditional applications that require high fidelity such as:
    1. cloning
    2. site-directed mutagenesis
    3. protein expression

- Next-generation sequencing of individual PCR amplicons in pooled libraries for SNP analysis requires the highest fidelity DNA polymerase. Platinum Taq High Fidelity, a blend of polymerases exhibiting poor fidelity, is commonly used for this application.

- KAPAHiFi™ HotStart has the highest fidelity and highest performance of any DNA polymerase currently available in the market.

Case Study

Cancer resequencing on Roche GS FLX Sequencer

The problem:

Resequencing pooled amplicon libraries on next-gen sequencers requires the highest possible fidelity during PCR or the results will be littered with false positive SNPs (variation created by the polymerase rather than a real mutation).

Experimental design:

KAPAHiFi™ and Invitrogen Platinum® Taq High Fidelity were each used to generate amplicons for a cancer resequencing project. 90 PCR amplicons, covering 5 candidate genes from 24 tumors, were generated using each enzyme. The resultant PCR products were concentration normalized and pooled for sequencing.

Case Study

Cancer resequencing on Roche GS FLX Sequencer

	KAPAHiFi™	Platinum® Taq High Fidelity
Type of Enzyme	Single engineered polymerase with strong proofreading activity	Blend of wild-type Taq polymerase and wild-type proofreading polymerase
Total Bases Sequenced	17,724,794	7,273,424
Bases Amplified/PCR Error	3,544,959	89,795
Error Rate	2.82 E-07	1.11 E-05

The data set for KAPAHiFi™ covered ~17.7 million raw sequenced bases revealing 20 unique SNPs and 5 unique PCR-induced errors.

The data set for Platinum Taq High Fidelity covered ~7.2 million raw sequenced bases revealing the same 20 SNPs and 81 unique PCR-induced errors.

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

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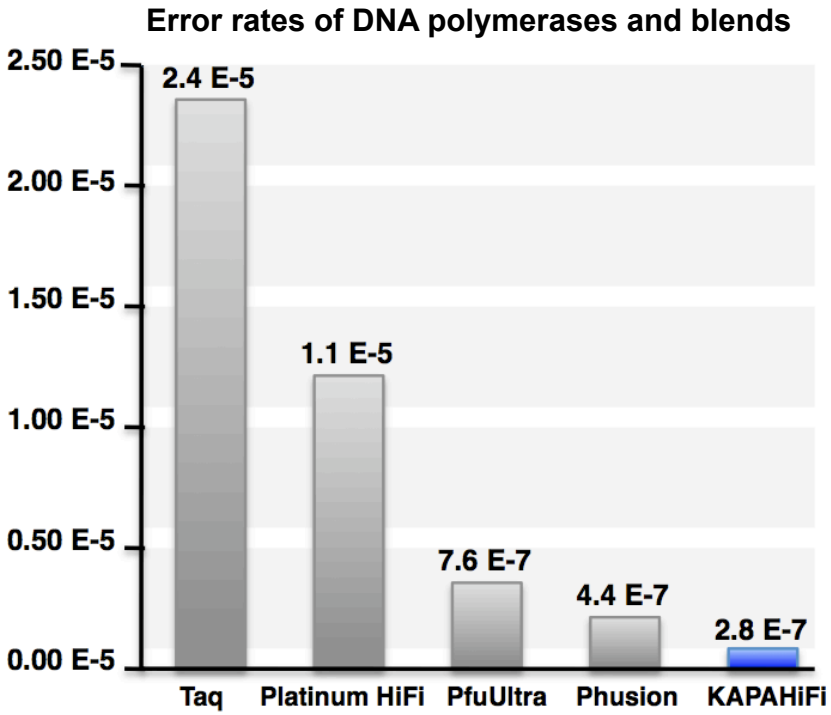
The error rate of KAPAHiFi™ is calculated at 1 error in 3.54×10^6 bases covered (2.82×10^{-7}) compared to 1 error in 8.98×10^4 bases covered (1.11×10^{-5}) with Platinum® Taq High Fidelity.

The lower error rate and higher yield of KAPAHiFi™ leads to significantly less false positives and increased coverage (higher yields).

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

Product Features

World's highest fidelity polymerase for PCR



KAPAHiFi™ HotStart is a highly engineered, single-enzyme with a strong 3' - 5' exonuclease (proofreading) activity and improved buffer system that results in extremely high fidelity.

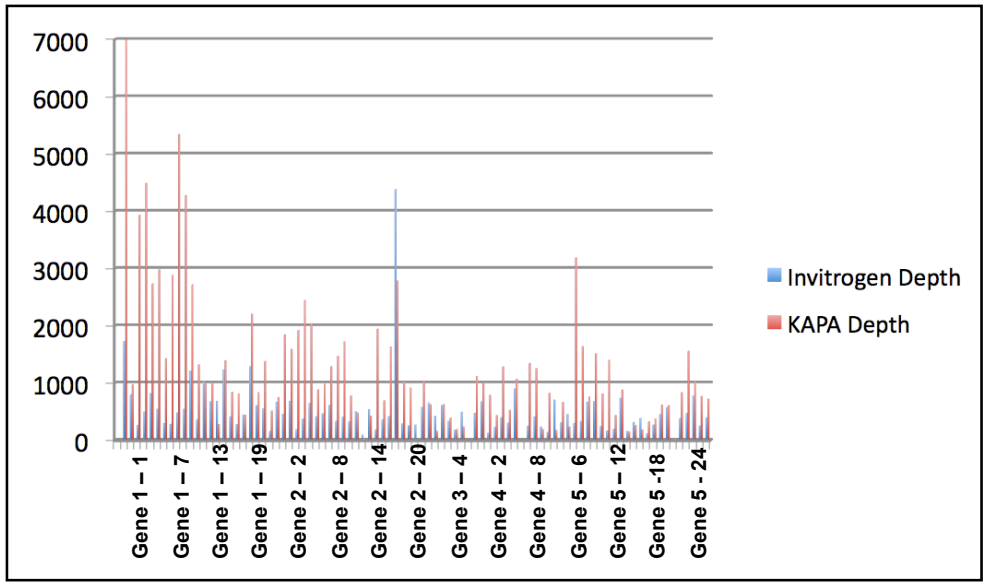
Error rate = 2.8 x 10-7

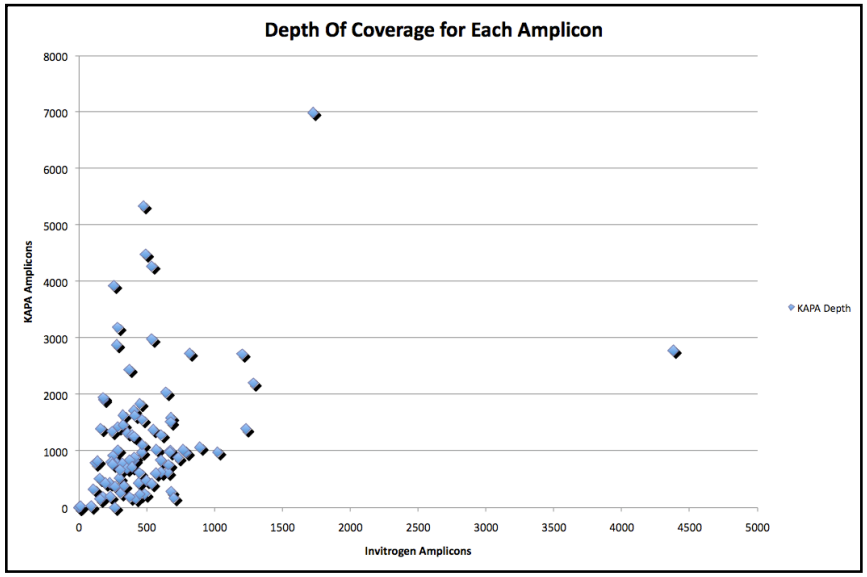
100x lower than Taq polymerase, 40x lower than long range blends (Platinum Taq High Fidelity, Roche Expand, Takara LATaq and ExTaq), 6x lower than wild-type Pfu, 3x lower than Pfu Ultra, and 2x lower than Phusion/ iProof.

Case Study

Cancer resequencing on Roche GS FLX Sequencer

Higher yields of KAPAHiFi HotStart dramatically increases depth of coverage





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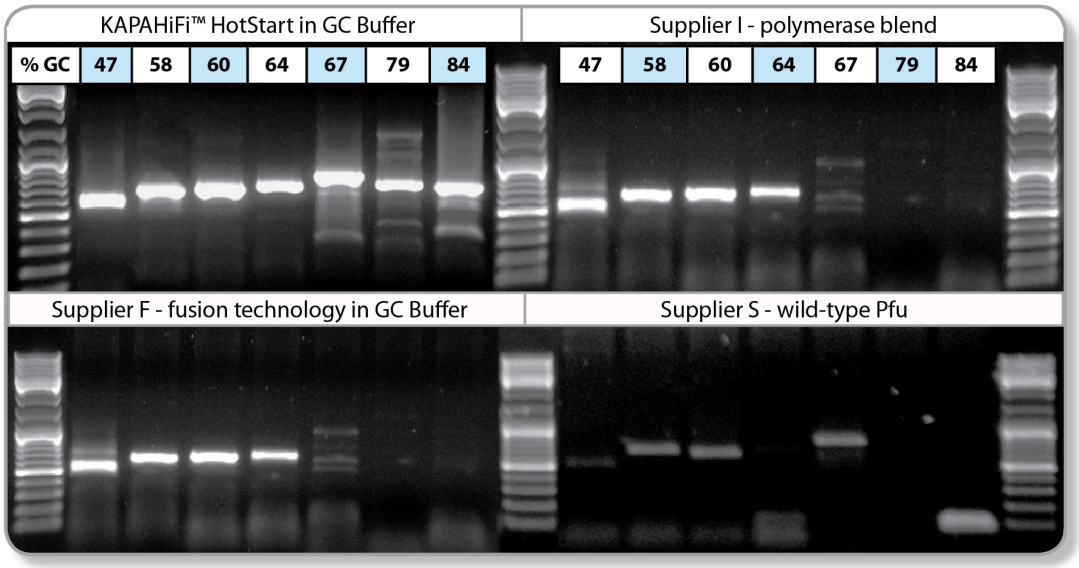
Product Features

Unrivaled success with difficult templates (AT- and GC-rich)



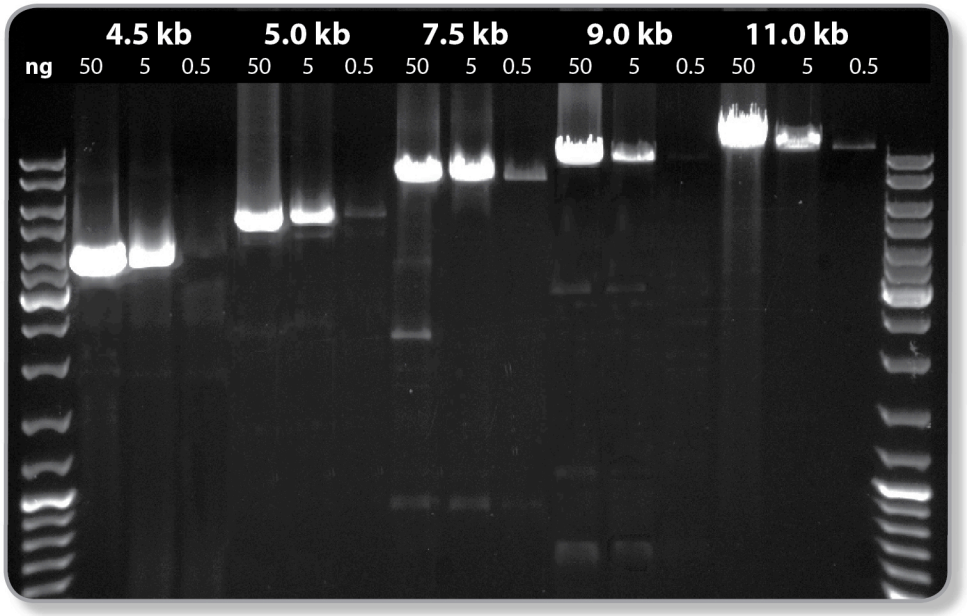
Amplification of AT- and GC-rich, single-copy human genomic targets. Seven single-copy gene fragments representing a range of GC content were used to compare the robustness of KAPAHiFi™ HotStart DNA Polymerase against a panel of competitor high fidelity DNA polymerases and polymerase blends. Amplicons range between 0.5 kb and 0.7 kb in length and ranged from 47% to 84% GC content. All reactions (25 µl each) contained 50 ng human genomic DNA as template and were performed using manufacturers' protocols and buffers, with standard 3-step cycling profiles (35 cycles). 12.5 µl of each reaction was loaded on the gel.

The complexity of human genomic DNA (GC content, repeats, etc…) can make amplification of even short fragments very difficult.

KAPAHiFi™ HotStart is supplied with an improved GC Buffer optimized for specifically difficult templates. The success rate is significantly higher than any competing high fidelity polymerase, including Phusion in its GC Buffer, Invitrogen Platinum Taq High Fidelity blend and wild-type Pfu.

Product Features

Long range and high fidelity from complex targets



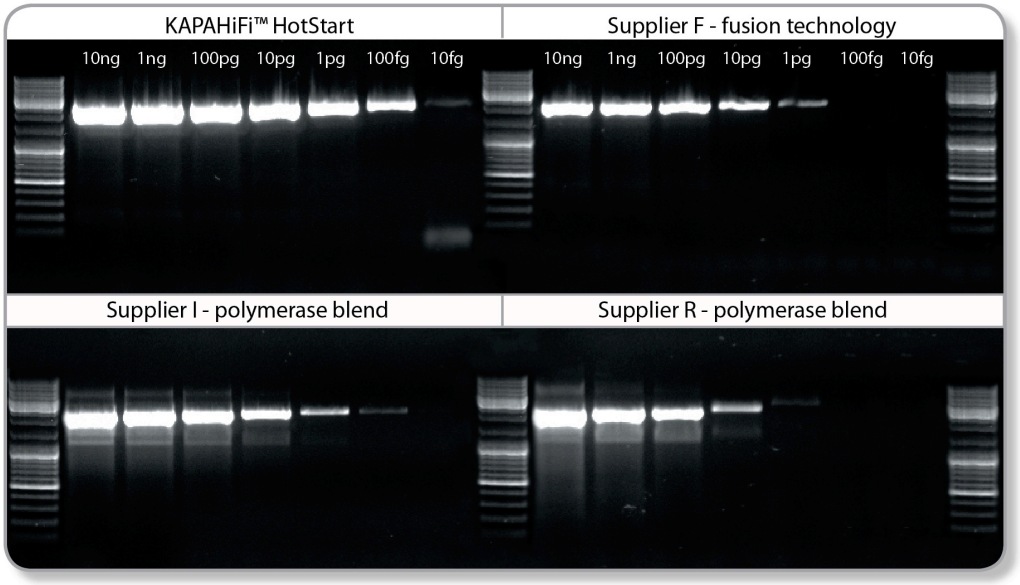
Amplification of hgDNA targets up to 11 kb. Each target was amplified from a descending range of template hgDNA concentrations (50 ng to 0.5 ng per reaction). Reactions (25 µl each) were performed using standard 3-step cycling profiles (35 cycles): 20 sec denaturation, 15 sec annealing, and 30 sec/kb extension time. Total reaction time for the 11 kb amplicon was 3h 50mins. 12.5 µl of each reaction was loaded on the gel.

KAPAHiFi™ HotStart allows for the amplification of long and complex templates (genomic DNA) while maintaining high fidelity.

11 kb from hgDNA from 500 pg of template.

Product Features

Extreme sensitivity



2 kb lambda phage target amplified from a 10 fold dilution series starting from 10 ng down to 10 fg using KAPAHiFi™ HotStart DNA Polymerase with Fidelity Buffer, fusion technology polymerase, and polymerase blend systems. All reactions (25 µl each) were performed using manufacturers' protocols and buffers, with standard 3-step cycling profiles (35 cycles). 12.5 µl of each reaction was loaded on the gel.

A major limitation for single-enzyme proofreading polymerases is poor sensitivity due to damaged nucleotides and primer degradation. The engineered KAPAHiFi™ HotStart DNA Polymerase exhibits dramatic improvements in sensitivity - outperforming fusion technology polymerases and polymerase blends.

10 femtograms of 2kb lambda.

Product Features

High speed for short and long amplicons

KAPAHiFi™ HotStart is capable of extension times of 30 sec/kb as compared with 1 min-2 min for many wild type high fidelity enzymes and polymerase blends and equivalent in speed to fusion polymerases (Phusion, Pfu Ultra II) and KOD.

Example: Total reaction time for the 11 kb amplicon was 3h 50mins compared with 7+ hours for long range polymerase blends.

Market Opportunity

Market Opportunity

- Phusion/iProof and Pfu Ultra II improved performance by attaching an accessory protein to Pfu that improves binding to DNA.

- KAPAHiFi™ HotStart has been engineered to have increased affinity to DNA without the need for accessory proteins. The processivity of KAPAHiFi™ HotStart is >100 nt per binding event.

- The improved processivity of KAPAHiFi™ HotStart confers significant improvements to sensitivity, speed, fidelity, and range.

- KAPAHiFi™ HotStart has industry-leading performance compared to all other high fidelity polymerases.

Market Opportunity

Conclusion:

KAPAHiFi HotStart DNA Polymerase couple's the world's highest fidelity with extreme robustness, sensitivity, and speed.

Application Note

Site-directed mutagenesis is a common application used in protein engineering and structure-function studies.

KAPAHiFi™ and KAPAHiFi™ HotStart DNA Polymerase:

- Extreme fidelity allows for the introduction of intended mutations without spurious mutations.
- Long range allows for the amplification of entire plasmid.
- High speed - reduce reaction times by up to 75%.

Application Note

Back-to-back site-directed mutagenesis allows for:

- Introduction of point mutations, insertions or deletions.
- Amplification step improves ratio of mutated DNA to parental background.

Common site-directed mutagenesis kits:

- Stratagene QuickChange
- Clontech Transformer
- Phusion Site-directed Mutagenesis Kit