Published in final edited form as:
*DNA Repair (Amst)*. 2010 September 4; 9(9): 958–963. doi:10.1016/j.dnarep.2010.06.007.

# Transcription-coupled Nucleotide Excision Repair of a Gene Transcribed by bacteriophage T7 RNA Polymerase in *Escherichia coli*

**Ann K. Ganesan*** and **Philip C. Hanawalt**
Department of Biology, Stanford University, Stanford, CA 94305-5020

## Abstract

Transcription-coupled nucleotide excision repair (TC-NER) removes certain kinds of lesions from the transcribed strand of expressed genes. The signal for TC-NER is thought to be RNA polymerase stalled at a lesion in the DNA template. In *Escherichia coli*, the stalled polymerase is dissociated from the lesion by the transcription repair coupling factor (Mfd protein), which also recruits excision repair proteins to the site resulting in efficient removal of the lesion. TC-NER has been documented in cells from a variety of organisms ranging from bacteria to humans. In each case, the RNA polymerase involved has been a multimeric protein complex. To ascertain whether a gene transcribed by the monomeric RNA polymerase of bacteriophage T7 could be repaired by TC-NER, we constructed strains of *Escherichia coli* in which the chromosomal *lacZ* gene is controlled by a T7 promoter. In the absence of T7 RNA polymerase, little or no β-galactosidase is produced, indicating that the *E. coli* RNA polymerase does not transcribe *lacZ* efficiently, if at all, in these strains. By introducing a plasmid (pAR1219) carrying the T7 gene 1 under control of the *E. coli* lac UV5 promoter into these strains, we obtained derivatives in which the level of T7 RNA polymerase could be regulated. In cultures containing upregulated levels of the polymerase, β-galactosidase was actively produced indicating that the T7 RNA polymerase transcribes the *lacZ* gene efficiently. Under these conditions, we observed that UV-induced cyclobutane pyrimidine dimers were removed more rapidly from the transcribed strand of *lacZ* than from the nontranscribed strand, supporting the conclusion that TC-NER occurred in this gene. This response was absent in an *mfd-1* mutant, indicating that the underlying mechanism may be similar to that for the bacterial RNA polymerase.

### Keywords

transcription-coupled nucleotide excision repair; bacteriophage T7 RNA polymerase; *lacZ*; T7 promoter; pAR1219



*Corresponding author, Telephone 1-650 723-2425, Fax 1-650 725-1848, anngan@stanford.edu.



Conflict of interest statement
The authors declare that there are no conflicts of interest.

## 1. Introduction

Nucleotide excision repair (NER) removes a variety of lesions from DNA. It is generally accepted that NER comprises two subpathways: transcription coupled nucleotide excision repair (TC-NER), and global genome nucleotide excision repair (GG-NER) (reviewed in [1]). The former repairs lesions in the transcribed strand of expressed genes, while the latter repairs lesions in the genome overall. When bacteriophage T7 damaged by UV light infects *E. coli*, the phage DNA can be repaired by the bacterial NER system, resulting in "host cell reactivation" of the phage [2]. It is not known, however, whether TC-NER can contribute to this reactivation.

In *E. coli*, TC-NER of damaged bacterial DNA requires RNA polymerase and Mfd, the product of the *mfd* gene, in addition to the UvrA, UvrB, and UvrC proteins, which recognize a DNA lesion and incise the strand containing it [1,3,4]. Mutants deficient in Mfd do not show TC-NER [5–7]. According to current ideas, lesions that block the RNA polymerase (e.g. cyclobutane pyrimidine dimers (CPDs)), are substrates for TC-NER. The blocked polymerase, however, occludes the lesion and prevents access by the incision complex [8–10]. The Mfd protein, also known as the transcription repair coupling factor, is required to displace the polymerase, allowing repair to occur. In addition to displacing the RNA polymerase, Mfd is thought to recruit UvrA to the site to initiate NER, thus enhancing the rate of repair [3,4,11,12]. TC-NER is detected experimentally when the transcribed strand of an expressed gene is repaired more rapidly than the nontranscribed strand of the gene [13,14].

The RNA polymerase of *E. coli* is a complex of several different proteins. In contrast, the RNA polymerase of T7 is a monomeric protein. The elongation complex of the T7 enzyme, like that of *E. coli*, can be stalled by a CPD in the transcribed strand of a gene [10]. Results obtained from studies of defined systems in vitro, however, indicate that the T7 polymerase can bypass CPDs more readily than can the multimeric polymerases [15,16]. Furthermore, unlike the *E. coli* RNA polymerase, the T7 polymerase does not appear to interact with the Mfd protein in vitro [10]. Therefore, as a test of the generality of the proposed mechanism for TC-NER, it was of interest to evaluate the possibility that a gene transcribed by T7 RNA polymerase might show this type of repair.

We constructed *E. coli* derivatives in which a T7 promoter controls the *lac* operon, and the T7 RNA polymerase is supplied by a plasmid carrying the T7 gene 1. Our results show that TC-NER can occur in the *lac* operon when it is transcribed by the T7 RNA polymerase; furthermore, in *mfd-1* derivatives, we did not observe TC-NER, indicating that even when a gene is transcribed by the T7 RNA polymerase Mfd is required for TC-NER.

## Materials and Methods

### 2.1. Bacteria

Table 1 lists the derivatives of *E. coli* K-12 used.

### 2.2 Plasmid

The plasmid used, pAR1219 [17], contains the T7 gene 1, which encodes the T7 RNA polymerase. The gene is regulated by the lac UV5 promoter. The plasmid also carries the *E. coli lacI* gene, which codes for the lac repressor protein. When bacteria carrying this plasmid are grown in the presence of an inducer like isopropyl-β-D-thiogalactopyranoside (IPTG), the inducer binds to the repressor, preventing it from associating with the UV5 promoter. In the absence of an inducer, the repressor can bind to the promoter and prevent synthesis of the T7 RNA polymerase.

### 2.3 Media

The minimal medium (MM) was Difco Minimal Broth Davis containing 0.4% glucose and 0.05% vitamin-free casamino acids. For plates, 1.5% Difco Bacto agar was added. The complex medium was LB [18]. For LB plates, 1.2% Difco Bacto agar was added. L-broth [19] containing 1 g/l of glucose was used for preparing P1vir phage.

For thymine-requiring strains, 10 μg/ml of thymine was added to all media.

When needed, kanamycin was incorporated into plates at a concentration of 25 μg/ml, chloramphenicol at a concentration of 30 μg/ml, ampicillin at a concentration of 30 μg/ml, and tetracycline at a concentration of 15 μg/ml. To select against *sacB*, bacteria were plated on Difco Minimal Broth Davis containing 0.2% glycerol, 5% sucrose and 0.05% vitamin-free casamino acids.

The T7 gene 1 carried on the plasmid, pAR1219, was induced by growing cultures in MM containing 1 mM IPTG.

### 2.4 Recombineering

To construct *E. coli* derivatives with the *lacZ* gene under control of the T7 promoter, we used recombineering [20] with NC397 as the starting strain [21, Supporting Information]. NC397 contains the *cat-sacB* cassette for counterselection upstream from *lacZ*, and the gene conferring kanamycin resistance upstream from the *cat-sacB* cassette. Using a 100 base lagging strand oligonucleotide (5'GGGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGAATTtaatacgactcactataggg ACAGGAAACAGCTATGACCATGATTACGGATTCACTGGCC) containing the T7 promoter sequence (lower case letters) flanked by 40 bases of kan on one side and 40 bases of *lacZ* on the other, we were able to replace the *cat-sacB* cassette with the T7 promoter (Fig. 1). The products of recombineering were sensitive to chloramphenicol, resistant to sucrose, and resistant to kanamycin. The resulting kan-T7p-lacZ sequence was transferred to SR108 by P1 transduction. Finally, the plasmid pAR1219 [17] was introduced by electroporation.

Oligonucleotides for recombineering and for PCR were purchased from Midland Certified Reagent Company, Inc. (www.mcrc.com).

### 2.5 Transduction

Lysates of P1vir were prepared, and transductions performed, essentially as described by Miller [22].

### 2.6 Electroporation

Late exponential phase cultures (25 ml) were pelleted by centrifugation, the pellets were washed with 25 ml of chilled, sterile water, and pelleted again. The pellets were resuspended in 1 ml chilled water and the suspensions were transferred to 1.5 ml microfuge tubes. The cells were pelleted once more and resuspended in 100 μl chilled water. To the cell suspension, 5 μl of pAR1219 (92 mg/ml) was added, and 40 μl of the mixture was placed in an electroporation cuvette (1 mm gap). An Eppendorf Electroporator 2510, set at 1800v, was used to deliver the pulse, after which the mixture was added to 1 ml of LB medium and incubated for 2 hours at 37° C before being plated on LB+ampicillin. Ampicillin-resistant derivatives that produced substantial amounts of β-galactosidase after growth with IPTG were isolated and tested for TC-NER.

### 2.7 DNA Sequencing

Results of recombineering were verified using primers recommended by D.L. Court (personal communication) for amplifying and sequencing the region around, and including, the T7 promoter (Fig. 2). Sequencing was done by Sequetech Corp. (Mountain View CA). Sequencher 4.6 (Gene Codes Corp., MI) was used to compare the sequences to the wild type sequence listed in EcoCyc [23].

### 2.8 Irradiation

The UV source was an unfiltered 15 w germicidal bulb emitting primarily 254 nm. The incident dose rate was 0.6 $J/m^2$/sec. In each experiment, the dose used was chosen to produce approximately one CPD per strand of the DNA restriction fragment in which repair was to be analyzed. Irradiated cultures were maintained in yellow light (>500 nm) to avoid photoreactivation.

### 2.9 Assays for β-galactosidase

Enzyme activity was assayed with o-nitrophenyl-β-D-galactoside (ONPG) essentially as described by Miller [18].

### 2.10 Repair assays

The frequencies of CPDs in individual strands of the *lac* operon were measured as previously described [24,25]. Exponentially growing cultures of *E. coli* in defined medium (MM) were irradiated and reincubated to allow repair to occur. Samples (5 ml) were removed from each culture at intervals, and mixed with equal volumes of ice cold NET (100 mM NaCl, 10 mM EDTA, 10 mM Tris-HCl). The cells were pelleted by centrifugation and stored at −80° C. Subsequently, the DNA was purified on QIAGEN Genomic-tip columns, treated with restriction enzymes and T4 endonuclease V (TEV), then analyzed by electrophoresis in alkaline agarose gels. After the DNA had been transferred from the gels to Amersham Hybond-$N^+$ membranes (GE Healthcare), each membrane was hybridized with a $^{32}$P-labeled strand-specific RNA probe for the restriction fragment of interest. Probes were made using the Promega Riboprobe System (Promega Corp., Madison, WI) with pZH-10 [14] as the template. The amount of radioactivity hybridized to the full length DNA fragment was measured using a phosphorimager (Bio-Rad GS-363 Molecular Imaging System) and associated software (Molecular Analyst, version 2.1).

CPD frequencies were calculated from the percentage of full length fragments (i.e. fragments that had no CPDs, the “0 class”), using the Poisson expression [24,25].

At least two biological experiments were performed with each strain for each DNA fragment examined. For statistical analysis and graphing of results we used Prism 4 (GraphPad Software, Inc.). Error bars on graphs represent SEM.

## 3. Results

### 3.1 Strain construction

To obtain derivatives of *E. coli* K-12 containing a gene located on the chromosome and transcribed by the T7 RNA polymerase, we replaced the *cat-sacB* cassette of NC397 with the T7 promoter using "recombineering" [20] (see Materials and Methods). This resulted in kanamycin-resistant derivatives in which the *lacZ* gene was controlled by the T7 promoter (Fig. 1). The kan-T7p-lac region from two of these (HL1134 and HL1172, respectively) was transferred to SR108 by P1 transduction using kanamycin to select the transductants. The plasmid, pAR1219 [17], was then electroporated into two of the transductants (HL1132 and

HL1173, respectively). Among the ampicillin-resistant electroporation products, four (HL1159, HL1160, HL1176, and HL1177) that produced substantial amounts of β-galactosidase when induced with IPTG were isolated. PCR and sequencing were used to verify that the T7 promoter was intact and in the expected location. Table 2 shows the β-galactosidase levels in strains grown with (Induced) and without (Not Induced) IPTG. The low levels of β-galactosidase in induced cultures of HL1132, which does not contain pAR1219, compared to those in its parent SR108, are consistent with the idea that in the absence of the T7 RNA polymerase, the *lacZ* gene in this strain is not transcribed well, and that the *E. coli* RNA polymerase does not recognize the T7 promoter efficiently, if at all. The high levels of β-galactosidase in induced cultures of HL1159 and HL1160 indicate that the *lacZ* gene in these strains is efficiently transcribed by the T7 RNA polymerase produced from pAR1219. Qualitatively similar results (not shown) were obtained with HL1176 and HL1177.

### 3.2 DNA Repair

To examine DNA repair in HL1159 and HL1160, we irradiated cultures growing exponentially in MM + IPTG and measured the removal of CPDs from individual strands of a 4.8 kb Bsu36 I - NruI fragment of the *lac* operon (Fig. 3). In previous work [14,25], a 6.6 kb ApaI - SacII fragment of the *lac* operon had been analyzed, but sequence changes in the strains we constructed precluded the use of this fragment. In both HL1159 and HL1160 the transcribed strand of the fragment was repaired more rapidly than the nontranscribed strand (Fig. 4), a response typical of TC-NER. In Fig. 4B the results obtained with HL1159 and HL1160 are combined because these strains are siblings with identical genotypes, and because they showed no significant differences in their repair responses. Similar results were obtained with HL1176 and HL1177, although fewer experiments were performed with these isolates (data not shown). Results obtained by analyzing the 6.6 kb fragment of the *lac* operon in the parental strain SR108, in which the *lac* operon is transcribed by the bacterial RNA polymerase, are shown for comparison (Fig. 4B).

In addition to analyzing the 4.8 kb fragment, we examined a smaller fragment (2.8 kb), produced by digestion with Bsu36 I and EcoRI. Unlike the 4.8 kb fragment, this fragment is entirely within the *lacZ* gene (Fig. 3). When DNA from cultures induced with IPTG was analyzed, we found, as before, that the transcribed strand of *lacZ* was repaired rapidly; however, when DNA from uninduced cultures was examined, we found that the transcribed strand was repaired slowly (Fig. 5). Thus, rapid repair of the transcribed strand of *lacZ* depends upon the gene being actively transcribed, as expected for TC-NER.

Having ascertained that TC-NER could be observed in the *lacZ* gene transcribed by the T7 RNA polymerase, we examined *mfd-1* derivatives. Using UNC361045 [10] as the donor, the *mfd-1* mutation linked to *zcf-117::Tn10* was introduced into HL1132 by P1 transduction. Tetracycline-resistant transductants were isolated and tested for *mfd-1* using UV sensitivity as the initial criterion. A UV-sensitive transductant (HL1167) was isolated. The plasmid p1219 was introduced into it by electroporation. Production of β-galactosidase was measured in the resulting strains (HL1179, HL1180, respectively). Both strains showed higher levels of β-galactosidase when induced with IPTG than when not induced (Table 2), consistent with transcription of *lacZ* by the T7 RNA polymerase. In these strains, no TC-NER of the *lacZ* gene was observed (Fig. 6). The repair results obtained for HL1179 and HL1180 were combined for presentation in Fig. 6 because they were siblings with identical genotypes and showed no significant differences in their repair responses.

## 4. Discussion

Our results show that a gene transcribed by the RNA polymerase of bacteriophage T7 can be repaired by TC-NER in *E. coli*. When the *lacZ* gene was transcribed by T7 RNA polymerase, CPDs were removed more rapidly from the transcribed strand than from the nontranscribed strand of the gene (Fig. 4). Rapid repair of the transcribed strand occurred only when the gene was actively transcribed (Fig. 5) and only when the wild type allele of *mfd* was present (Fig. 6). These responses are the hallmarks of TC-NER in *E. coli* [14].

The signal for TC-NER is thought to be RNA polymerase blocked at a DNA lesion. For purified T7 RNA polymerase in vitro, many "bulky" lesions in DNA, including CPDs, are only partial blocks to transcription [15,16,26]; nevertheless, except for the 20 minute samples, the amount of repair of CPDs in the transcribed strand of *lacZ* was essentially the same whether the gene was transcribed by the T7 RNA polymerase or the *E. coli* RNA polymerase (Fig. 4B).

The mechanism by which the T7 RNA polymerase promotes TC-NER may be similar to the one used by the *E. coli* polymerase. In wild type *E. coli* K-12 the Mfd protein is required for TC-NER, and Mfd-deficient mutants do not show this response [5–7]. The Mfd protein interacts with the β subunit of the *E. coli* RNA polymerase when the polymerase is blocked at a lesion, such as a CPD, in the transcribed strand of a gene [11,27]. Mfd dissociates the RNA polymerase from the lesion, allowing repair to occur. In addition, it enhances the rate of repair in the transcribed strand of the gene [3,4,12]. The mechanism by which this occurs is not well understood, but Mfd has a UvrB homology module [10] that may interact with UvrA in the $UvrA_2B_2$ complex [28], and facilitate the binding of UvrB to DNA. Studies of the purified Mfd protein in vitro indicated that, although it interacted with the *E. coli* RNA polymerase, it did not interact with the T7 polymerase, and it did not displace the T7 polymerase stalled at a lesion in DNA [10]. It therefore seemed unlikely that TC-NER mediated by the T7 RNA polymerase would depend upon Mfd; however, our results with the *mfd-1* derivatives show that Mfd is required for T7 RNA polymerase-dependent TC-NER (Fig. 6). The common requirement for Mfd may indicate that TC-NER in *E. coli* involves similar mechanisms whether the bacterial or the phage RNA polymerase recognizes the lesions; however, there may be another factor (or factors) required by the T7 polymerase, but not the bacterial polymerase, that was missing from the reactions in vitro.

## Abbreviations

| | |
|---|---|
| **NER** | nucleotide excision repair |
| **TC-NER** | transcription-coupled nucleotide excision repair |
| **GG-NER** | global genomic nucleotide excision repair |
| **IPTG** | isopropyl-β-D-thiogalactopyranoside |
| **ONPG** | *o*-nitrophenyl-β-D-galactopyranoside |

## Acknowledgments

This work was supported by grant CA-090915 to P.C.H. from the National Cancer Institute (NIH). We thank Dr. D. L. Court (NCI) for essential bacterial strains and advice; Dr. F. W. Studier (Brookhaven National Laboratory) for pAR1219, Dr. B. Van Houten (University of Pittsburgh), and Dr. G. B. Sancar (University of North Carolina) for helpful discussions, and Dr. G. Spivak (Stanford) for critical reading of the manuscript.

## References


1. Hanawalt PC, Spivak G. Transcription-coupled DNA repair: two decades of progress and surprises. Nat. Rev. Mol. Cell. Biol. 2008; 9:958–970. [PubMed: 19023283]
2. Kuemmerle NB, Masker WE. In vitro packaging of UV radiation-damaged DNA from bacteriophage T7. J. Virol. 1977; 23:509–516. [PubMed: 330877]
3. Savery NJ. The molecular mechanism of transcription-coupled DNA repair. Trends. Microbiol. 2007; 15:326–333. [PubMed: 17572090]
4. Deaconescu AM, Savery N, Darst SA. The bacterial transcription repair coupling factor. Curr. Opin. Struct. Biol. 2007; 17:96–102. [PubMed: 17239578]
5. Selby CP, Sancar A. Gene- and strand-specific repair in vitro: Partial purification of a transcription-repair coupling factor. Proc. Natl. Acad. Sciences. USA. 1991; 88:8232–8236.
6. Selby CP, Witkin EM, Sancar A. *Escherichia coli mfd* mutant deficient in "mutation frequency decline" lacks strand-specific repair: in vitro complementation with purified coupling factor. Proc. Natl. Acad. Sciences. USA. 1991; 88:11574–11578.
7. Mellon I, Champe GN. Products of DNA mismatch repair genes *mutS* and *mutL* are required for transcription-coupled nucleotide excision repair of the lactose operon in Escherichia coli. PNAS. 1996; 93:1292–1297. [PubMed: 8577757]
8. Selby CP, Sancar A. Transcription preferentially inhibits nucleotide excision repair of the template strand in vitro. J. Biol. Chem. 1990; 265:21330–21336. [PubMed: 2250027]
9. Donahue BA, Yin S, Taylor JS, Reines D, Hanawalt PC. Transcript cleavage by RNA polymerase II arrested by a cyclobutane pyrimidine dimer in the DNA template. Proc. Natl. Acad. Sci. U. S. A. 1994; 91:8502–8506. [PubMed: 8078911]
10. Selby CP, Sancar A. Molecular mechanism of transcription-repair coupling. Science. 1993; 260:53–58. [PubMed: 8465200]
11. Selby CP, Sancar A. Structure and function of transcription-repair coupling factor. II. Catalytic properties. J. Biol. Chem. 1995; 270:4890–4895. [PubMed: 7876262]
12. Assenmacher N, Wenig K, Lammens A, Hopfner KP. Structural Basis for Transcription-coupled Repair: the N Terminus of Mfd Resembles UvrB with Degenerate ATPase Motifs. J. Mol. Biol. 2006; 355:675–683. [PubMed: 16309703]
13. Mellon I, Spivak G, Hanawalt PC. Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. Cell. 1987; 51:241–249. [PubMed: 3664636]
14. Mellon I, Hanawalt PC. Induction of the *Escherichia coli* lactose operon selectively increases repair of its transcribed DNA strand. Nature. 1989; 342:95–98. [PubMed: 2554145]
15. Smith CA, Baeten J, Taylor JS. The ability of a variety of polymerases to synthesize past site-specific cis-syn, trans-syn-II, (6-4), and Dewar photoproducts of thymidylyl-(3'-->5')-thymidine. J. Biol. Chem. 1998; 273:21933–21940. [PubMed: 9705333]
16. Kalogeraki VS, Tornaletti S, Hanawalt PC. Transcription arrest at a lesion in the transcribed DNA strand in vitro is not affected by a nearby lesion in the opposite strand. J. Biol. Chem. 2003; 278:19558–19564. [PubMed: 12646562]
17. Davanloo P, Rosenberg AH, Dunn JJ, Studier FW. Cloning and expression of the gene for bacteriophage T7 RNA polymerase. PNAS. 1984; 81:2035–2039. [PubMed: 6371808]
18. Miller, JH. Experiments in Molecular Genetics. Cold Spring Harbor Laboratory; 1972.
19. Maloy, SR. Experimental Techniques in Bacterial Genetics. Jones and Bartlett; 1990.
20. Thomason L, Court DL, Bubunenko M, Costantino N, Wilson H, Datta S, Oppenheim A. Recombineering: genetic engineering in bacteria using homologous recombination. Chapter 1. Curr. Protoc. Mol. Biol. 2007 Unit 1.16.
21. Svenningsen SL, Costantino N, Court DL, Adhya S. On the role of Cro in lambda prophage induction. Proc. Natl. Acad. Sci. U. S. A. 2005; 102:4465–4469. [PubMed: 15728734]
22. Miller, JH. A Short Course in Bacterial Genetics. Cold Spring Harbor Laboratory Press; 1992.

23. Keseler IM, Collado-Vides J, Gama-Castro S, Ingraham J, Paley S, Paulsen IT, Peralta-Gil M, Karp PD. EcoCyc: a comprehensive database resource for *Escherichia coli*. Nucleic. Acids. Res. 2005; 33:D334–D337. [PubMed: 15608210]
24. Spivak G, Hanawalt PC. Determination of damage and repair in specific DNA sequences. Methods:. A. Companion. to. Methods. in. Enzymology. 1995; 7:147–161.
25. Ganesan AK, Smith AJ, Savery NJ, Zamos P, Hanawalt PC. Transcription coupled nucleotide excision repair in *Escherichia coli* can be affected by changing the arginine at position 529 of the beta subunit of RNA polymerase. DNA Repair. (Amst). 2007; 6:1434–1440. [PubMed: 17532270]
26. Scicchitano DA. Transcription past DNA adducts derived from polycyclic aromatic hydrocarbons. Mutat. Res. 2005; 577:146–154. [PubMed: 15922365]
27. Selby CP, Sancar A. Structure and function of transcription-repair coupling factor. 1. Structural domains and binding properties. J. Biol. Chem. 1995; 270:4882–4889. [PubMed: 7876261]
28. Malta E, Moolenaar GF, Goosen N. Dynamics of the UvrABC nucleotide excision repair proteins analyzed by fluorescence resonance energy transfer. Biochemistry. 2007; 46:9080–9088. [PubMed: 17630776]
29. Hanawalt, PC. Photophysiology: Current Topics. Academic Press; 1968. Recovery from Photochemical Damage; p. 203-251.
30. Sancar A, Franklin KA, Sancar GB. Escherichia coli DNA photolyase stimulates uvrABC excision nuclease in vitro. Proc. Natl. Acad. Sci. U. S. A. 1984; 81:7397–7401. [PubMed: 6390436]
31. Sancar GB, Smith FW. Interactions between yeast photolyase and nucleotide excision repair proteins in *Saccharomyces cerevisiae* and *Escherichia coli*. Mol. Cell. Biol. 1989; 9:4767–4776. [PubMed: 2689865]
32. Mazon G, Philippin G, Cadet J, Gasparutto D, Fuchs RP. The alkyltransferase-like ybaZ gene product enhances nucleotide excision repair of O(6)-alkylguanine adducts in *E. coli*. DNA Repair. (Amst). 2009; 8:697–703. [PubMed: 19269902]
33. Shi YB, Gamper H, Hearst JE. Interaction of T7 RNA polymerase with DNA in an elongation complex arrested at a specific psoralen adduct site. J. Biol. Chem. 1988; 263:527–534. [PubMed: 3275650]
34. Minko IG, Kurtz AJ, Croteau DL, Van Houten B, Harris TM, Lloyd RS. Initiation of repair of DNA-polypeptide cross-links by the UvrABC nuclease. Biochemistry. 2005; 44:3000–3009. [PubMed: 15723543]

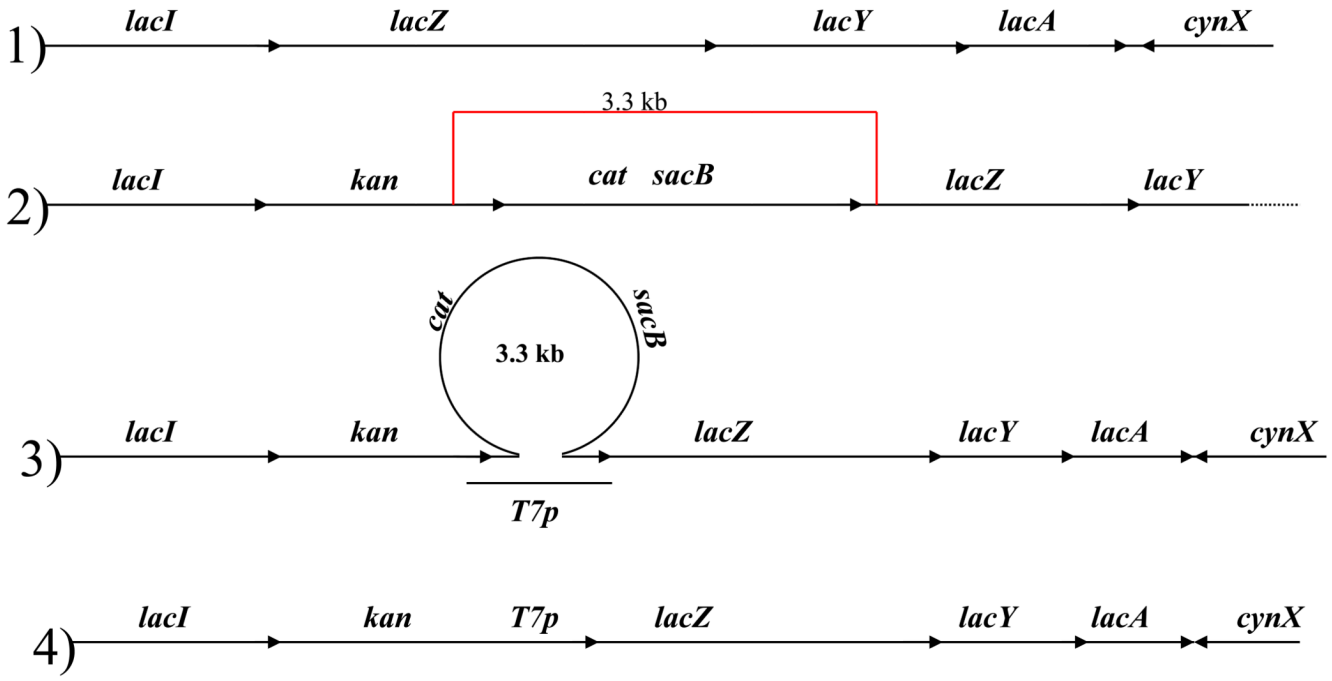


**Fig 1.**

Strain construction. 1) represents the normal *lac* region (present in SR108); 2) the chromosome arrangement in NC397; 3) alignment of the recombineering oligonucleotide with the chromosome of NC397; 4) the chromosome arrangement after successful recombineering (e.g. HL1134).

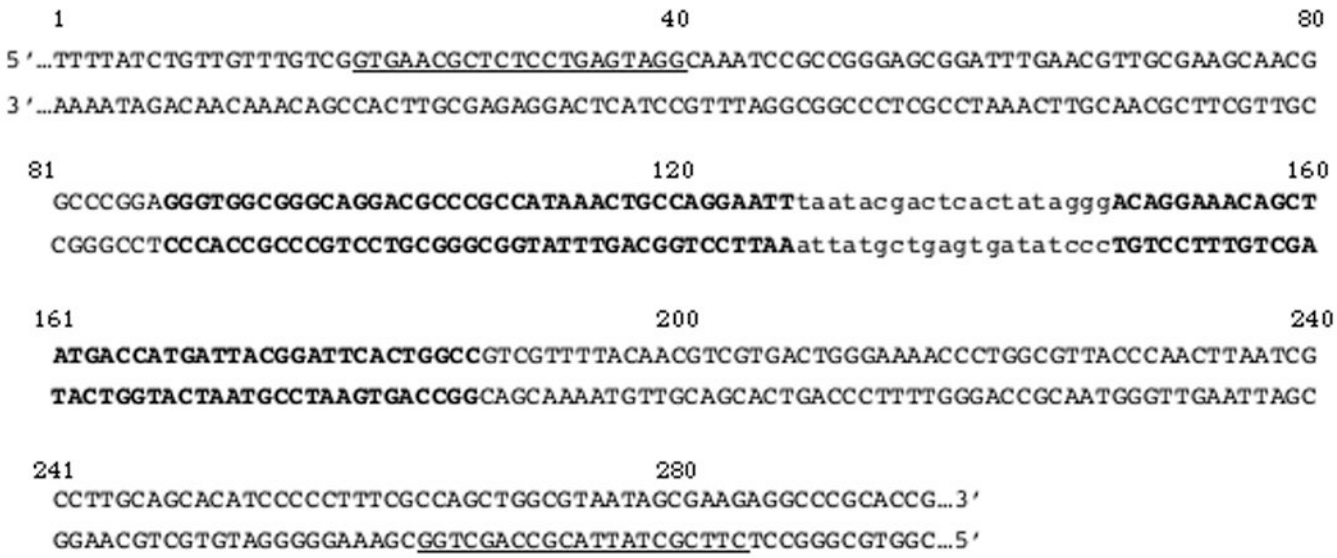


**Fig 2.**

Sequences used in this study. Base pairs 1 through 127 are from the kanamycin resistance gene. Base pairs 148 through 296 are from *lacZ*. Underlined sequences are those of the primers used for PCR. The bold letters denote the flanking sequences used for recombineering. Lower case letters indicate the T7 promoter sequence.

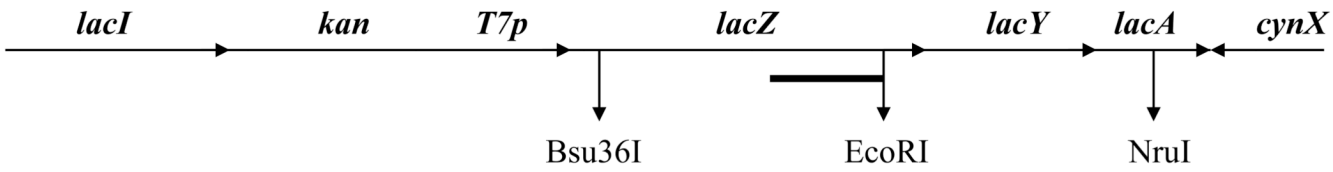


**Fig. 3.**

Restriction fragments and hybridization probe used. The *lac* region of HL1159 and HL1160 is illustrated. The 4.8 kb Bsu36I - NruI fragment, and the 2.8 kb Bsu36I - EcoRI fragment, were detected using 1 kb strand specific probes produced from pZH-10 (heavy line).

Figure 4A

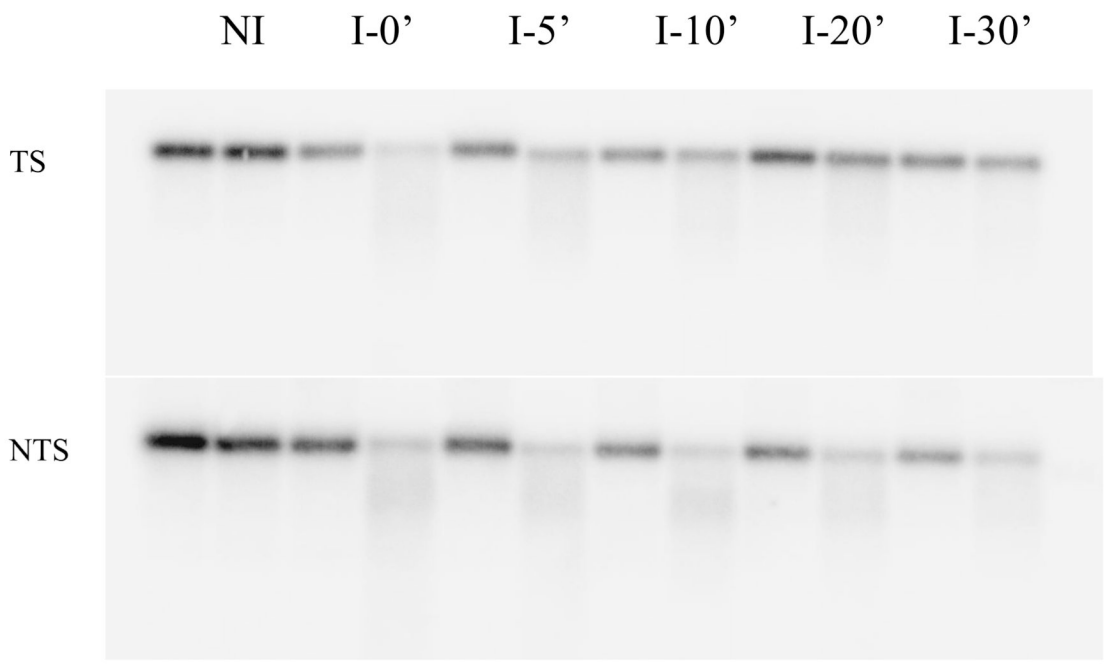

Figure 4B

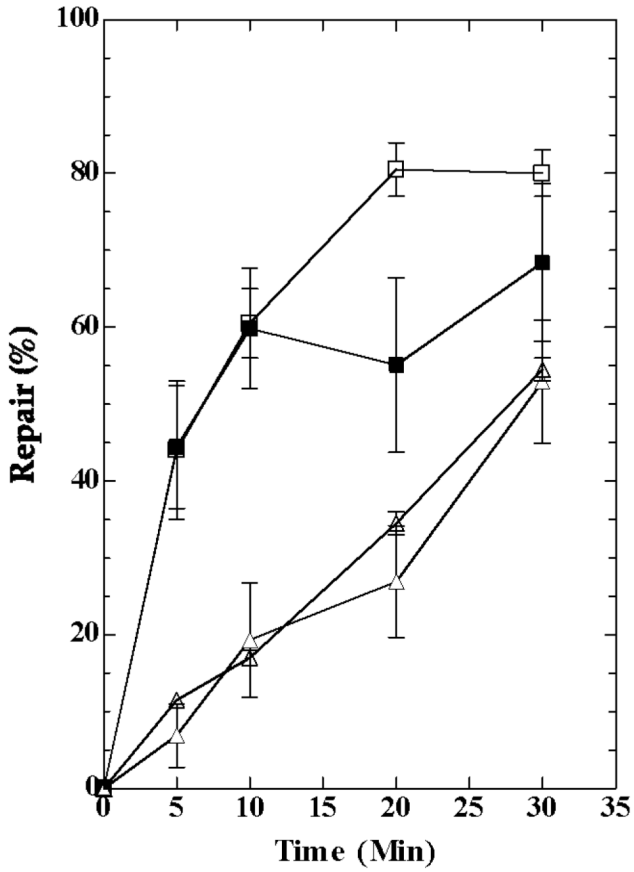


**Fig. 4.**

Repair of CPDs in the *lac* operon of cultures induced with IPTG. A) Image showing repair in the 4.8 kb band of the *lac* operon obtained by analyzing DNA from HL1159 irradiated with 54 J/m$^2$. B) HL1159 and HL1160 were irradiated with 54 J/m$^2$, and the 4.8 kb fragment of the *lac* operon was analyzed. Results from the two strains were combined. HL108 was irradiated with 40 J/m$^2$ and the 6.6 kb fragment of the *lac* operon analyzed. Symbols: squares, transcribed strands; triangles, nontranscribed strands; solid symbols, HL1159 and HL1160 (combined data); open symbols, SR108

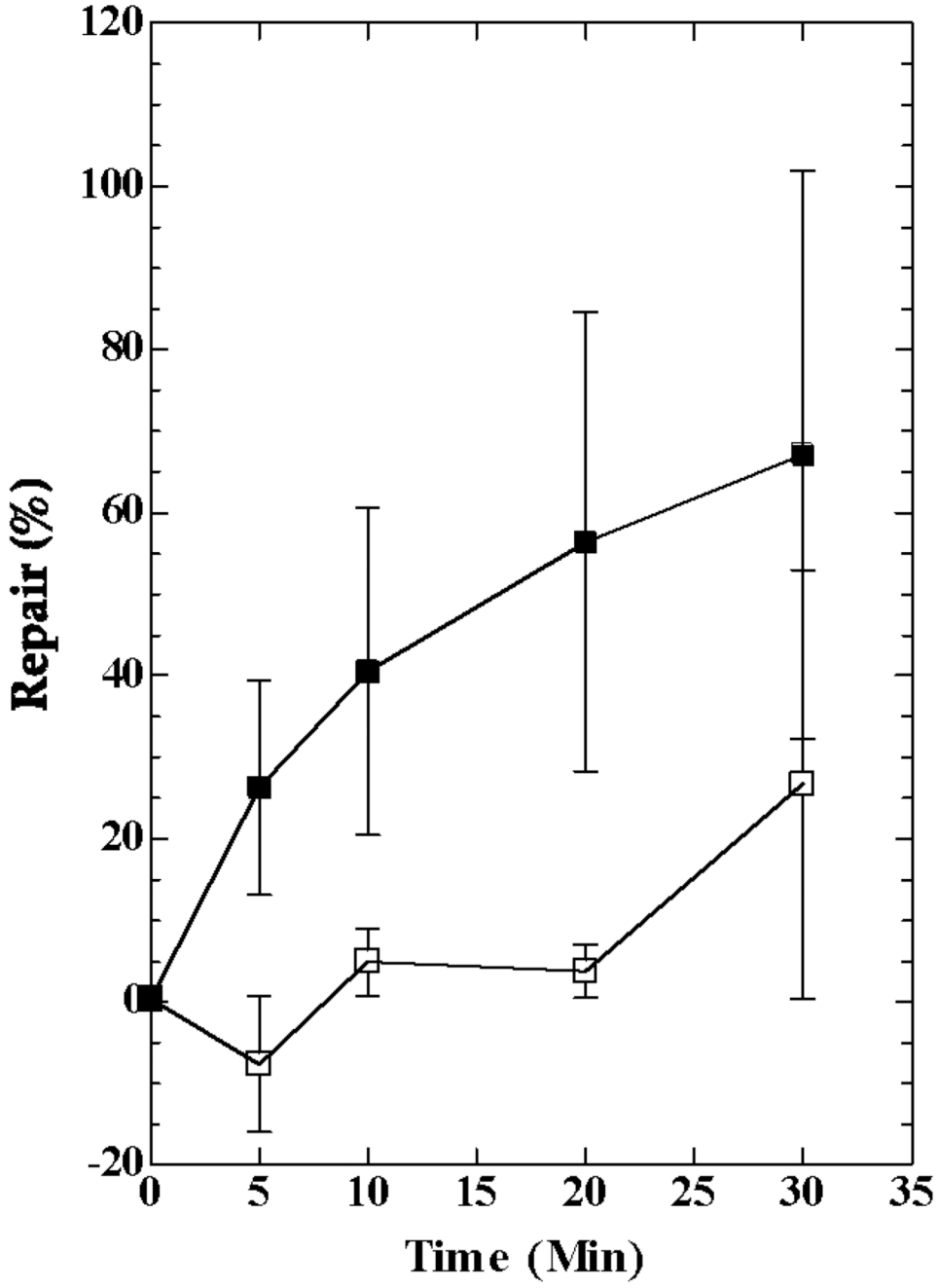


**Fig. 5.**

Repair of CPDs in the transcribed strand of the 2.8 kb DNA fragment of the *lac* operon in cultures of HL1159 irradiated with UV (72 J/m$^2$). Symbols: solid squares, induced cultures; open squares, uninduced cultures

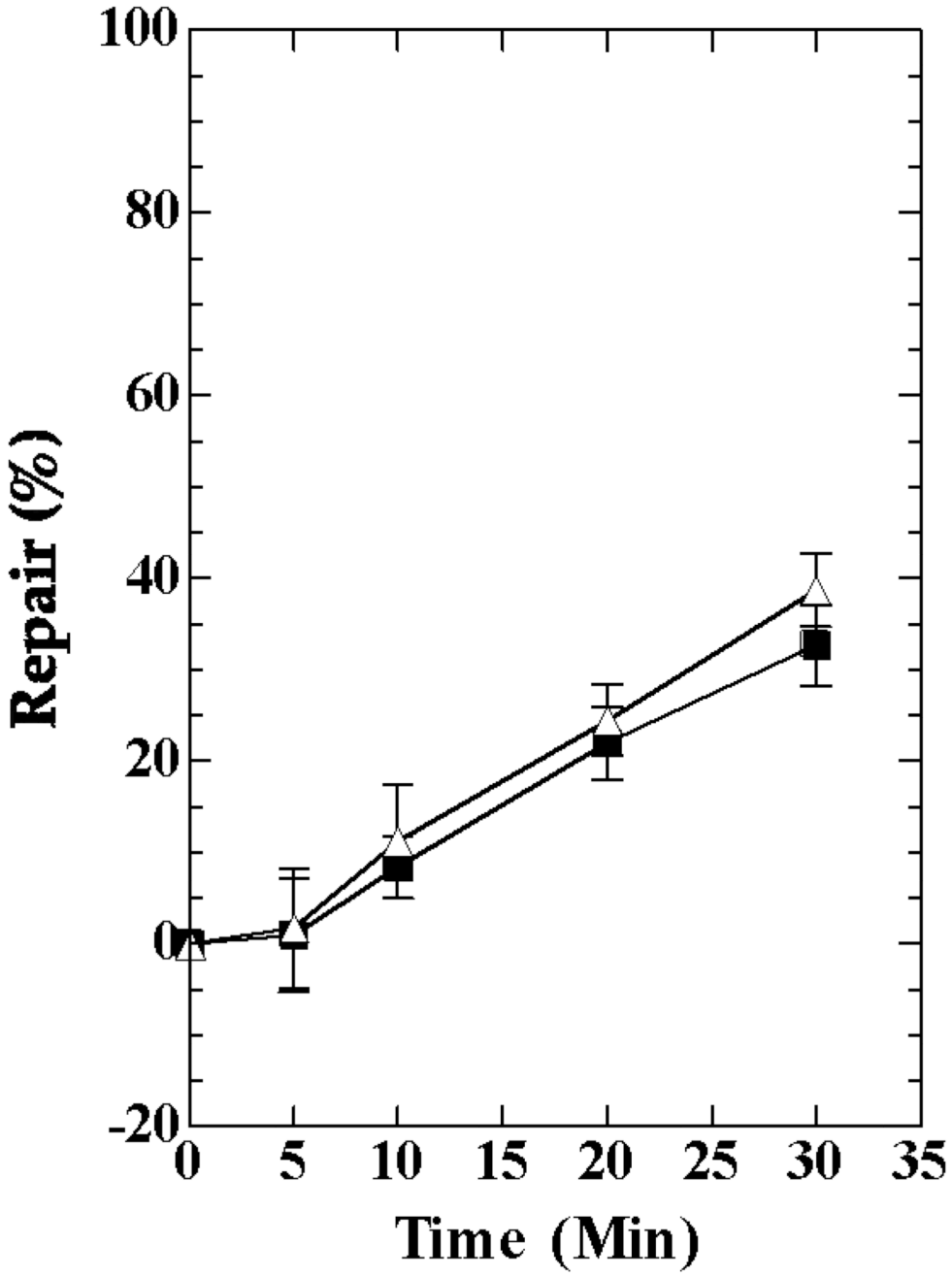


**Fig. 6.**

Repair of CPDs in the *lac* operon of *mfd* strains induced with IPTG. HL1179 and HL1180 were irradiated with UV (72 J/m$^2$) and the 4.8 kb fragment of the *lac* operon was analyzed. Results from the two strains were combined. Symbols: squares, transcribed strands; triangles, nontranscribed strands.

## Table 1

*E.coli* K-12 derivatives used in these experiments. Strains with HL numbers were produced for this paper.

| Designation | Genotype | Source |
|---|---|---|
| SR108 | F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1 | J. Cairns |
| NC397 | F-, pglΔ8, gal490, ΔCI857, Δ (cro, bioA), (In NC397 the *cat/sacB* cassette replaces the *lacZ* promoter (the *lacI* promoter, 5' end of lacI and RBS of *lacZ* remain), a kanamycin resistance gene and transcription terminator are inserted directly upstream of *cat/sacB*.) | D.L. Court |
| UNC3610-45 | F- λ-, thr-1, ara-14, leuB6, Δ(gpt-proA)62, lacY1, tsx-33, supE44, galK2, rac-, hisG4(Oc), rfbD1, mgl-51, rpsL31, kdgK51, xyl-5, mtl-1, argE3, thi-1, zcf-117::Tn10, mfd-1 | C. Selby |
| HL1132 | F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ | |
| HL1134 | F-, pglΔ8, gal490, λCI857, Δ(cro, bioA), kan-T7p-lacZ | |
| HL1159 | F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ / pAR1219 | |
| HL1160 | F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ / pAR1219 | |
| HL1166 | F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ, zcf-117::Tn10 | |
| HL1167 | F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ, zcf-117::Tn10, mfd-1 | |
| HL1172 | F-, pglΔ8, gal490, λCI857, Δ (cro, bioA), kan-T7p-lacZ | |
| HL1173 | F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ | |
| HL1176 | F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ / pAR1219 | |
| HL1177 | F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ /pAR1219 | |
| HL1179 | F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ, zcf-117::Tn10, mfd-1 / pAR1219 | |
| HL1180 | F- λ-, thyA36, deoC2, IN(rrnD-rrnE)1, kan-T7p-lacZ, zcf-117::Tn10, mfd-1 / pAR1219 | |

**Table 2**

β-galactosidase activity of strains grown with (Induced) or without (Not Induced) 1 mM IPTG. Activity is expressed as pmols of ONPG hydrolysed/min/$10^7$ colony forming units.

| Strain | Induced | Not Induced |
|---|---|---|
| SR108 | 6060 | 8 |
| HL1132 | 1 | 2 |
| HL1159 | 15561 | 77 |
| HL1160 | 5119 | 40 |
| HL1179 | 8422 | 40 |
| HL1180 | 6912 | 39 |