Invitrogen™
life technologies

Platinum® *Pfx* DNA Polymerase

Fidelity data – Questions and Answers

Platinum® *Pfx* DNA Polymerase is a proprietary enzyme preparation containing recombinant DNA polymerase from a *Pyrococcus sp.,* combining the superior performance of Platinum® technology with a novel high fidelity "proofreading" DNA polymerase. It is precomplexed with specific monoclonal antibodies to inhibit DNA polymerase and 3´ exonuclease activities during PCR assembly and the initial denaturation step. Platinum® *Pfx* DNA Polymerase amplifies genomic templates up to 12 kb and plasmid templates up to 20 kb, and is ideal for demanding PCR applications such as site-directed mutagenesis and expression cloning.

How does Platinum® *Pfx* DNA Polymerase fidelity compare to other DNA polymerases?

We determined the fidelity of leading DNA polymerases using the *rps*L Fidelity Assay described below (1). Table 1 shows the average mutation frequency and average error rate for *Taq* DNA Polymerase, *PfuTurbo*® DNA Polymerase and Platinum® *Pfx* DNA Polymerase. Platinum® *Pfx* DNA Polymerase shows a lower mutation frequency and error rate, resulting in a greater relative fidelity than the other enzymes assayed.

DNA Polymerase	Average Mutation Frequency* (% ± SD)	Average Error Rate** (10^{-6} ± SD)	Relative Fidelity
Taq DNA Polymerase	4.37 ± 1.40	41.7 ± 19.0	1X
PfuTurbo® DNA Polymerase	0.27 ± 0.07	2.44 ± 0.42	17X
Platinum® *Pfx* DNA Polymerase	0.15 ± 0.03	1.58 ± 0.46	26X

TABLE 1. Comparison of fidelity of leading DNA polymerases.

The average of five independent *rps*L Fidelity Assays is shown. Two 25-cycle amplifications and three 15-cycle PCR amplification products were analyzed.

* Mutation Frequencies were calculated using the equation: MF = (Ap^r Sm^r colonies) / [Ap^r colonies × dilution factor × 100 %]. Specific MF calculations were as follows: *Taq:* 2,467/56,446; *PfuTurbo®:* 1,068/395,469; Platinum® *Pfx:* 876/584,230.

** Error Rates were calculated using the equation: ER = Mutation Frequency / (Template Doublings × 130 bp).

Template Doublings were determined using the equation: TD = (amount of PCR product) / (amount of starting product). Template Doublings for all three polymerases ranged from 11 to 12 for 25 PCR cycles, and 6 to 7 for 15 cycles.

Why is the *rps*L Fidelity Assay used by Invitrogen?

As shown in figure 1 (on back), the *rps*L Fidelity Assay is a functional assay based upon amplification of the *E. coli rps*L gene, which encodes a protein that is a target of streptomycin. Since PCR-induced mutations within this gene render cells resistant to streptomycin, mutation frequencies of specific DNA polymerases can be determined using simple antibiotic selection. First, the target PCR fragment containing *rps*L is amplified and purified. Then the fragment is self-ligated, followed by transformation into *E. coli*, and plating onto selective medium containing ampicillin and ampicillin plus streptomycin. The number of *rps*L mutants is counted relative to total transformants to determine mutation frequencies which are then used to calculate relative fidelity values. The advantage of this system is greater accuracy due to:

- *Lower background* – Spontaneous mutation frequency for *rps*L is 10^{-6} (compared to 10^{-3}–10^{-4} for *lac*Z, a common target in other fidelity assays), enabling easier selection.

- *Positive selection* – Assay allows for the direct selection of *rps*L mutants on streptomycin-containing plates, versus the extensive platings required for other assay targets such as *lac*Z, which can introduce error and lead to inaccurate counting.

See reverse side for additional information.

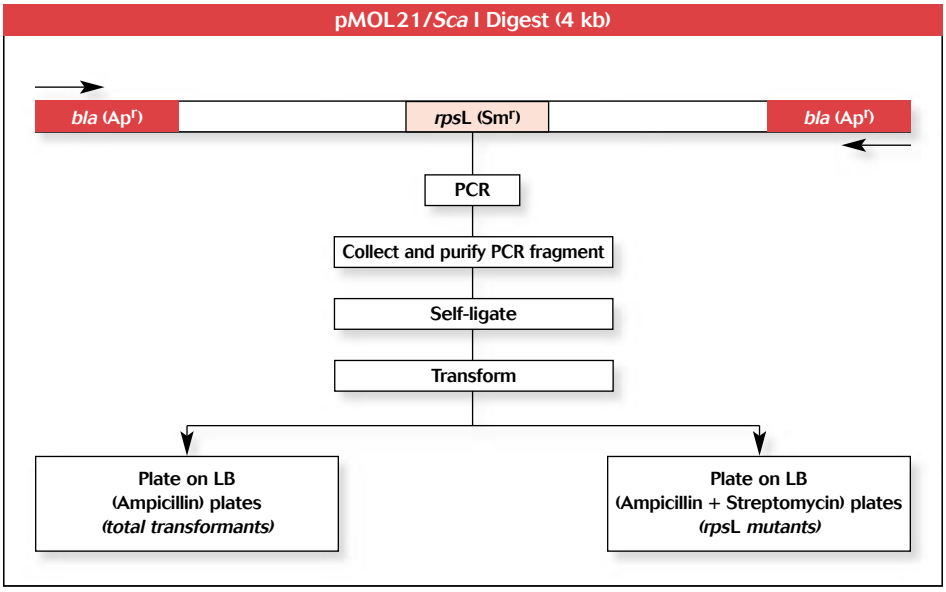

FIGURE 1. Strategy of the *rps*L Fidelity Assay.

Why do these fidelity data differ from other claims and other assay results using the same enzymes?

There are two main reasons for differing fidelity values and error rates:

• *Variation between different assays* – There are multiple means by which fidelity can be assayed. Different DNA polymerase mutation frequencies will be generated depending upon which target sequence and assay system are used. However, relative relationships between the enzymes tested should remain similar from one assay to the next.

• *Variation within the same assay* – When the *rps*L Fidelity Assay was performed by a separate group, the results varied. Although absolute fidelity values were not identical, the relationships between the individual enzyme results were similar. This may be due to different PCR conditions, or more simply, to the variation inherent in biological assays.

Reference:

1. Mo, J.Y., Maki, J., and Sekiguchi, M. (1991) *J. Mol. Biol.* 222, 925.

Ordering information

Description	Cat. No.	Size
Platinum® *Pfx* DNA Polymerase	11708-013	100 rxns
[Includes: 10X *Pfx* Amplification Buffer, 10X PCR_x Enhancer Solution, and 50 mM magnesium sulfate]	11708-021	250 rxns
	11708-039	500 rxns

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