Rapid repeated cloning of mutant lac repressor genes

(Restriction DNA; M13lacIZ vector; F'lac × M13lac recombination; α-complementation; plasmid; I° and L8 promoter mutations; lacI system; nucleotide sequence analysis)

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SUMMARY

We have developed a procedure to efficiently recover lac repressor mutations (lacI−) from F'lac onto a single-stranded M13 phage vector. The recovery is based on homologous recombination between F'lac and an M13lac vector. This vector, mRS81, carries the entire Escherichia coli lacI gene as well as the adjacent α-complementation region of the lacZ gene, inserted in the AvaI site of the M13 ori region. It also carries a single point mutation in lacZ−α which abolishes its α-complementing ability. Recovery of lacI− genes from F is based on the conversion of this lacZ−α phage to lacI−Z+α by recombination with F'lacI−Z+. This double exchange restores its α-complementing ability in the absence of any inducer of the lac operon. Detection requires a lacI−α-complementation host, which was also constructed in this study. The procedure was developed to obtain rapid nucleotide sequence information on large collections of lacI mutants for the purpose of studying mutational mechanisms and specificities.

INTRODUCTION

Studies of the molecular nature of mutation require the development of methods permitting the repeated recovery of mutant alleles of a gene. This is also desirable in studies of gene regulation and gene product function where knowledge can be gained from studies of mutant genes. The E. coli lacI gene, coding for the repressor of the lac operon, is an example of a gene for which the study of mutant genes has been important in defining functional domains, interactions and structural aspects of the lac repressor (Miller, 1979). The lacI gene has also been extensively used as a tool to study the process of origin of replication; RF, replicative form; Rif, rifampicin; Sm, streptomycin; ss, single-stranded; wt, wild type; XGal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; [ ] designates plasmid-carrier state.
of mutation itself. This is typically done by the production of a mutational spectrum of lac Φ mutants. These mutants, characterized by a defective lac repressor are easily selected on the basis of their constitutive expression of the lac operon. Knowledge of the exact nature and location of a large number of mutational changes should provide valuable insight into the mechanisms of the mutational process under study. This approach has so far been largely confined to the subclass of nonsense mutations which can be assigned to exact locations by purely genetic techniques. Although substantial information has been gained from such studies (Coulondre et al., 1977; 1978; Todd and Glickman, 1982; Foster et al., 1983; Kunz and Glickman, 1984; Miller and Low, 1984), nonsense mutations constitute in many cases only a minor fraction of the total mutations; a full understanding of mutational processes therefore requires that mutational spectra be recorded by nucleotide sequencing. In this paper we describe a rapid procedure by which hundreds of different mutant lacI genes can be transferred simultaneously from F'lac, where lacI- mutants are commonly selected, to an M13 phage vector for the purpose of direct nucleotide sequence determination. The transfer is based on in vivo genetic recombination between F'lac and an M13 vector carrying the entire lacI gene as well as the α-complementation region of the adjacent lacZ gene. The transfer procedure is characterized by a high efficiency and a very low background of nonrecombinant lacI- phage.

MATERIALS AND METHODS

(a) Enzymes and biochemicals

Restriction endonucleases AvaI, AvaII and BamHI were purchased from Bethesda Research Laboratories. T4 DNA ligase and calf intestine alkaline phosphatase were from Boehringer Mannheim Biochemicals. All enzymes were used according to the manufacturers’ specifications. XGal and IPTG were from Bachem Fine Chemicals.

(b) Media

All platings of bacteria and bacteriophages (listed in Table I) were performed on minimal media, containing Vogel-Bonner (1956) salts, glucose (0.2%), thiamine (5 μg/ml), and amino acids as required (50 μg/ml). XGal (40 μg/ml) was contained in the bottom agar and, when required, IPTG was added to the plating mixture (bacteria plus top agar) or to the bacterial culture immediately prior to plating to give a final concentration in the plate of 15 μg/ml. 100 μg Rif/ml and 200 μg Sm/ml (Sigma) were included in the plates where necessary. LB, used for liquid crosses, contained Difco Bacto tryptone (10 mg/ml), Difco Yeast Extract (5 mg/ml) and NaCl (10 mg/ml).

(c) DNA preparations

The preparation of bacteriophage stocks and ss bacteriophage DNA was as described by Messing (1983). Replicative-form DNA and plasmid DNA were prepared as described by Birnboim (1983).

(d) α-Complementation

α-Complementation, scored as the formation of blue plaques on a lacZ- ΔM15 strain (Messing, 1983), was performed on minimal-media plates containing XGal with or without IPTG as described in section b above. In strain NR9099, α-complementation does not require IPTG, although the rate of blue color formation is enhanced when IPTG is present. Plaque color was determined after 24–36 h of incubation at 37°C.

(e) M13 × F'lac recombination

Approx. 5 x 10⁶ phage (mRS81) and 5 x 10⁷ bacteria carrying the F'lac of interest were grown together (moi 0.1) in 1 ml of LB at 37°C with shaking. Sufficient phage growth and recombinants can be detected after 4–6 h, but the cultures were routinely grown overnight. The next morning the cells were pelleted (4000 rev./min, 20 min) and a sample of the supernatant was removed for plating. Usually 100 μl of a 10⁻⁵ and a 10⁻⁶ dilution of this supernatant were plated on XGal plates using NR9099 as the indicator, to obtain between 10⁵ and 10⁶ plaques per plate. Recombinants were identified by their blue color after 24 h. For further analysis, blue plaques were picked and purified by replating on NR9099. After
this point the recombinants were assigned relative phage color values, tested for suppressibility and, where required, grown up for nucleotide sequence analysis.

(f) Nucleotide sequence analysis

Sequencing was performed by the dideoxy chain termination method of Sanger et al. (1977) on ss DNA preparations. Synthetic 14- or 15-mers complementary to specific positions along the lacI and lacZ gene sequences (P-L Biochemicals or New England Biolabs) were used as primers. The resulting sequences were compared to the published sequences of the lacI gene (Farabaugh, 1978) and the lacZ gene (Reznikoff and Abelson, 1978).

RESULTS AND DISCUSSION

(a) Rationale

Mutant lacI genes (lacI−) are selected on the episomal factor F′ lac. To make the mutant genes available for nucleotide sequencing, we sought to transfer them onto an ss phage vector by means of genetic (homologous) recombination. Recombinational transfers to bacteriophage M13 have been described previously for chromosomal his genes (Artz et al., 1983; Barnes and Tuley, 1983). Such a system would require: (i) a starting ss phage vector already carrying the E. coli lacI + gene to provide the necessary homology, (ii) a selective procedure to distinguish the resulting recombinant lacI− phages from the starting lacI + phage, and (iii) safeguards against any frequent occurrence of spontaneous lacI− phages, originating by spontaneous mutation in the phage rather than by recombinational exchange.

On this basis we designed the recombinational recovery scheme as depicted in Fig. 1. It describes the simultaneous transfer, by recombination, of the adjacent lacI and lacZα genes from F′ to M13, according to the formula:

\[ F′ lacI− Z + \times M13 lacI + Z − α \rightarrow M13 lacI− Z + α. \]

The resulting recombinant M13 lacI− Z + α phage can be easily distinguished from its parental phage as well as phages carrying other combinations of lacI and lacZ genes. This distinction is based on the (negative) control that the lacI gene (coding for the lac repressor) exerts on α-complementation by the lacZ gene residing on the same bacteriophage. Thus, when measuring α-complementation as blue plaque formation in the presence of XGal, lacI− Z + α phages are derepressed and produce blue plaques, whereas the parental lacI + Z − α phage remain colorless. This test is to be performed in the absence of the inducer IPTG and in an α-complementation host which is itself lacI−. An essential feature of this system is the requirement of a double change from lacI + Z − α to lacI− Z + α. This requirement effectively prevents contributions from spontaneous lacI− phages arising in the parental population.

In the following sections we describe the various steps in the construction of a successful lacI + Z − α vector. We also describe the construction of the lacI− α-complementation host.

Fig. 1. Homologous recombination of M13 lacI + Z − α with F′ lacI− Z + to yield M13 lacI− Z + α: a recovery system for mutant lacI genes. This is a schematic representation and no specific recombinational model is implied. Recombinational transfers might involve only part of the indicated genes, depending on the exact location of the mutation of interest; for more details regarding the extent of recombinational transfer see sections e and g of RESULTS AND DISCUSSION and Fig. 4.
Fig. 2. Construction of M13mRS1 and M13mRS58. Phage mRS1 was constructed by inserting a 2.4-kb AvaI fragment from plasmid pMC1, carrying the lacI and lacZa genes, into the AvaI site of M13. Phage M13mRS58 (lacI+Za) was constructed from M13mRS1(lacI+Za) and M13mp2T90(lacZ-a) by exchange of the large AvaII-BamHI fragment. As a consequence of the difference in cloning sites for M13mRS1 and M13mp2 [AvaI site at position 5825 and an HaeIII site at position 5868 of the M13 wild-type sequence, respectively (Van Wezenbeek et al., 1980)], M13mRS58 has the region between the two sites deleted (43 bp). Arrows denote direction of transcription, or in case of the M13 ori, the direction of replication. (RI, EcoRI; H, HincII).
TABLE I
Strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotypea</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S90C</td>
<td>Δ(pro-lac), ara, thi, strA</td>
<td>Miller et al. (1977)</td>
</tr>
<tr>
<td>S90C[pMC1]p</td>
<td>as S90C, but carrying plasmid pMC1</td>
<td>Calos et al. (1978)</td>
</tr>
<tr>
<td>JM83</td>
<td>Δ(pro-lac), ara, thi, strA, (φ80d lacIQZ - ΔM15)</td>
<td>P-L Biochemicals</td>
</tr>
<tr>
<td>KA796 (= P90C)</td>
<td>Δ(pro-lac), ara, thi [F' pro-lacIQlacZL8]</td>
<td>Miller et al. (1977)</td>
</tr>
<tr>
<td>NR3835</td>
<td>Δ(pro-lac), ara, thi, trpE9777 [F' pro-lacIQlacZL8]</td>
<td>Todd and Glickman (1982)</td>
</tr>
<tr>
<td>Δ169</td>
<td>Δ(pro-lac), ara, thi, galeE, valR (φ80d lacZ - Δ lacl)</td>
<td>Schmeissner et al. (1977)</td>
</tr>
<tr>
<td>Δ169R</td>
<td>Δ169, but no longer galactose sensitive</td>
<td>This paper</td>
</tr>
<tr>
<td>NR8099</td>
<td>NR8057[F' pro-lacIQ4043lacZ - ΔM15]</td>
<td>This paper</td>
</tr>
<tr>
<td>NR9100</td>
<td>S90C[F' (pro-lac)QlacZL8]] from NR3835</td>
<td>This paper</td>
</tr>
<tr>
<td>NR9101</td>
<td>S90C[YI (pro-lac)QlacZL8]] from NR3835(lacIQo20)</td>
<td>This paper</td>
</tr>
<tr>
<td>NR8079</td>
<td>Δ(pro-lac), ara, thi, nalA, rif, metB, argEam, supB [F' pro-lacIQ4043lacZ - ΔM15] (XA10B from Miller et al. (1977), but F' from NR9099)</td>
<td>This paper</td>
</tr>
<tr>
<td>Bacteriophage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M13</td>
<td>wild type</td>
<td>Van Wezenbeek et al. (1980)</td>
</tr>
<tr>
<td>M13mp2(T90)</td>
<td>lacZ - αT90(ochre)</td>
<td>Kunkel (1984)</td>
</tr>
<tr>
<td>M13mRS1</td>
<td>M13lacI + Z - αUV5</td>
<td>This paper</td>
</tr>
<tr>
<td>M13mRS58</td>
<td>M13lacI + Z - αT90</td>
<td>This paper</td>
</tr>
<tr>
<td>M13mRS65</td>
<td>M13lacIQZ + zL8</td>
<td>This paper</td>
</tr>
<tr>
<td>M13mRS81</td>
<td>M13lacIQZ - zT129,L8</td>
<td>This paper</td>
</tr>
</tbody>
</table>

a The symbols IQ (lacp) and L8 and UV5 (both lacZp) denote the presence of promoter mutations. The IQ mutation is a C → T change at position (~35) of lacI (Calos, 1978); the L8 mutation is a (~66) G → A change affecting the CRP-cAMP binding site of lacZp (Dickson et al., 1977).
b Plasmid pMC1 (Calos et al., 1978) was a kind gift from Dr. M. Calos, Stanford University. The lac portion of this plasmid is derived from λh80d lacUV5c1857S7 and carries a wild type lacI promoter and the UV5 lacZ promoter.
c Number refers to position in lacZ where 1 is the first transcribed base. Phage M13mp2(T90) is M13mp2 with a G → T base change at position 90 (T. Kunkel, personal communication). Likewise M13mRS81 has a C → T substitution at position 129.

(b) Construction of M13 mRS1 (M13lacI + Z + α)

The source for lac DNA was plasmid pMC1 (Fig. 2). This pMB9 derivative contains a 2.7-kb HincII partial fragment from a ΔlacUV5 phage, carrying the entire lacI gene and the first (α-complementation) part of the lacZ gene (Calos et al., 1978). The 789-bp HincII fragment carrying the terminal part of lacI and the first part of lacZ is the fragment contained in the M13mp phage series (Messing et al., 1977).

A restriction analysis of pMC1 revealed the presence of a 2.4-kb AαI fragment which contains, together with some surrounding DNA, the entire lacI.
F’lac* obtained in one other experiment at low frequency (5%).

The gene and the lacZα-complementation region. This AvaI fragment was inserted into the unique AvaI site in the intergenic ori region of M13 and the desired product was identified by screening for blue plaques in the presence of XGal and IPTG after transfection into α-complementation host CSH50 (Fig. 2). This attempt was successful (39 blue plaques were found among 800 total). The blue plaques so obtained appeared relatively stable, white plaques appearing among them with a frequency of approx. 1 in 1000. Restriction analysis confirmed the insertion of a single 2.4-kb AvaI fragment into unit-length M13, and showed that 24 isolates out of 24 tested had the orientation depicted in Fig. 2, i.e., transcription of lacZ occurs clockwise, away from the M13 origin, as is the case in the M13mp series of phages.

(c) Construction of M13lac1 + Z - α

It was considered desirable that the lacZ- phenotype of the lac1 + Z - α vector be conferred by a single point mutation not to adversely affect the recombination frequency between homologous lacZ genes. Therefore, a known point mutation in lacZ was transferred into mRS1 from a sequenced mp2-lacZ - CI isolate (Kunkel, 1984) by replacing the large AvaII-BamHI fragment of mRS1 with the corresponding AvaII-BamHI fragment of mp2-lacZ - CI (Fig. 2). This replacement yields a white plaque phenotype in an α-complementation assay. One isolate, M13mRS58, carrying an ochre mutation in lacZα (Table I), was selected and tested for recombinational ability. Preliminary results (not shown) indicated that mRS58, when grown on an F’lacZ - α-containing host, recombinated efficiently with F’lac to become lacZ + α. However, a test for concomitant transfer of the lacI gene required a lacI - α-complementation host (described below). No lacZ + phages could be detected after growth in a recA - F’lacZ + strain.

(d) Construction of an F’lacZ - Z - ΔM15

For the successful scoring of lacI - Z + α recombinant phages among their parental lacZ + Z - α phages, an α-complementation host is needed which is lacI - . Only in this configuration does α-complementation completely depend on the lacI gene carried by the bacteriophage. We therefore constructed an F’lacI - , lacZ - ΔM15. This could not be easily done by using existing F’lacZ - ΔM15 strains such as

![Diagram](Fig. 4. Recombination between M13mRS58 and F’lacI - lacZ - ΔM15 to yield lacZ + α-phage. The arrows indicate the extent to which recombination, once initiated in lacZ, travels into lacI. This model provides a physical basis for the various plaque-color phenotypes that were observed and which are represented in the panel on the right. The percentages are based on an experiment in which the progeny of the two crosses was plated on NR9099 in the presence of IPTG. Dark blue plaques (lacZ + α phage) were observed at similar frequencies in the two strains (7.2 x 10^-4 and 9.0 x 10^-4). Individual blue plaques were replated on NR9099 in the absence of IPTG. We examined 37 plaques from each strain, and obtained 22 light blue and 15 colorless plaques for the cross with F’lacI +, and 9 light blue and 28 dark blue plaques for the cross with F’lacZ - ΔM15. Out of these 28 dark blue plaques 13 proved suppressible (S-dark) in host NR8079 which carries an ochre suppressor (Table I). It was concluded that complete transfers (including the f0 promoter) lead to a colorless phenotype in case of a cross with F’lacI + and a suppressible dark-blue phenotype in case of a cross with F’lacZ - ΔM15. Nucleotide sequence analysis of the f0, oc2, and L8 regions of phages belonging to each of the categories confirmed all assignments. This includes the darker blue isolates from the cross with}
CSH50 or JM101, since these carry tra− mutations, which abolish conjugal transfer. Construction was therefore started with strain JM83 as outlined in Fig. 3. JM83 carries the (lacI', lacZ ΔM15) operon on a defective prophage in its chromosome. A wt F'(pro'--lac') was transferred into this strain and F'(pro'--lac'--Z + ΔM15) recombinants were selected following homogenotization and selection of white colonies on plates containing XGal and IPTG. Transfer of the resulting F' into KA796 yielded a strain which could be z-complemented by M13mp2 phage in the presence of IPTG. The lacI− derivative of this F' was selected following transfer to strain Δ169R which carries lacI− and lacZ+ genes chromosomally. The resulting conjugant is white when plated on XGal media in the absence of IPTG. Blue colonies that occur at low frequencies are presumably spontaneous lacI− mutants. Several of these blue colonies were purified and their F' episomes were crossed into NR8057. These conjugants were tested for the following properties: white colonies on XGal in both the presence and absence of IPTG, z-complementation with M13mp2 in the absence of IPTG, and z-complementation with mRS1 (lacI + Z + z) in the presence but not in the absence of IPTG. Several isolates satisfied these criteria; one, denoted NR9099, was used throughout the rest of this study.

(e) Recovery of mutant lacI genes from F'lac

The recombinational rescue scheme, outlined in Fig. 1, was tested by growing phage M13mRS58 on strain NR9101 that contained an F' (pro− lac) carrying a known lacI nonsense mutation, oc20 (Coulondre et al., 1977). The resultant progeny phage were then plated on NR9099 in the absence of IPTG. The same experiment was performed, in parallel, with the wt F' strain NR9100 as a control. The results of this experiment were unexpected, blue plaques being obtained from either cross. Mainly light blue plaques were recovered from the cross with F' lacI+, both light and dark blue plaques from the cross with F' lacIoc20. We realized that a more careful consideration was required of the lacI and lacZ promoters that might be found on the recombinant phages. Starting phage M13mRS58 carries the wt promoters for lacI and lacZ, whereas the F'lac commonly used for mutational studies of the lacI gene carries two mutant promoters, the fO ("up") lacI promoter, and the L8 ("down") promoter for lacZ (Miller et al., 1977). In Fig. 4 we have depicted a model describing how recombinational transfer from F to M13mRS58, once initiated, for instance, in lacZ, might travel various distances into lacI. Assuming that recombinational transfer in many cases is indeed not complete, we can explain the light blue plaques observed in both crosses by a lacI + Z + z genotype, in which the recombinant phage still carries the wt lacI promoter. This notably weak promoter might not produce enough repressor to completely repress z-complementation. We proved that this hypothesis was correct by directly sequencing the promoter regions of all the classes of recombinants depicted in Fig. 4 (for more details see in the legend). We concluded that for a successful functioning of the recovery system a starting phage vector was required that already carried the (stronger) fO promoter. The model in Fig. 4 predicts the occurrence of 'white' lacI + Z + z (fO, L8) recombinants from the cross with F' lacI+. Several of these "complete-transfer" recombinants were recovered after initial plating on XGal-IPTG plates, following by replating of individual blue plaque isolates on XGal plates in the absence of IPTG. Such phages constitute a convenient starting point for the construction of the desired lacI + Z + z (fO, L8) vector.

(f) Selection for M13mRS81 [lacI + Z + z (fO, L8)]

Phage M13mRS65 (Table I) is one of the recombinant phages obtained from a cross with F' lacI+ as described above; on host NR9099, M13mRS65 produces blue plaques in the presence of IPTG but not in its absence. Sequence analysis confirmed the expected lacI + Z + z (fO, L8) genotype. Spontaneous lacI− z derivatives of this phage (white plaques in the presence of IPTG) were selected and tested for the following properties: pure white phenotype, low reversion frequency to lacI + Z + z and high recombination frequency with F' lacI. One of the several phages which satisfied these three criteria, M13mRS81, was selected and its lacZa region sequenced. A single bp change was observed (C → T at position 129). This phage was used for further recombination studies.
Recovery of \( lac I^- \) mutations with M13mRS81

The M13 derivative mRS81 carries the \( f^O \) and L8 mutations found necessary for the direct recovery of host \( lac I \) mutations. This phage was used in the crosses with \( F' lac I^+ \) and \( F' lac I^- (oc20) \) mutants as described in section e. The results are presented in Table II. Now all the \( lac Z^+ \alpha \) plaques recovered in the \( lac I^- (oc20) \) cross depend on the presence of IPTG to display blue color. In contrast, 75% of the \( lac Z^+ \alpha \) recombinants obtained in the \( lac I^- (oc20) \) cross were \( lac I^- \) and produced blue plaques whether IPTG was present or not. The remaining 25% were \( lac I^+ \) and as such depend on the presence of IPTG for blue plaque formation. This result is completely consistent with the model described in Fig. 4. A second experiment was done in which direct plating was performed on plates without IPTG. For the \( F' lac I^- \) cross, no blue plaques were scored among 100000 total phage. In case of the \( F' lac I^- (oc20) \) cross, 70 blue plaques were recovered among 100000 plaques. The recovery procedure was then tested with five known \( lac I^- \) mutants, namely am11, am17, am20, am36, and oc20 (Coulondre et al., 1977). From each cross, five recombinant phage were purified and their DNAs sequenced. In all cases the recombinant blue (\( lac I^- \)) phage carried the expected mutation (T → A at position 213 for am11, G → T at position 542 for am17, C → A at position 606 for am20, T → A at position 984 for am36, and G → T at position 518 for oc20). It was concluded that with M13mRS81 mutant \( lac I \) genes can be reliably and efficiently recovered from \( F' lac \) on an ss vector.

Further comments

Calos and Miller (1981) developed a recovery method in which \( lac I \) mutations were crossed into the multicopy plasmid pMC1, followed by the segregation of pure mutant plasmid clones, a process called homogenotization. Mutations were identified by Maxam–Gilbert sequencing. The M13 method described herein offers the substantial advantage associated with the handling and sequencing of ss DNA. Secondly, the double selection for \( lac I^- Z^+ \alpha \) has a distinct advantage over the single selection for \( lac I^- \) only (as in the case of pMC1). Simultaneous selection for \( lac Z^+ \alpha \) constitutes a direct selection for recombinants among the total population since the \( lac Z^- \) mutation of mRS81 does not revert at measurable rates. Therefore the danger of inadvertently collecting a spontaneous \( lac I^- \) mutation that arose on the vector is strongly diminished. We estimate that in the M13 system described here, perhaps less than 1 in 1000 isolates could be of such origin. This very low frequency allows us to confidently screen large collections of \( lac I^- \) mutations for the purpose of determining mutational specificities. Subsequent studies have already indicated that even large deletion or insertion mutations, which effectively disrupt homology and reduce the recombination frequency, can be reproducibly transferred onto M13 for sequence analysis (Schaaper et al., unpublished data). For example, IS1 insertion mutations, which are the result of a 768-bp insertion into the gene, are transferred to MRS81 with an average frequency of slightly below \( 10^{-5} \), which is well within the range of detection. Although presently we have only tested the recovery from \( F \), the usefulness of the method almost certainly extends beyond this application. In view of the general recombination proficiency of

<table>
<thead>
<tr>
<th>Cross</th>
<th>Frequency (( \times 10^4 )) of blue plaques (+IPTG)</th>
<th>Distribution of plaque phenotypes upon replating (−IPTG)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>White</td>
</tr>
<tr>
<td>mRS81 × ( F' lac I^+ )</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>mRS81 × ( F' lac I^- (oc20) )</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>
E. coli, recovery from plasmids, phage \( \lambda \) vectors, or the bacterial chromosome should be equally possible. In subsequent papers we will report on a nucleotide sequence analysis of 200 spontaneous lacI\(^{-}\) mutations as well as over 400 UV-light-induced mutations.

NOTE ADDED IN PROOF

Miller et al. [J. Mol. Biol. 182 (1985) 65–68] recently described an improvement of their method of recovering lacI\(^{-}\) mutations onto plasmids by using \( \lambda \)-complementation and a double lacI and lacZ selection, in a fashion analogous to the one presented in this communication.

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REFERENCES


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