

Polymerases Leave Fingerprints: Analysis of the Mutational Spectrum in *Escherichia coli* *rpoB* To Assess the Role of Polymerase IV in Spontaneous Mutation

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We compared the distribution of mutations in *rpoB* that lead to rifampin resistance in strains with differing levels of polymerase IV (Pol IV), including strains with deletions of the Pol IV-encoding *dinB* gene, strains with a chromosomal copy of *dinB*, strains with the F'128 plasmid, and strains with plasmid amplification of either the *dinB* operon (*dinB-yafNOP*) or the *dinB* gene alone. This analysis identifies several hot spots specific to Pol IV which are virtually absent from the normal spontaneous spectrum, indicating that Pol IV does not contribute significantly to mutations occurring during exponential growth in liquid culture.

Damage-inducible polymerases (20, 22; for reviews, see references 9 and 16), such as the SOS-induced polymerase IV (Pol IV) and Pol V in *Escherichia coli*, not only bypass certain noncoding lesions but also increase replication errors across from normal bases (19, 20, 23). Their discovery has led to the suggestion that a significant fraction of spontaneous mutations in growing cells under normal conditions might be due to errors caused by basal levels of error-prone polymerases (18). The *dinB*-encoded Pol IV is the leading candidate, since the overexpression of *dinB* on high-copy plasmids leads to increases in base substitutions and frameshifts, particularly –1 frameshifts (11, 12, 23). Moreover, several studies have shown an approximately twofold decrease in spontaneous mutations in strains with an inactivating allele of *dinB* that also reduces the expression of three genes downstream of *dinB-yafNOP* (14, 18), although this effect is not present if only *dinB* is inactivated (14). The expression of *dinB* and *yafNOP* is increased after SOS induction by DNA-damaging agents (4), and these four genes have been shown to be part of an operon (14).

We decided to examine the spectra of base substitution mutations in strains with differing levels of *dinB* expression, since a comparison of detailed genetic fingerprints of these strains might reveal patterns specific to processes involving and not involving Pol IV. We recently characterized a system using mutations in the *rpoB* gene that yield the rifampin resistance (Rif^r) phenotype at 37°C in order to analyze the base substitution profiles of mutagens and mutators (10). We have now characterized 77 mutations in *rpoB*. Each of the six base substitutions is monitored with a set of 9 to 17 sites. In the study reported here, we looked at cells that carry a single copy of the *dinB* operon on the chromosome and compared the mutational spectrum of these cells with those of strains with deletions of the *dinB* gene, strains that carry a second copy of the *dinB* operon on an F' plasmid, and strains that carry a multicopy

plasmid with an insert containing the *dinB* operon in one case and just the *dinB* gene in another case. We showed that some mutational hot spots are specific for the overexpression of the *dinB* operon and that others are found in the spectrum of wild-type strains but not after the amplification of the *dinB* operon. A comparison of the different spectra leads us to conclude that spontaneous mutations occurring during exponential growth in the absence of SOS induction or a *dinB*-overexpressing plasmid do not contain a significant contribution from *dinB*-Pol IV-induced mutations.

Table 1 shows the strains used in this work. We examined the frequencies of Rif^r mutants that result from mutations in *rpoB* in strains P90C, CC107, EW90, EW99, EW100, and EW101. EW90 carries a deletion of *dinB* (1) that is nonpolar for the expression of the other genes in the *dinB* operon, and CC107 (5) is a derivative of P90C (15) that has an F'128 with a mutated *lac* operon, *proAB*, and the *dinB* operon (11). The expression of *dinB* from the F' plasmid has been reported to be three times higher than that from the chromosome (11), so CC107 would have four times the *dinB* level of P90C. EW100 is a CC107 derivative carrying a multicopy plasmid that overexpresses the *dinB* operon, and EW101 is the same as EW100 except that the plasmid insert contains only the *dinB* gene. EW99 carries the plasmid without any insert. We did not detect any difference in mutation frequencies among CC107, P90C, or EW90, as shown in Table 2. EW100 and EW101 have six- to sevenfold higher Rif^r frequencies than the EW99 control (Table 2).

We analyzed mutations by direct DNA sequencing (for the methods used, see reference 10) and in a number of cases supplemented the sequence analysis with oligonucleotide hybridization (3, 17). In these cases, colonies were spotted onto LB Omnitrays (Nunc, Rochester, N.Y.), grown overnight at 37°C, and transferred to nylon membranes (Hybond N) from Amersham (Piscataway, N.J.). The membranes were placed in denaturing solution (1.5 M NaCl, 0.5 M NaOH) for 7 min and then in neutralizing solution (1.5 M NaCl, 0.5 M Tris-HCl [pH 7.2], 0.001 M EDTA) for 6 min. The membranes were baked at 80°C for 2 h and prehybridized (5× SSPE [1×

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TABLE 1. *E. coli* K-12 strains used in this study

Strain	Derivation	Plasmid	Genotype	Reference
P90C			<i>ara</i> $\Delta(gpt-lac)5$ <i>thi</i>	15
CC107	F'128 <i>lacIZ proA</i> ⁺ <i>B</i> ⁺		<i>ara</i> $\Delta(gpt-lac)5$ <i>thi</i>	5
EW90 ^a			<i>ara</i> $\Delta dinB61::ble$ $\Delta(gpt-lac)5$ <i>thi</i>	This work
EW99 ^b	CC107	pCR2.1-TOPOcam	<i>ara</i> $\Delta(gpt-lac)5$ <i>thi</i>	This work
EW100 ^c	EW99	<i>dinB-yafNOP</i>	<i>ara</i> $\Delta(gpt-lac)5$ <i>thi</i>	This work
EW101 ^d	EW99	<i>dinB</i>	<i>ara</i> $\Delta(gpt-lac)5$ <i>thi</i>	This work
EM90			<i>ara</i> $\Delta(gpt-lac)5$ <i>mutS::mini-Tn10 thi</i>	This work
EC90			<i>ara</i> $\Delta dinB61::ble$ $\Delta(gpt-lac)5$ <i>mutS::mini-Tn10 thi</i>	This work

^a Contains $\Delta dinB61::ble$, a nonpolar deletion from strain AR30 (1) transferred to a Rif^r derivative of P90C by Pat Foster. We converted this strain to a Rif^s derivative (EW90) by two P1 transductions, crossing in a Tn10 in *argE* and then crossing it out selecting for Arg⁺ and screening for Rif^r. The relevant region of the *rpoB* gene was sequenced for verification.

^b Contains a plasmid derived from pCR2.1-TOPO (Invitrogen, Carlsbad, Calif.) by inserting the *cat* gene.

^c Contains a 5.2-kb fragment derived by partial Sau3AI digestion of *E. coli* DNA and cloned into the BamHI site. This fragment contains *mbhA*, *dinB*, *yafNOP*, and *b0235*.

^d Contains a 1.5-kb fragment insert encoding only the *dinB* gene amplified by PCR with forward primer 5'-GGGGATAAAGTGGTACCGCCGCTGGT-3' and reverse primer 5'-CCGCAACCGGTGGATCCATAAAGTATTTAGC-3' and subsequently cloned into the KpnI-BamHI site.

SSPE is 0.18 M NaCl, 10 mM NaH₂PO₄, and 1 mM EDTA {pH 7.7}, 0.5% sodium dodecyl sulfate [SDS], 5× Denhardt's solution, 100 µg of herring sperm DNA/ml) during shaking at 47°C for 1 h. ³²P-end-labeled probes (5 pmol of oligonucleotide strand, 10 U of T4 polynucleotide kinase, 5× T4 polynucleotide kinase buffer, 10 µCi of [γ -³²P]ATP/µl) were denatured at 100°C for 5 min, and 1 µl was added to hybridization solutions for each oligonucleotide tested. Following hybridization overnight at 47°C, membranes were washed twice in 2× SSPE–0.1% SDS at room temperature for 10 min, once in 1× SSPE–0.1% SDS at 47°C for 15 min, and once in 0.1× SSPE–0.1% SDS at 47°C for 10 min. Autoradiography was carried out with a phosphorimaging screen.

The oligonucleotides were complementary to the mutant and wild-type sequences at the following base mutation sites in *E. coli* (corresponding base pair mutations from the wild type to the mutant are indicated in boldface type): 437 (T→G), 5'-GTGTTATCGTTTCCAGCT-3'; 443 (A→T), 5'-ATCGT TTCCAGCTGCA-3'; 1534 (T→C), 5'-GCCAGCTGTCTCA GTTTAT-3'; 1538 (A→T), 5'-AGCTGTCTCAGTTTATGG A-3'; 1546 (G→A), 5'-TCAGTTTATGGACCAGAA-3'; 1547 (A→G), 5'-GTTTATGGACCAGAAACAAC-3'; 1576 (C→A), 5'-CTGAGATTACGCACAAACG-3'; 1576 (C→T), 5'-TGA GATTACGCACAAACG-3'; 1714 (A→C), 5'-TCGGTCTGA TCAACTCTCT-3'; 1721 (C→T), 5'-TGATCAACTCTCTGT CCGT-3'.

Table 3 shows the sequence results for 1,057 mutations in *rpoB* from the strains described above, as well as from EM90 and EC90, *mutS* (mismatch repair-deficient) derivatives of P90C and EW90, respectively. We can make several comparisons on the basis of Table 3. The sites that are most prominent in the two wild-type spectra, those of CC107 and P90C (with and without F'128, respectively), are well represented in the spectrum of EW90 (which has a deletion of *dinB*). (Note that the CC107 sample is twice the size of both the P90C and the EW90 samples.) For example, the five most frequent changes in the wild-type (CC107 and P90C) samples are AT→GC at sites 1547 and 1534, GC→AT at site 1576, AT→TA at site 443, and AT→CG at site 1714. The first four of these changes are also prominent in the spectrum of EW90 (which has a deletion of *dinB*), and the fifth one (AT→CG at site 1714) is still represented in EW90. Therefore, the polymerases operating in

the absence of Pol IV are capable of producing the hot spots seen in the wild-type spectra. Only the GC→AT transition at site 1576 is lacking in prominence in the spectrum of P90C (the wild type without the F'128), but this mutation is also very prominent in EW90, the *dinB* deletion derivative of P90C. Moreover, the distributions of mutations in a *mutS* background, lacking mismatch repair, are very similar in strains with and without the *dinB* deletion (Table 3).

We can also compare the mutational profiles of strains CC107, P90C, and EW90 with that of EW100, the strain carrying *pdinB-yafNOP* and yielding an elevated level of *rpoB* mutations (Table 2). (It should be noted that the distribution of mutations in CC107 containing the plasmid without an insert [EW99; data not shown] is the same as that for CC107 alone.) A comparison of CC107 and EW100 (with the same sample size of approximately 300 mutations each) shows that although there are marked similarities with regard to some of the hot spots, there are also several clear differences. Most notably, the GC→TA transversion at site 1576 is the most prominent change for EW100 with 62 occurrences, compared with 4 occurrences for CC107. The other transversion at this site, GC→CG, is also more prominent in EW100 than in CC107. In addition, the AT→CG transversion at position 437 is represented by 17 occurrences in the EW100 sample, but it is absent from the spectra of CC107 and P90C (with a sample size of 146). Thus, there are three hot spots present in the Pol IV-overproducing strain (EW100) that are essentially absent from the wild-type (CC107) strain. On the other hand, the

TABLE 2. *rpoB* frequencies and rates of mutations^a

Strain	<i>f</i> (10 ⁻⁸) ^b	μ (10 ⁻⁸) ^b
CC107	7.6 (5.2–8.8)	1.5 (1.1–1.7)
P90C	11 (9.1–14)	1.8 (1.5–2.2)
EW90	7.1 (4.2–9.8)	1.3 (0.82–1.7)
EW99	3.5 (2.0–6.8)	0.65 (0.41–1.1)
EW100	22 (14–30)	3.6 (2.5–4.7)
EW101	26 (14–44)	4.3 (2.6–6.8)

^a The *rpoB* mutation frequency (*f*) per cell was calculated by dividing the median number of mutants by the average number of cells in a series of cultures, and the mutation rate (μ) per replication was calculated from these values by the method of Drake (7).

^b Mean value with 95% confidence limit (6) in parentheses.

TABLE 3. Distribution of mutations in *rpoB*

Site (bp)	Amino acid change	Base pair change	No. of occurrences for:					
			EW100	CC107	P90C	EW90	EM90	EC90
443	Q148R	AT→GC	2	5	4	0	0	0
1522	S508P	AT→GC	2	0	0	1	1	0
1532	L511P	AT→GC	1	3	0	0	2	0
1534	S512P	AT→GC	13	17	13	11	22	11
1538	Q513R	AT→GC	1	0	0	0	3	0
1547	D516G	AT→GC	46	40	16	27	36	67
1552	N518D	AT→GC	2	1	2	1	2	1
1577	H526R	AT→GC	0	0	1	0	8	0
1598	L533P	AT→GC	0	1	1	5	1	0
1703 ^a	N568S	AT→GC	0	0	1	0	0	0
1715	I572T	AT→GC	0	1	1	8	0	0
1520	G507D	GC→AT	0	0	0	0	0	0
1535	S512F	GC→AT	5	6	1	1	0	0
1546	D516N	GC→AT	23	5	0	1	5	0
1565	S522F	GC→AT	4	7	0	1	1	0
1576	H526Y	GC→AT	12	25	2	11	1	0
1585	R529C	GC→AT	1	3	0	0	1	0
1586	R529H	GC→AT	2	3	0	0	0	0
1592	S531F	GC→AT	7	7	0	0	0	0
1595	A532V	GC→AT	0	0	0	0	0	0
1600	G534S	GC→AT	0	0	0	0	0	0
1601	G534D	GC→AT	0	0	0	3	0	0
1609	G537S	GC→AT	0	0	0	0	0	0
1610 ^a	G537D	GC→AT	0	0	0	1	0	0
1691	P564L	GC→AT	4	7	0	2	1	0
1708	G570S	GC→AT	0	0	0	0	0	0
1721	S574F	GC→AT	3	10	4	0	0	0
2060 ^b	R687H	GC→AT	0	0	0	0	0	0
443	Q148L	AT→TA	4	38	27	9	0	0
1532	L511Q	AT→TA	0	3	4	0	0	0
1538	Q513L	AT→TA	2	11	8	0	0	0
1547	D516V	AT→TA	2	1	3	5	0	0
1568	E523V	AT→TA	0	0	0	0	0	0
1577	H526L	AT→TA	1	0	1	2	0	0
1598	L533H	AT→TA	2	3	0	0	0	0
1714	I572F	AT→TA	4	7	2	7	3	1
1715	I572N	AT→TA	1	2	9	16	0	0
437	V146G	AT→CG	17	0	0	1	0	0
443	Q148P	AT→CG	0	0	0	2	0	0
1525	S509R	AT→CG	0	1	0	2	0	0
1532	L511R	AT→CG	0	3	4	4	0	0
1534	S512A	AT→CG	0	0	0	0	0	0
1538	Q513P	AT→CG	3	2	2	6	0	0
1547	D516A	AT→CG	0	1	1	0	0	0
1577	H526P	AT→CG	2	3	0	0	0	0
1598	L533R	AT→CG	0	0	0	1	0	0
1687	T563P	AT→CG	0	0	6	4	0	0
1714	I572L	AT→CG	28	31	8	6	0	0
1715	I572S	AT→CG	2	13	12	10	0	0
436	V146F	GC→TA	4	2	0	1	0	0
442	Q148K	GC→TA	0	0	0	0	0	0
444	Q148H	GC→TA	0	1	2	0	0	0
1527	S509R	GC→TA	0	3	0	0	0	0
1535	S512Y	GC→TA	1	1	1	0	0	0
1537	Q513K	GC→TA	1	1	0	0	0	0
1546	D516Y	GC→TA	1	2	0	0	0	0
1565	S522Y	GC→TA	1	1	0	0	0	0
1576	H526N	GC→TA	62	4	1	1	1	0
1578	H526Q	GC→TA	0	0	0	0	0	0
1586	R529L	GC→TA	0	0	0	0	0	0
1592	S531Y	GC→TA	2	0	0	0	0	0
1595	A532E	GC→TA	0	0	0	1	0	0
1600	G534C	GC→TA	0	4	2	0	0	0

Continued on following page

TABLE 3—Continued

Site (bp)	Amino acid change	Base pair change	No. of occurrences for:					
			EW100	CC107	P90C	EW90	EM90	EC90
1601	G534V	GC→TA	0	1	1	1	0	0
1708	G570C	GC→TA	0	0	1	0	0	0
1721	S574Y	GC→TA	1	5	3	1	0	0
1585 ^b	R529S	GC→TA	0	0	0	0	0	0
444	Q148H	GC→CG	0	0	0	0	0	0
1527	S509R	GC→CG	0	1	1	1	0	0
1574	T525R	GC→CG	0	2	0	0	0	0
1576	H526D	GC→CG	20	5	0	0	0	0
1578	H526Q	GC→CG	0	0	0	1	0	0
1585	R529G	GC→CG	0	0	0	0	0	0
1600	G534R	GC→CG	0	0	0	0	0	0
1601	G534A	GC→CG	0	1	0	0	0	0
1691	P564R	GC→CG	0	0	0	0	0	0
1709	G570A	GC→CG	0	0	0	0	0	0
1716	I572M	GC→CG	0	0	0	0	0	0
2059	R687G	GC→CG	0	0	0	0	0	0
Not found			0	4	1	1	0	0
Total ^c			289	298	146	156	88	80

^a First described in this study.
^b Shows temperature effects between 30 and 42°C and may not yield Rif^r colonies at 37°C.
^c The results for 77 sites were considered for the total, but the number increases to 79 with the inclusion of sites 2060 and 1585.

AT→TA transversion at 443 appears to occur frequently in all of the strains that lack the Pol IV-overexpressing plasmid but not in the strain (EW100) with the overexpressing plasmid (38 occurrences in CC107 versus 4 occurrences in EW100 for the same sample size).

Figure 1 displays the data from Table 3 by position so that different base substitutions at the same site can be visualized

together while the mutation type can still be distinguished. It can be seen that position 1576 is the most prominent hot spot in the EW100 (*pdinB-yafNOP*) spectrum, with the total number of mutations there accounting for 30% of all the base substitutions in the EW100 profile. In contrast to the predominance of GC→TA and GC→CG transversions at site 1576 in the *pdinB-yafNOP* (EW100) spectrum, mutations at this site in

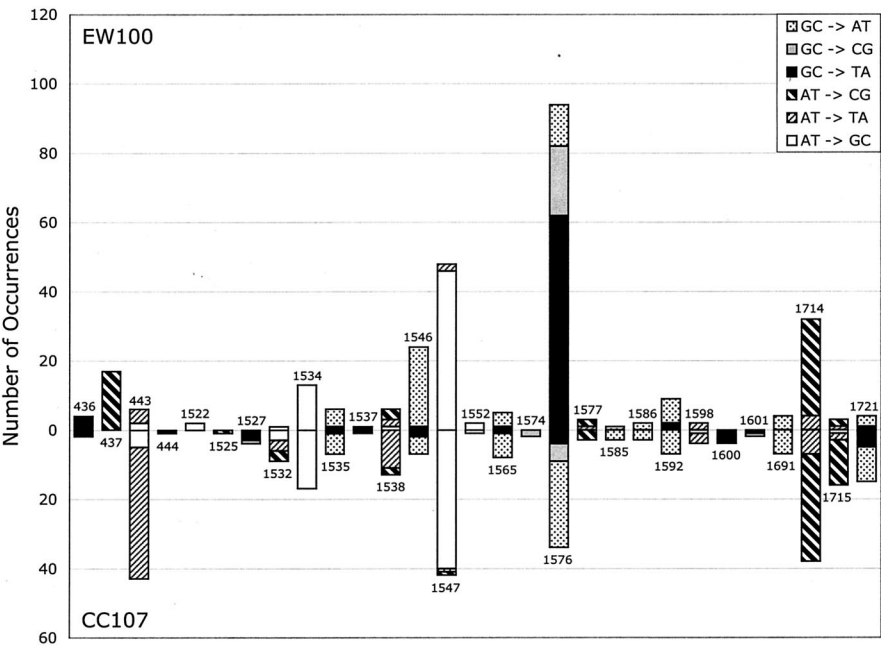


FIG. 1. Distribution of mutations in *rpoB* that lead to Rif^r in the wild-type strain (CC107) and in a derivative (EW100) carrying *dinB-yafNOP* on a multicopy plasmid. Similar sample sizes (294 for CC107 and 289 for EW100) were used. Different base substitutions at the same site are indicated by different patterns. The positions of the sites are indicated with numbers above and below (see Table 3) and are not to scale.

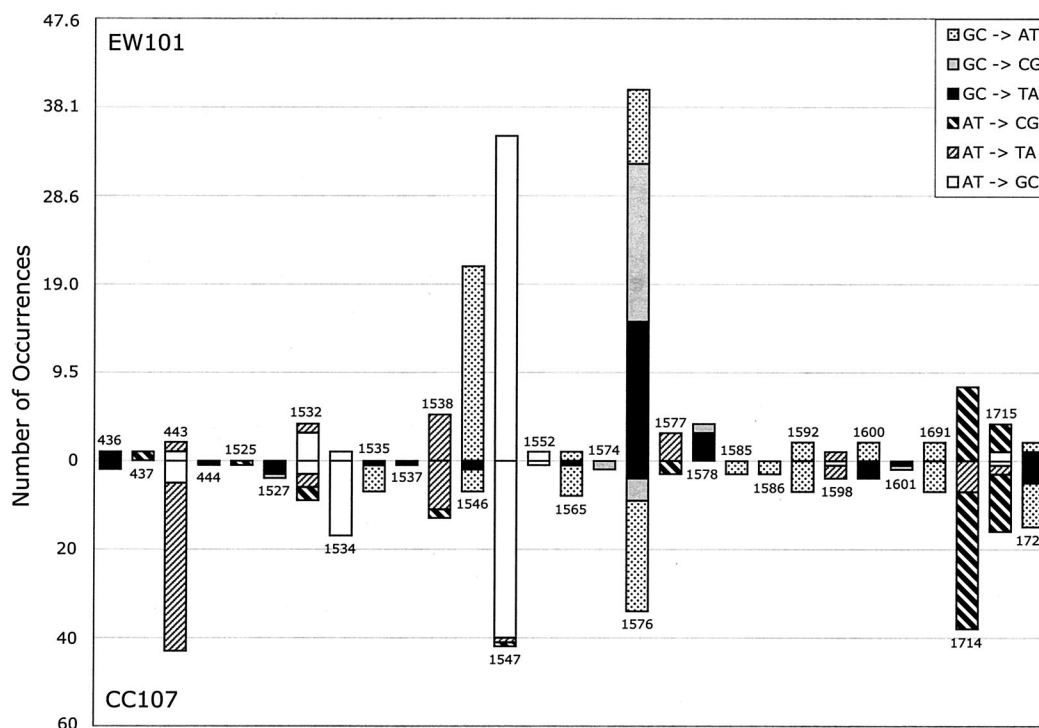


FIG. 2. Distribution of mutations in *rpoB* that lead to Rif^r in the wild-type strain (CC107) and a derivative (EW101) carrying the *dinB* gene on a multicopy plasmid. Because different sample sizes are used, the peak heights here represent percentages of the sample size (140 for EW101 and 294 for CC107, with the heights of the EW101 peaks being effectively normalized [by multiplying by 2.1] to fit the CC107 sample size). The vertical axis indicates actual numbers, but the scales of the peak heights differ. This approach allows a visual comparison with Fig. 1. Note that the transversions at site 1576 are prominent in EW101, as they are in EW100 (Fig. 1). In EW101, at site 1576, GC→CG transversions occur in 17 of 140 instances, compared with 5 of 294 instances for CC107, and GC→TA transversions occur in 15 of 140 instances, compared with 4 of 294 instances for CC107.

the wild-type (CC107) spectrum are mostly GC→AT transitions. Although there are many similarities between these two spectra, they clearly differ at positions 437 and 443, in addition to the pronounced difference in transversions at 1576. We also found no difference in the distributions of mutations in EW100 (carrying *pdinB-yafNOP*) and EW101 (carrying *pdinB*) (Fig. 2).

Taken together, the data in Table 3 and Fig. 1 and 2 argue that spontaneous base substitutions in wild-type strains such as P90C and CC107 do not contain significant contributions from the *dinB*-encoded Pol IV or products of the other *dinB* operon genes (*yafNOP*). The underrepresentation of the prominent hot spots in *rpoB* that are specific to the *pdinB-yafNOP*-containing strain (EW100) in the spectrum of mutations from CC107 allows us to place an upper limit on the contribution from Pol IV and putative products of the *yafNOP* genes at no more than 10%. The data also indicate that there is no difference in the base substitution mutational spectra of strains with and without the F'128 plasmid, even though there is a fourfold difference in Pol IV levels. It should be noted that all of these determinations are for mutations occurring in growing cells. For adaptive mutations occurring in the stationary phase (2, 8), a requirement for Pol IV has been reported (13, 21).

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