

Pfu DNA Polymerase: A High Fidelity Enzyme for Nucleic Acid Amplification



By Michael Slater, Susanne Selman, Bill Mogilevsky, Harryl Ammons and Jim Hartnett
Promega Corporation

Corresponding author: e-mail to m Slater@promega.com

Pfu DNA Polymerase exhibits the lowest error rate of any thermostable DNA polymerase studied (1-3). For routine PCR, where simple detection of an amplification product or estimation of the product's size is important, Taq DNA polymerase is the obvious enzyme to choose. However, when the amplified product is to be cloned, expressed or used in mutagenesis studies, Pfu DNA Polymerase is a much better enzyme of choice for PCR. Pfu DNA Polymerase is also used in blends with Taq DNA polymerase, or amino-terminally truncated versions of Taq DNA polymerase, to amplify longer stretches of DNA in PCR with greater accuracy than Taq DNA polymerase alone (1).

INTRODUCTION

Pyrococcus furiosus Vc1 (DSM3638) was discovered in geothermally heated marine sediments in Vulcano, Italy (4), and grows optimally at 100°C. The DNA polymerase was isolated from *P. furiosus* and shown to possess a 3'→5' proofreading exonuclease (5). It was also shown that the *Pfu* DNA Polymerase^(a) could be used in PCR and that it possessed dramatically better fidelity than *Taq* DNA polymerase. The gene encoding the *Pfu* DNA polymerase was cloned, sequenced and shown to be homologous to the alpha-like DNA polymerase family (6), and not the Pol I-like family of which *Taq* DNA polymerase is a member. The *Pfu* DNA polymerase gene encodes a polypeptide of 775 amino acids with a predicted molecular weight of 90,109Da (7). We have purified the enzyme from the native organism and verified its fidelity and performance in PCR. [Figure 1](#) shows the purity and relative sizes of Promega's and another supplier's enzyme.

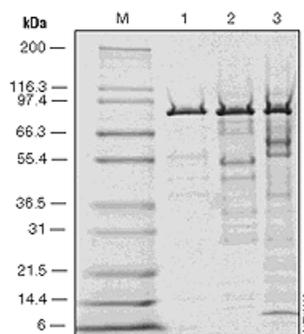


Figure 1. Comparison of Promega's *Pfu* DNA Polymerase protein to that of another supplier by SDS-PAGE. The greater purity of Promega's *Pfu* DNA Polymerase is evident by the prominent protein band of ~90kDa in lane 1. Equal activity units of each enzyme preparation were resolved on a Tris-Glycine 4-20% Novex™ SDS-PAGE gel (220V for 60 minutes in standard Laemmli buffer). Lane 1, Promega's *Pfu* DNA Polymerase (native); lanes 2 and 3, separate lots of another supplier's native *Pfu* DNA polymerase. Lane M, Novex™ Mark 12™ Marker (10µl).

FIDELITY

The low error rate of *Pfu* DNA Polymerase in PCR, documented and confirmed by several different methods ([Table 1](#)), is roughly 10⁶ per base pair (bp) duplicated. Error rate is commonly expressed as the mutation rate per bp duplicated, and accuracy as the inverse of error rate. In other words, accuracy is the average number of nucleotides the polymerase incorporates before making an error. One of the higher reported fidelity values for *Taq* DNA polymerase is 8 x 10⁶ (1). In that study, the fidelity of many thermostable DNA polymerases was measured using the same method, which demonstrated the superior accuracy of *Pfu* DNA polymerase for PCR applications requiring high fidelity (1; [Table 2](#)).

Table 1. Published Fidelity Values for <i>Pfu</i> DNA Polymerase.		
Error Rate x 10 ⁻⁶ (Mutation rate per bp	Accuracy x 10 ⁵ (Error rate ⁻¹)	

duplicated)	¹⁾	Method
0.65 ± 0.31	15	Direct measurement of PCR-generated point mutant DNA by denaturing constant capillary electrophoresis (2).
2.0	5	Measurement of p53 activity in yeast (<i>S. cerevisiae</i>) after amplification and transformation of the p53 gene (3).
1.3 ± 0.2	7.7	Measurement of <i>lacI</i> activity in bacteria (<i>E. coli</i>) after amplification and transformation of the <i>lacI</i> gene (1).

Table 2. Reported Fidelity for Several Different Thermal Stable DNA Polymerases Using the Forward Mutation Assay (1).		
DNA Polymerase	Error Rate x 10⁻⁶	Accuracy x 10⁵
<i>Pfu</i>	1.3 ± 0.2 S.D.	7.7
Deep Vent _R TM	2.7 ± 0.2 S.D.	3.7
<i>Tli</i> (Vent _R [®])	2.8 ± 0.9 S.D.	3.6
<i>Taq</i>	8.0 ± 3.9 S.D.	1.3
<i>Ultma</i> [®]	55.3 ± 2.0*	0.2
*Range of duplicates.		

We compared the fidelity of native *Pfu* DNA Polymerase from Promega with that of another supplier using a PCR-based forward mutation assay of the *E. coli lacI* gene (Figure 2). The method is similar to that used by Cline *et al.* (1). Each PCR was prepared using the supplied reaction buffer and according to the respective manufacturer's recommendations. Reactions were performed in duplicate as described in Table 3. We found the accuracy of Promega's native *Pfu* DNA Polymerase to be 15 times greater than that of the other supplier's native *Pfu* DNA polymerase. The increased accuracy of Promega's *Pfu* DNA Polymerase can be attributed to the difference in the pH of the reaction buffers provided by each supplier, and is consistent with the data demonstrating the influence of pH on *Pfu* DNA polymerase accuracy presented in Cline *et al.* (1).

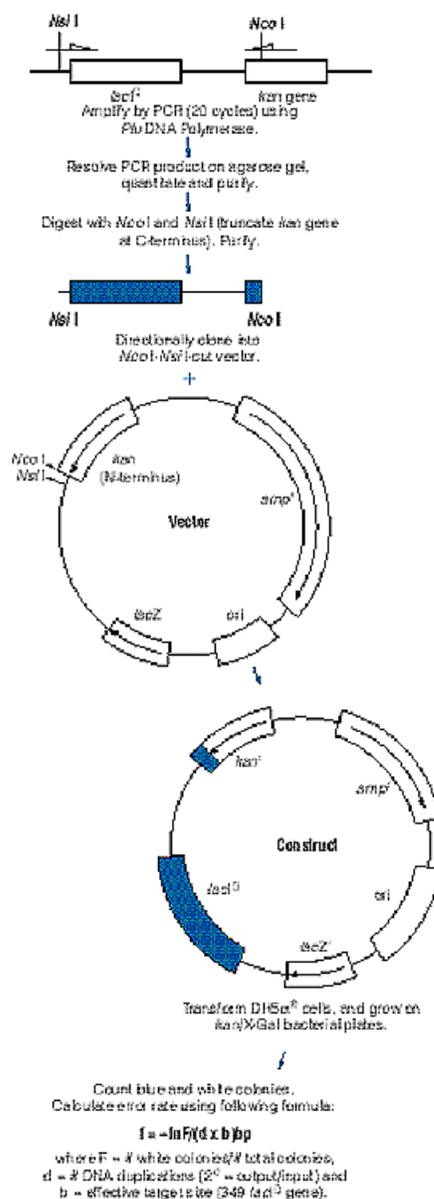


Figure 2. Measurement of enzyme fidelity by a PCR-based forward mutation assay. Enzyme fidelity was measured by amplifying the *lacI^Q* gene and assessing its function in *E. coli*. A mutation introduced during PCR in the *lacI^Q* gene will result in derepression of the *lacZ* gene and give a blue colony phenotype on X-gal plates. While the *lacI^Q* gene is 1,080 bases long, only 349 single-base substitutions in that region result in a mutant phenotype (Cline *et al.* [1]). The first step in the forward mutation assay is amplification of a 1.8kb region containing the *lacI^Q* gene and the carboxy-terminus of the *kan* gene. Twenty cycles of PCR were performed using *Pfu* DNA polymerase, 200μM each dNTP and buffer containing 2.0mM Mg²⁺. One-tenth of the amplification product was subjected to electrophoresis on an agarose gel, and the expected 1.8kb PCR product was quantified using a Molecular Dynamics FluorImager™. The PCR amplification product was excised from the gel, purified with Wizard® PCR Preps DNA Purification Resin (Cat.# A7170) and digested with *Nco* I and *Nsi* I. The *Nco* I-*Nsi* I fragment (1,776bp), also purified on Wizard® PCR Preps, was ligated into an *Nco* I-*Nsi* I digested 4.3 kb vector containing a carboxy-terminal truncated *kan* gene. Successful ligation of the PCR product to the vector restores a functioning *kan* gene. Each construct in the resulting pool of ligation products contains either a wildtype or a mutant *lacI^Q* gene. The ratio of mutant and wildtype constructs varies with the accuracy of the DNA polymerase used in the amplification. The ligated mixture was transformed into competent DH5alpha® cells, and the transformed culture was plated on kanamycin/X-gal plates. The blue and white colonies were counted to calculate the error rate as indicated. Error rate formula adapted from Lundberg *et al.* (5).

Table 3. Comparison of Fidelity for Promega's and Another Supplier's Native *Pfu* DNA Polymerase*.

Supplier	Yield	<i>lacI</i>	Error Rate (x 10 ⁻⁶)	Accuracy (x 10 ⁵)
Promega	5,166ng	0.36%	0.9	11

	5,431ng	0.40%	1.0	10
Other	2,838ng	5.4%	15	0.67
	2,875ng	5.6%	16	0.63

*Two separate trials were performed per supplier. DNA Polymerases were tested in the supplier's provided buffer. Two nanograms of template DNA were used per trial. Greater than 25,000 colonies were scored for the LacI phenotype.

PERFORMANCE

The detection sensitivity of Promega's *Pfu* DNA Polymerase was compared with a leading supplier's native *Pfu* DNA polymerase in a standard PCR protocol using decreasing amounts of human genomic DNA as template (Figure 3). The primers amplified a 1.2kb fragment of the human alpha-1-antitrypsin gene. After 40 cycles, the amplified product could be visualized on agarose gel starting from 30pg of template DNA (equivalent to 10 copies of genomic DNA). Both enzyme preparations exhibited equivalent performance.

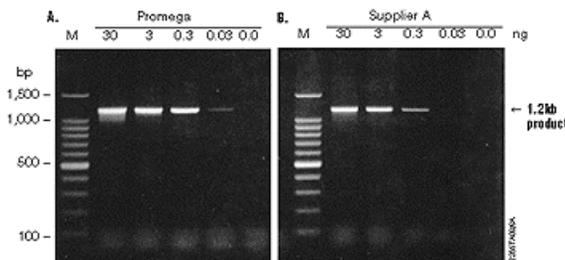


Figure 3. Comparison of amplification sensitivity of Promega's native *Pfu* DNA Polymerase to that of another supplier. A 1.2kb fragment of the human alpha-1-antitrypsin gene was amplified using Promega's (Panel A) or another supplier's polymerase (Panel B). Promega's *Pfu* DNA Polymerase was capable of amplifying 30pg of genomic template, the equivalent of 10 copies of this gene. After 40 cycles of amplification, 10 μ l aliquots of the 50 μ l amplification reactions were analyzed on a 1% agarose gel followed by ethidium bromide staining. The amounts of Human Genomic DNA (Cat.# G3041) template were: Lane 1, 30ng; lane 2, 3ng; lane 4, 300pg; lane 4, 30pg; and lane 5, no template. Lane M contains Promega's 100bp DNA Ladder (Cat.# G2101).

In order to demonstrate the capability of Promega *Pfu* DNA Polymerase to amplify different templates, we performed PCR on DNA isolated from mouse blood and tissues (Figure 4). Blood containing the anti-coagulants acid citrate dextrose (ACD), citrate, EDTA, heparin and Alsever's solution, was used. DNA samples were isolated using a modified protocol for the Wizard[®] Genomic DNA Purification Kit (see "Isolation and Analysis of Human Genomic DNA," which begins on page 20 of this issue, for more details on the modified protocol).

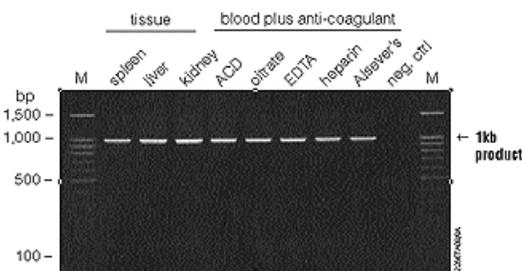


Figure 4. Compatibility of Promega's *Pfu* DNA Polymerase for amplification of DNA from varied sources. Amplification was performed on a 1,000bp fragment of mouse glyceraldehyde 3-phosphate dehydrogenase (G3PDH) gene using DNA isolated from mouse tissue and blood. DNA was purified using the Wizard[®] Genomic DNA Purification Kit protocol. Twenty nanograms of DNA was used as template in each amplification. Tissue sources and blood anti-coagulants are indicated in the figure. Lane M contains 100bp DNA Ladder (Cat # G2101).

CLONING

Because *Pfu* DNA polymerase possesses proofreading activity, it does not have the terminal transferase-like activity demonstrated by *Taq* DNA polymerase, which often places a nontemplate A residue at the 3'-end of amplimers (8). *Pfu* DNA Polymerase produces blunt-ended amplification products (8). Purified, blunt-ended fragments may be modified by a second incubation with *Taq* DNA polymerase, in the presence of dATP, to facilitate cloning into Promega's pGEM[®]-T Easy Vector^(b,c) (Figure 5). This cloning method was tested using a 1,200bp fragment generated by amplification with *Pfu* DNA Polymerase. This procedure can generate 80-90% positive transformants. Ligation reactions using nontailed amplified DNA resulted in no positive (white, recombinant) colonies. Restriction digestion of selected recombinants with *Not* I verified the presence of the insert fragment (data not shown). We recommend

that the amplification reaction products be purified prior to modification to eliminate nonspecific reaction products, primers and primer-dimers. Promega's Wizard[®] PCR Preps DNA Purification System^(d) (Cat.# A7170) provides a convenient method for purifying amplification products.

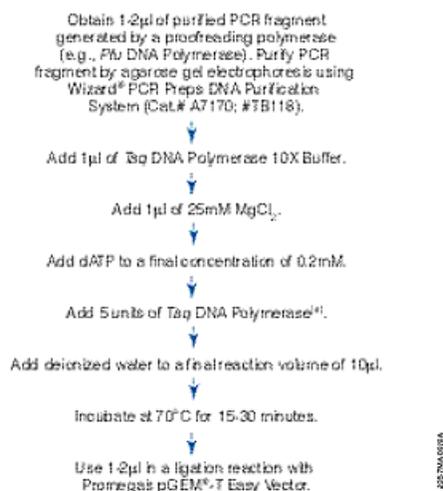


Figure 5. A-tailing protocol used to prepare a blunt-ended PCR product for T-vector cloning.

APPLICATION

Proofreading DNA polymerases such as *Pfu* have strong 3'→5' exonuclease activity, which can rapidly remove bases from the 3' end of PCR primers. Truncation of the oligonucleotides may result in an increase in the level of nonspecific amplification, as evidenced by the presence of a smear on an agarose gel. This nonspecific amplification can be attributed to truncated primers annealing at undesirable positions on the target DNA. When using *Pfu* DNA Polymerase, it is important to perform a hot start. Techniques such as withholding the enzyme or magnesium until the tubes are placed in a thermal cycler, or assembling the reactions on ice and rapid transfer to a preheated thermal cycler, can minimize this problem. Primers can also be protected by introducing phosphorothioate bonds at their 3' termini (9).

SUMMARY

Promega is proud to offer native *Pfu* DNA Polymerase for high fidelity amplifications desired for cloning, mutagenesis, mutation analysis and long PCR. Our *Pfu* DNA Polymerase was developed and manufactured under the quality standards of ISO 9001. The quality controls performed on our *Pfu* DNA Polymerase include extensive assays for contaminating exonucleases, endonucleases, general nucleases and the ability to amplify a 1.2kb DNA segment from 100 copies of a nonrepeated chromosomal target in genomic DNA. The native enzyme provides exceptional accuracy and reproducible performance in high fidelity PCR.

REFERENCES

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Ordering Information

Product	Size	Cat.#
<i>Pfu</i> DNA Polymerase	100 units	M7741
	500 units	M7745

Pfu DNA Polymerase is supplied with 10X Reaction Buffer containing 20mM MgSO₄.

Related Products

Product	Size	Cat.#
Taq DNA Polymerase	100 units	M1661
	500 units	M1665
pGEM [®] -T Easy Vector, System I	20 reactions	A1360
pGEM [®] -T Easy Vector, System II	20 reactions	A1380

This system is provided with JM109 Competent Cells, High Efficiency.

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