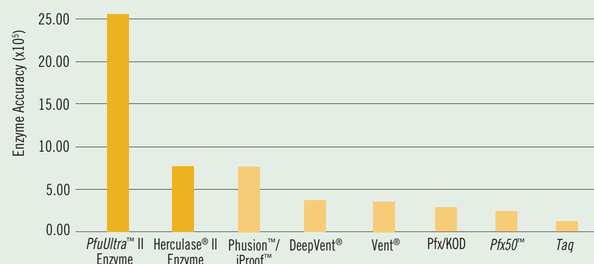
Our new breed is the center of attention.

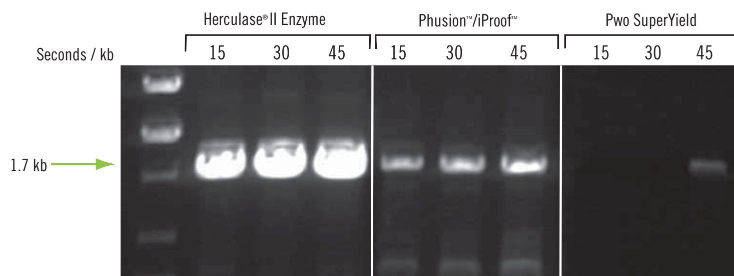
PfuUltra™ II enzyme for highest fidelity · Herculase® II enzyme for superior yield

Our next generation of high fidelity *Pfu*-based fusion enzymes sets a new standard in high fidelity PCR performance. Engineered for industry-leading fidelity *plus* 12x enhanced processivity, our new *PfuUltra*™ II Fusion HS DNA Polymerase and Herculase® II Fusion DNA Polymerase deliver superior yield, excellent reliability, and faster overall run times.

Our *PfuUltra*™ II Fusion HS DNA Polymerase offers the highest fidelity.
Error rates were determined by the *lac*I fidelity assay.



Our Herculase® II Fusion DNA Polymerase produces superior yield in as short as 15 second/kb extension time.



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Ask us about these great products:

***PfuUltra*™ II Fusion HS DNA Polymerase**	40 rxn	600670
Herculase® II Fusion DNA Polymerase	40 rxn	600675

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STRATAGENE
An **Agilent Technologies** Company

New Class of High-Fidelity *Pfu*-based Fusion Enzymes

Two New High-Fidelity Fusion DNA Polymerases for Different Applications:

+ *PfuUltra*™ II Fusion HS DNA Polymerase[a] for highest fidelity
+ Herculase® II Fusion DNA Polymerase[b] for superior yield

OUR HIGH-FIDELITY FUSION DNA POLYMERASES ARE A NEW GENERATION OF HIGH-PERFORMANCE ENZYMES. WITH ENHANCED PROCESSIVITY AND EXCLUSIVE ENZYME IMPROVEMENT ADDITIVES, OUR FUSION ENZYME FORMULATIONS PROVIDE EXTREME ACCURACY, SUPERIOR YIELD, AND EXCELLENT PCR RELIABILITY, WITH THE ADDITIONAL BENEFIT OF FAST CYCLING TIMES. YOU CAN NOW RELY ON OUR NEW GENERATION ENZYMES FOR THE BEST PERFORMANCE IN YOUR CLONING, SITE-DIRECTED MUTAGENESIS, AND EXPRESSION APPLICATIONS.

Enhanced Processivity and Improved Template Integrity

Our new generation *Pfu*-based fusion DNA polymerases set a new standard in high-fidelity PCR performance. We have dramatically increased the processivity of *Pfu*-based enzymes by fusing both our *PfuUltra*™ and Herculase® DNA polymerases with a high affinity double-stranded DNA binding domain. This domain serves to better anchor the DNA polymerase, preventing early dissociation from the DNA template. The processivity of our *PfuUltra*™ II Fusion HS DNA Polymerase has improved 12-fold over the *Pfu* DNA polymerase (Figure 1 and Table 1). The *PfuUltra* II enzyme is also shown to be >5-fold more processive than other fusion enzymes including Phusion™ and *Pfx50*™ enzymes. This improved processivity allows the incorporation of more nucleotides per binding event, which enhances PCR yield and shortens the extension time, hence saving you time and improving template integrity by minimizing exposure to extreme cycling temperatures.

Extreme Speed

The improved processivity of our *PfuUltra* II and Herculase II enzymes permits the use of much shorter PCR extension times. This improved speed means quicker time-to-results, and higher throughput. While typical proofreading polymerases require 1-2 minutes per kilobase extension times depending upon the target length, the *PfuUltra* II and Herculase II fusion DNA polymerases perform exceptionally well with short extension times (seconds per kilobase) (Table 2). Now you are limited only by the ramping and heating capability of thermal cyclers. As demonstrated in Figure 2, the Herculase II fusion DNA polymerase can produce high yield of a 900 bp fragment with as short as one second extension time.

Herculase® II Fusion DNA Polymerase

The Herculase® II Fusion DNA Polymerase, with its special formulation and the addition of our exclusive ArchaeMaxx® factor, provides superior yield for both routine and challenging PCR amplification with the additional benefit of fast cycling times. Some PCR targets are problematic to amplify due to their content or structure. Our Herculase II fusion DNA polymerase helps you overcome these PCR challenges with successful amplification of complex and GC-rich templates. Thus, our Herculase II fusion DNA polymerase provides you a reliable and economical solution to resolve your PCR challenges.

Maximum Yield and High Sensitivity

- Robust yield on all targets with short cycling times
- Successful amplification of difficult GC-rich targets
- High sensitivity for amplification of low amounts of DNA

Superior Yield with Fast Cycling Times

Our new Herculase® II Fusion DNA Polymerase is more robust than other PCR enzymes and produces unrivaled amplicon yields. This great performance is a result of the highly processive Herculase II enzyme as well as our proprietary ArchaeMaxx PCR enhancing factor and specially optimized Herculase II buffer. In routine applications, such as in the amplification of 6 kb and 1.7 kb genomic fragments, the Herculase II fusion DNA polymerase produced superior yields with extension times as short as 15 sec/kb. Other fast or high-yield proofreading DNA polymerases failed to amplify or produced lower yield (Figure 6 and front cover).

Successful Amplification of GC-Rich/Complex Targets

Our Herculase II fusion DNA polymerase is also ideal for amplification of difficult targets. Its special enzyme formulation and optimized buffer system enables the enzyme to amplify difficult targets with great yields using fast cycling conditions. As shown in Figure 7, it easily amplifies targets that contain as high as 84% GC content while competitors' enzymes failed or produced low yield. DMSO is provided separately as a PCR adjunct, and can be added when amplifying difficult targets without adversely affecting enzyme fidelity.

High Sensitivity with Low Abundance Targets

The Herculase II fusion DNA polymerase amplifies DNA fragments over a wide range of template lengths and with great sensitivity. It amplified a 3.9 kb fragment of the Hα1AT gene from as little as 1 ng input genomic DNA (Figure 8). In comparison, competitor's enzymes were less sensitive and less specific. Therefore, our Herculase II fusion DNA polymerase enables you to amplify limited amounts of DNA with great accuracy.

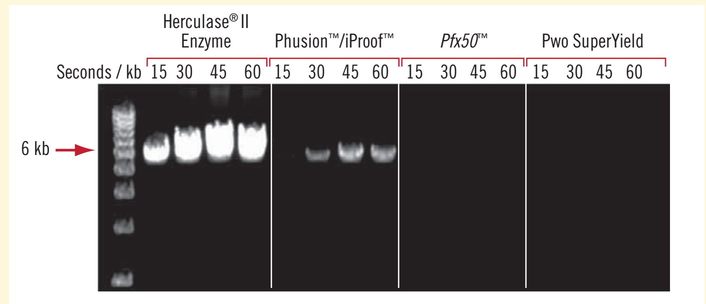

Figure 6
Robust Yields Achieved with Fast Cycling Times
The Herculase® II Fusion DNA Polymerase produced superior yields of a 6 kb fragment in amplifications employing human genomic DNA and extension times of 15, 30, 45, and 60 sec/kb. Experiments were conducted under identical conditions using each enzyme's recommended buffer.

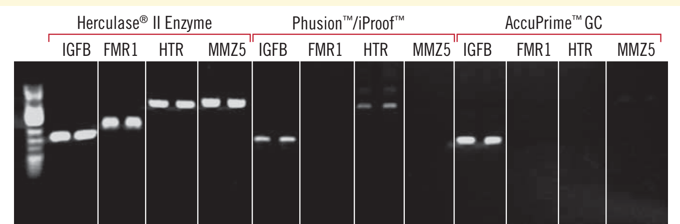

Figure 7
Herculase® II Fusion DNA Polymerase Excels in Amplifying GC-Rich Targets
Our Herculase® II Fusion DNA Polymerase easily amplifies targets that contain as high as 84% GC content while other competitors' enzymes failed or produced low yield. Human genomic DNA targets were as follows: IGFB, human insulin-like growth factor (79% GC, 250 bp); FMR1, fragile X mental retardation syndrome protein (84% GC, 300 bp); HTR, hydroxytryptomine receptor C2 fragment (65% GC, 540 bp); MMZ5, ZIC5, zinc family member 5 protein (68% GC, 562 bp). All reactions were conducted using manufacturer's recommended buffer and cycling conditions for GC-rich targets.

Figure 8
Herculase® II Fusion DNA Polymerase with Superior Sensitivity
A 3.9 kb fragment of the gene Hα1AT was amplified from human genomic DNA with DNA input amount varied from 0, 1, 5, 10, 50, 100, 200, and 300 ng, respectively. The Herculase® II Fusion DNA Polymerase amplified the specific target DNA fragment with as low as 1 ng input DNA. In comparison, competitors' enzymes were less sensitive and less specific. All reactions were conducted using manufacturer's recommended buffer and cycling conditions.

Herculase® II Fusion DNA Polymerase

Herculase® II Fusion DNA Polymerase	40 rxn	600675
	200 rxn	600677
	400 rxn	600679

Extreme Reliability of Fusion Enzymes

The improved processitivity of our high-fidelity fusion DNA polymerases, together with our proprietary ArchaeMaxx® factor, enhance the reliability of *PfuUltra* II and Herculase II enzymes. Our exclusive ArchaeMaxx factor eliminates dUTP poisioning which causes proofreading enzymes to stall[1]. Higher reliablity ensures success when amplifying diverse clone collections which demand high fidelity, specificity, and throughput. This application is demonstrated in Figure 3. The *PfuUltra* II fusion HS DNA polymerase amplified 91 of 94 randomly selected clones from a human cDNA library with significantly high yields. Amplification success rates of *PfuUltra* II and Herculase II DNA polymerases were 97%, and 99%, respectively while that of cloned *Pfu* DNA Polymerase was 87% and with lower yield.

Enzyme	Median Processivity (nt)	Processivity versus *Pfu* (fold)
***PfuUltra*™ II Fusion HS DNA Polymerase**	185	12.3
Phusion™ DNA Polymerase	30-35	2.3
***Pfx50*™ DNA Polymerase**	35	2.3
Cloned *Pfu* DNA Polymerase	15	1.0

Table 1
Enhanced Processivity of *PfuUltra*™ II Fusion HS DNA Polymerase
Median processivity valus as determined in Figure 1.

	Number of Cycles	Temperature	*PfuUltra*™ II Fusion HS DNA Polymerase	*PfuTurbo*™ DNA Polymerase
Initial Activation	1	95°C	2 min	2 min
Denaturation	30	95°C	20 sec	30 sec
Annealing	30	Tm-5°C	20 sec	30 sec
Extension	30	72°C	**15 sec/kb**	1 min/kb
Final Cycle	1	72°C	3 min	10 min

Table 2
Recommended Cycling Conditions (Genomic Targets <10 kb)

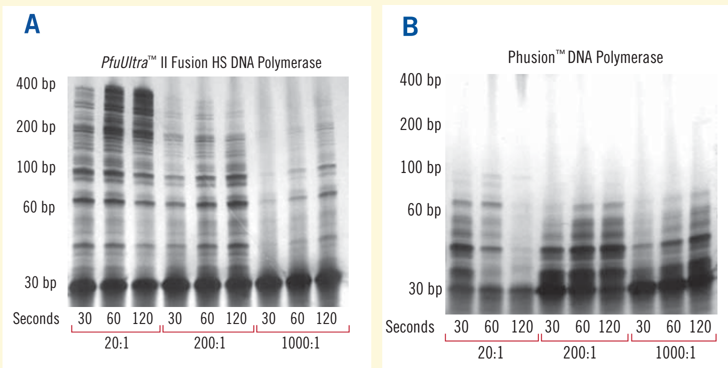

Figure 1
Enhanced Processivity of *PfuUltra*™ II Fusion HS DNA Polymerase
The *PfuUltra*™ II Fusion HS DNA Polymerase is over 5-fold more processive than the Phusion™ enzyme. A ^{33}P-labeled primer was annealed to ssM13mp18 DNA and incubated with 800 µM total dNTP in each enzyme's respective buffer at 72°C. DNA synthesis was then initiated by the addition of DNA polymerase at the indicated substrate: enzyme molar ratios of 20:1, 200:1, and 1000:1. Samples taken at various times were quenched in gel loading buffer, and analyzed on a 10% TBE-Urea gel. When the product length no longer changes with an increase in reaction time or a decrease in enzyme concentration, those reactions were used to determine median processivity. Median processivity values are shown in Table 1.

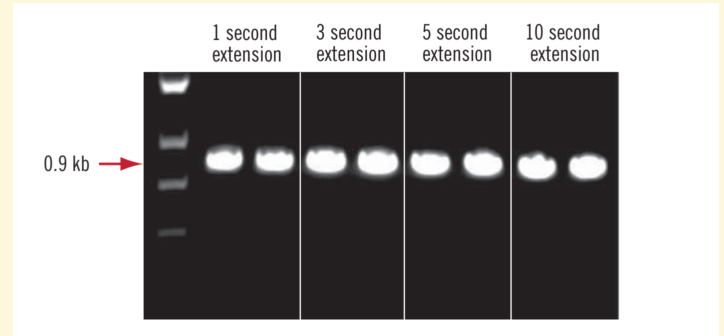

Figure 2
Dramatically Reduced Extension Time
The Herculase® II Fusion DNA Polymerase amplifies a 900 bp fragment from genomic DNA with extension time as short as one second.

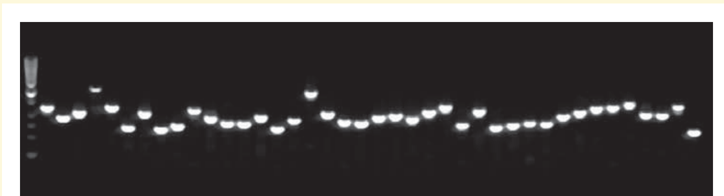

Figure 3
Greatest Success Rate with our *PfuUltra*™ II Fusion HS DNA Polymerase
A random set of 94 clones from a human cDNA library was amplified from bacterial colonies. The amplicon sizes varied between 1-3 kb. The *PfuUltra*™ II Fusion HS DNA Polymerase amplified 91 of 94 randomly selected clones (97%) with high yields and fast cycling time.

PfuUltra™ II Fusion HS DNA Polymerase for Highest Fidelity

Stratagene is the leader in the development of high fidelity PCR enzymes and novel PCR performance-enhancing additives. In our new *PfuUltra*™ II Fusion HS DNA Polymerase, we coupled the polymerase fusion technology with our engineered *PfuUltra* DNA polymerase, hotstart antibodies (HS), and proprietary ArchaeMaxx® PCR enhancing factor to achieve extreme accuracy, high specificity, and long target-length capability while dramatically reducing overall PCR extension times.

Highest Fidelity and Unrivaled Reliability

- Highest accuracy of any PCR enzyme
- Greater PCR success and reliability
- High specificity hotstart (HS) formulation
- Shorter run times for faster time-to-result

Industry Leading Fidelity

Our *PfuUltra* II fusion HS DNA polymerase is the new industry standard in ultra-high fidelity. Our fidelity testing method[2] demonstrates that the *PfuUltra* II fusion HS DNA polymerase exhibits an accuracy that is over 3-fold higher than any other proofreading enzyme, and has fidelity equal to that of our original *PfuUltra*™ Hotstart DNA Polymerase. The calculated PCR error rate for the *PfuUltra* II enzyme is 1 error in 2,500,000 nucleotides which is 20-fold fewer errors than with *Taq* DNA polymerase. Figure 4 shows the high accuracy of the *PfuUltra* II enzyme as well as that of other proofreading DNA polymerases using our testing method. We continually evaluate high-fidelity enzyme claims using this published method so that relative differences can be compared.

Amplify Long Targets in a Fraction of the Time

The *PfuUltra* II fusion HS DNA polymerase amplifies the widest variety of template sizes compared to other proofreading DNA polymerases. Because it requires short cycling times, genomic templates up to 19 kb in length can be amplified in a fraction of the time (Figure 5). Faster cycling not only saves you time, but also improves template integrity by minimizing exposure to extreme PCR temperatures. In contrast, most other commercial fusion enzymes exhibit limited target-length capability, such as up to 6 kb exhibited by the Phusion enzyme (Figure 5). It is critical for you to use high fidelity enzymes for amplifying long targets, since mutation frequency increases linearly with amplicon size. However, with conventional proofreading enzymes, amplifying long targets could take overnight (e.g., 10 hours for a 10 kb genomic DNA fragment using 2 min/kb extension times). In comparison, with the *PfuUltra* II polymerase, the same 10 kb target can be amplified in under 2 hours (15 sec/kb extension times).

High Specificity Hotstart Version

Unlike Phusion™/iProof™ enzymes and other fusion-based enzymes, our *PfuUltra* II fusion HS DNA polymerase is formulated with hotstart antibodies that neutralize both polymerase and exonuclease activities during reaction set-up, thus preventing mispriming and reducing background. The hotstart feature enhances specificity and facilitates robotic assembly.

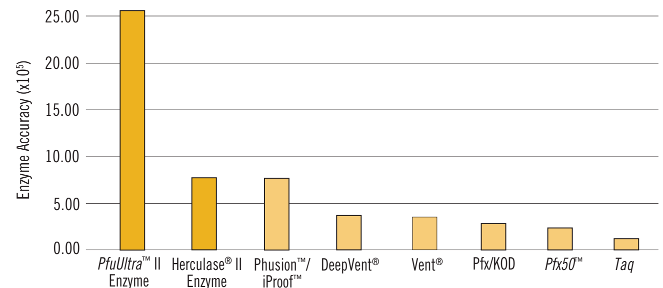

Figure 4
***PfuUltra*™ II Fusion HS DNA Polymerase, the Highest Fidelity PCR Enzyme**
The accuracy rate of the *PfuUltra*™ II enzyme and other proofreading DNA polymerases is shown using error rates determined from our testing method. We continually evaluate high fidelity enzymes using this published method, so that relative differences can be compared. (Enzyme accuracy is equal to 1/error rate).

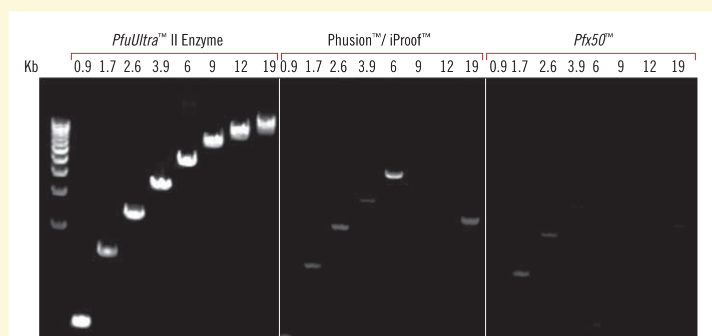

Figure 5
***PfuUltra*™ II Fusion HS DNA Polymerase Amplifies a Wide Range of Targets**
The *PfuUltra*™ II Fusion HS DNA Polymerase amplifies a broad range of genomic DNA targets up to 19 kb in length. In contrast, other commercial enzymes exhibit limited target-length capability.

***PfuUltra*™ II Fusion HS DNA Polymerase**

PfuUltra™ II Fusion HS DNA Polymerase	40 rxn	600670
	200 rxn	600672
	400 rxn	600674

Reference
1.Hogrefe, et al., (2002), *PNAS* 99:596:601.
2.Cline, J., Braman, J.C. and Hogrefe, H.H. (1996) *NAR* 24:3546-3551.

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a. U.S. Patent Nos. 6,734,293, 6,489,150, 6,444,428, 6,183,997, 5,948,663, 5,866,395, 5,545,552 and patents pending
b. U.S. Patent Nos. 6,734,293; 6,489,150; 6,444,428; 6,183,997; 5,948,663; 5,866,395; 5,556,772; 5,545,552; and patents pending

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Printed in USA, January 29, 2016
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