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Plasmid Insertion Mutagenesis and *lac* Gene Fusion with Mini-Mu Bacteriophage Transposons

BEATRIZ A. CASTILHO,[1] PAUL OLFSON,[2] AND MALCOLM J. CASADABAN[1,3]*

Committee on Genetics,[1] Department of Microbiology,[2] and Department of Biophysics and Theoretical Biology,[3] The University of Chicago, Chicago, Illinois 60637

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Small bacteriophage Mu transposable elements containing the *lac* operon structural genes were constructed to facilitate the isolation and use of Mu insertions and *lac* gene fusions. These mini-Mu elements have selectable genes for either ampicillin or kanamycin resistance and can be used to form both transcriptional and translational *lac* gene fusions. Some of the mini-Mu-*lac* elements constructed are deleted for the Mu A and B transposition genes and form stable insertions that cannot undergo transposition unless complemented for these functions. A procedure was developed for selecting mini-Mu insertions specifically into plasmids, including commonly used high-copy-number cloning vectors such as pBR322. Mu insertions in pBR322 were found to be distributed around the plasmid, but insertions in certain regions occurred more frequently than in others.

Several properties of bacteriophage Mu make it very useful for genetic manipulations (8, 23, 31, 55). It is a temperate phage which replicates by a process that involves DNA transposition. Transposition to new sites can be induced at a high rate by lytic growth, or it can be repressed in the lysogenic state. In addition, Mu has a headful mechanism for packaging its DNA so that adjacent DNA sequences, up to a total of approximately 38 kilobase pairs (kb) including the Mu genome, can be taken up into a viral particle and carried to a new cell upon infection (10). New DNA sequences can be incorporated between the ends of the Mu genome and carried, within the limits of DNA packaging, continually as a part of the phage to new cells and genomic locations. Segments of the Mu genome can be deleted without affecting phage growth, and even larger segments can be removed or altered without affecting intracellular transposition. If removed, both of these functions can be complemented by a nondefective helper Mu prophage as long as the DNA sequences at each Mu end are maintained (54).

For forming gene fusions, gene segments can be incorporated in the Mu genome close enough to the right end that transcription and translation can proceed from sequences outside of Mu across a small right-end segment into the incorporated sequence. The Mu d*lac* phage (16) (Fig. 1) has the *lacZYA* operon such that transcription from a promoter near the Mu insertion can proceed into the Mu dI phage to express the *lac* genes. The Mu dII*lac* phage (14a) has a similar structure but allows both transcription and translation (of the resulting transcript) to proceed to express the *lac* genes with the formation of enzymatically active hybrid β-galactosidase (*lacZ*) proteins that have their amino-terminal amino acids derived from sequences outside of Mu. These types of gene fusions are useful in the study of gene regulation (2) and protein structure (28, 35, 43, 44).

Here we describe the construction of a series of mini-Mu-*lac* gene fusion elements that have several advantages over the previously described Mu dI1 and Mu dII301 *lac* gene fusion elements. These mini-Mu elements have an additional selectable drug resistance gene (Km^r). Some are missing the Mu transposition genes but can be complemented for these functions to form insertions that are genetically stable and incapable of subsequent transposition. The small size of these elements also permits the use of the Mu transduction process to select Mu insertions, including insertions in high-copy-number plasmids.

MATERIALS AND METHODS

Strains are listed in Table 1. Bacterial growth conditions and genetic manipulation procedures including conjugation (42), standard DNA cloning procedures (39), and methods for handling bacteriophage Mu (9, 13, 16) have been described previously. Antibiotics and the concentrations used were ampicillin (25 μg/ml), kanamycin (20 μg/ml), spectinomycin (40 μg/ml), streptomycin (100 μg/ml), and tetracycline hydrochloride (10 μg/ml). All Mu elements used contained the *c*ts62 temperature-sensitive repressor mutation so that lytic growth could be induced from the lysogenic state. Mu lysogenic strains were grown at 30°C or below unless otherwise stated. Since Mu lysates can be unstable, Mu phage were stored as lysogens and lysates were freshly prepared by heat induction.

RESULTS

Overview of the genetic manipulations. An additional selectable drug resistance marker for kanamycin resistance was first introduced into the original Ap^r Mu dI1 and Mu dII301 *lac* fusion phage. The Mu dII Km^r Ap^r recombinant phage was then inserted into the pSC101 plasmid in which deletions of internal restriction fragments were made by DNA cloning technology to form mini-Mu dII prophage. Some of these were moved to the chromosome along with a complementing Mu *c*ts prophage to form strains which are convenient for inducing lysates. Mini-Mu dI transcription fusion-forming phage were constructed by recombination of Mu dI1 with mini-Mu dII phage. Next, the mini-Mu phage were tested for growth, transduction, and *lac* fusion formation.

Procedures for localizing mini-Mu insertions into plasmids were then developed. Recombination defective *recA*$^-$ ver-

* Corresponding author.

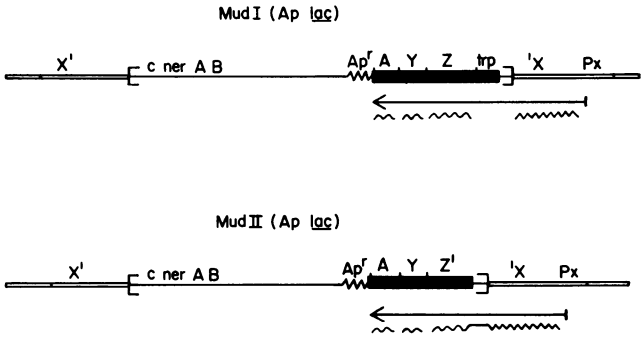

FIG. 1. Mu-*lac* gene fusion phage. Indicated are the original 37.2-kb Mu dI1 (Ap *trp*'CBA'*lac*'ZYA) *lac* transcription (16) and the 35.6-kb Mu dII301 (Ap *lac*'ZYA) *lac* transcription *lacZ* translation (14a) fusion-forming phage. These phage are defective (d) for Mu phage growth but are proficient for intracellular transposition. They differ only to the right of the *lac* sequences. Mu dI has a segment from the *trp* operon from the W209 *trp-lac* fusion which is probably missing the first two codons of *lacZ* and which probably uses the translation initiation site from or within the *trpA* gene (M. Berman, personal communication). Mu dII has a 116-base-pair segment from the Mu right end joined to the eighth codon of *lacZ*, with a *Bam*HI site formed at the joint. Transcription and translation from outside of Mu on the right can proceed across this small Mu segment into the *lacZ* gene to form enzymatically active hybrid proteins. The relevant early Mu repressor gene *c* and the transposition-replication genes A and B are indicated on the left side of Mu. An unselected and irrelevant IS*121* insertion sequence which transposed from the *E. coli* chromosome was found to be present in the middle of the original Mu dI1 phage (45) and is retained in Mu dII301 (Fig. 2). The phage are drawn as insertions into a hypothetical gene X with promoter Px which initiates transcription (arrows) toward the *lac* sequences to encode proteins indicated by the squiggly lines below.

sions of the mini-Mu lysogens were made to minimize the formation of multimeric forms of plasmids. Insertions into pBR322 were examined in detail.

Km^r derivatives of Mu-*lac* fusion phage. A Km^r-selectable marker was introduced into Mu dI1 (Ap^r) and Mu dII301 (Ap^r) by recombination with the Mu pf7701 (Km^r) phage (Table 1). Recombinants were obtained by infecting the MAL113 and POL3 lysogens of Mu dI1 and Mu dII301 with a lysate of Mu pf7701. Km^r Ap^r transductants were selected and scored for the presence of recombinants. About half of the transductants were recombinants as judged by their failure to release plaque-forming phage. The Mu dI and Mu dII phage are defective for phage growth because they are missing essential Mu genes, whereas Mu pf7701 is not defective for growth. Recombinants between the two would also be defective. The other half of the transductants presumably became Km^r by a new insertion of Mu pf7701 in the chromosome. The recombinant nature of these Mu dI7701-1 (strain BAC1) and Mu dII7701-301 (strain PO3) elements was verified by the cotransduction of the Ap^r and Km^r markers by lysates prepared from these strains after introduction by conjugation of a complementing Mu *c*ts prophage present on the F' episome from strain EC601.9. The physical structure of Mu dII7701-301 is discussed below and shown in Fig. 2.

Insertion of Mu dII7701-301 into pSC101. Mu dII7701-301 was inserted into the intermediate-copy-number plasmid pSC101 (21) by first incorporating it into an F' *ara* episome and then by using the process of plasmid mobilization to select Mu dII insertions into pSC101, as has been described for the isolation of Mu *c*ts insertions into pSC101 (19).

The F' *ara* from strain EFO was introduced by conjugation, with selection for Ara$^+$ Km^r, into strain PO3, which has a Mu dII7701-301 insertion in the *ara* genes (as an *ara-lac* fusion). Recombinant F' *ara* episomes, which had picked up the *ara*::Mu dII7701-301 insertion by homologous recombination, were selected by mating with strain M8820S. Km^r and Spc^r exconjugants were picked and scored for Leu$^+$ (*leu* is also carried on the episome), Ap^r, Str^s, and the ability to transfer the episome again, along with all its markers, to the *rpsL* strain M8820 Mu *c*ts. Expression of *lac* was also shown to be still induced by L-arabinose, as indicated by the formation of red Lac$^+$ colonies on lactose MacConkey agar when L-arabinose was added as inducer at 0.1%.

The pSC101 plasmid was introduced into one of these F' *ara*::Mu dII7701-301 strains (named PO401) by transformation. These cells were grown at 37°C to induce Mu transposition which would occasionally mediate the joining of the pSC101 plasmid to the F'::Mu dII episome by a cointegrate transposition structure. Such a cointegrate structure could then be transferred by conjugation to strain M8820 and selected as Tc^r, Km^r, and Str^r cells on lactose MacConkey indicator agar, with 0.1% L-arabinose as inducer. About half of the exconjugants formed white colonies, implying that the F' *ara*::Mu dII7701-31 episome had not been established and had presumably been separated from the cointegrate pSC101 by homologous recombination within the duplicated Mu sequences. These white colonies were also Leu$^-$ and thus did not have the episome. Plasmid DNA (as pPO1669) prepared from three of these strains had structures expected for Mu dII7701-301 insertions in pSC101, as judged by 0.7% agarose gel electrophoresis after digestion with the enzymes *Bam*HI, *Bgl*II, *Eco*RI, and *Hin*dIII (Fig. 2).

Internal deletions to form mini-Mu dIIs. Figure 2 shows the structures of Mu dII7701-301 in pSC101. The 9.9-kb pSC101 sequences are not indicated. Deletions of internal *Bam*HI or *Bgl*II fragments were made by digesting pPO1669 plasmid DNA, ligating the resulting fragments, and using this resulting mix to transform Mu-immune MC1040 cells. Tc^r transformants were selected at 30°C and scored for the Ap, Km, and Lac markers. Although the *lac* genes on pPO1669 are not fused to any known gene, they are expressed at a low level which can be detected on M63-glucose-XG media. There are no *Bgl*II sites on pSC101, but there is a site for *Bam*HI. This site, however, is in the Tc^r determinant and was retained by the selection for Tc^r. Plasmid structures were checked by gel analysis. Three useful Mu dII deletions are shown in Fig. 2. Mu dII1681 retains *lac* and the Km^r marker, whereas Mu dII1678 retains *lac* and Ap^r. Mu dII4041 is the smallest but is missing the *lac* segment.

To make an even smaller mini-Mu dII, an additional deletion was made by removing a *Hin*dIII fragment from Mu dII1681 (in plasmid pPO1681) to form Mu dII1734, as described above for the *Bam*HI and *Bgl*II deletions. This deletion, however, removes the Mu A and B genes necessary for transposition and replication. Prophage with this deletion can be complemented for these genes to transpose but at a lower rate than the mini-Mu phage that are A$^+$ and B$^+$, as reported below.

Mini-Mu dII transposition and placement into the chromosome. The mini-Mu dII prophage were checked for transposition from pSC101. The MC1040 strain background in which they were in contained a Mu *c*ts prophage so that transposition and phage growth could be induced directly by heating to 42°C. The resulting lysates were used to transduce strain M8820 to Ap^r or Km^r. Mu dII1678, Mu dII1681, and Mu dII4041 all gave transductants as did the parental Mu dII7701-301, Mu dII301, and Mu dI1 phage. Of the transduc-

TABLE 1. Strains, phage, and plasmids

Strain, phage, and plasmid	Description or genotype	Source or reference
Strain		
BAC1	MC4100 with *araB*::Mu dI7701-1	MAL113 × Mu pf7701
BAC101	M8820TRMucts with Mu dII4041	Infection
CT152	F⁻ *gal lac rpsL* Mu pf7701	M. Howe
D7053	Hfr Cav *thr araD139 trp met*	12
D7053.3	D7053 with *ara*::(Mu *cts*)*3*	Lysogeny
EFO	F′KLF-1 *thr*⁺ *araC*⁺*O*⁺*I*⁺*B*⁺*A*⁺*D*⁺ *leu*⁺ F⁻ *araD139* Δ(*araCOIBA-leu*)*7697*	14
EC601.9	F′ts114 *lacI*::Mu *cts62* *lacP*⁺*O*⁺*Z*⁺*Y*⁺*A*⁺ F⁻Δ(*lacIPOZYA-argF*)*U169 melB*? *glp*?	13[a]
M8820	F⁻, *araD139* Δ(*ara-leu*)*7697* Δ(*proAB-argF-lacIPOZYA*)*XIII rpsL*	12[a]
M8820Mu	M8820 with Mu	Lysogeny
M8820Mucts	M8820 with Mu *cts*	Lysogeny
M8820TR	M8820 with *recA56 srl*::Tn*10* (Tc^r)	P1 transduction
M8820TRMucts	M8820TR with Mu *cts*	Lysogeny
M88205S	M8820 with *spcA rpsL*	P1 transduction
M8820SMucts	M8820S with Mu *cts*	Lysogeny
MAL103	F⁻ Mu dI1 *ara*::(Mu *cts*)*3* Δ(*proAB-argF-lacIPOZYA*) *XIII rpsL*	16
MAL113	MC4100 with *araB*::Mu dI (an *ara-lac* fusion)	16
MAL315	Mu dII301 *malK* or *T*::Mu *cts* *araD139* Δ(*ara-leu*)*7697* Δ(*proAB-argF-lacIPOZYA*)*XIII rpsL*, unknown auxotroph	14a
MALII1681	MAL315 with Mu dII1681 (Km^r) in place of Mu dII301	× Mu dII1681
MALII1681TR	MALII1681 with *recA56 srl*::Tn*10* (Tc^r)	P1 transduction
MALII1734	MAL315 with Mu dII1734 (Km^r) in place of Mu dII301	× Mu dII1734
MALII1734TR	MALII1734 with *recA56 srl*::Tn*10* (Tc^r)	P1 transduction
MC1000	F⁻ *araD*⁻ Δ(*ara-leu*)*7697* Δ(*lacIPOZY*-A?) *X74 galU galK rpsL*	13
MC1040	F⁻ *araD139 ara*::(Mu cts)3 Δ(*lac*)*X74 galU galK rpsL*	D7053.3 × MC1000
MC4100	F⁻ *araD139* Δ(*lacIPOZYA-argF*)U169 *rpsL thi*	13[a]
POI1678	POI1681 with Mu dI1678 (Ap^r) in place of Mu dI1681	× Mu dI1678
POII1678	POII1681 with Mu dII1678 (Ap^r) in place of Mu dII1681	× Mu dI1678
POI1681	MAL103 with Mu dI1681 (Km^r) in place of Mu dI1	× Mu dI1681
POI1681TR	POI1681 with *recA56 srl*::Tn*10* (Tc^r)	P1 transduction
POII1681	M8820 with Mu dII1681 *ara*::(Mu *cts*)3 *araD*⁻ *leu*⁺ *lac*⁺ *pro*⁺	Mu dII1681 lysogen × D7053.3
POII1681TR	POII1681 with *recA56 srl*::Tn*10* (Tc^r)	P1 transduction
POI1734	MAL103 with Mu dI1734 (Km^r) in place of Mu dI1 (Ap^r)	× Mu dI1734
POII1734	MC1040(Mu *cts*) with Mu dII1734 *lac*⁺	(D7053.3 × Mu dII1734) × MC1040
PO3	MC4100 with *araB* or *A*::Mu dII7701-301	POL3 × Mu pf7701
POL3	MC4100 with *araB* or *A*::Mu dII301 (an *ara-lac* fusion)	Lysogeny
PO401	M8820S Mu *cts* with F′KLF-1 *thr*⁺ *araB* or *A*::Mu dII7701-301 *leu*⁺	(EFO × PO3) × M8820SMucts
Phage		
Mu	Mu *c*⁺	A. Bukhari
Mu *cts*	Mu *cts62*	M. Howe
Mu pf7701	Mucts62::Tn*5* (Km^r) (ΔIS*50* right) Δ(445-3) a plaque-forming Km^r phage	M. Howe, CT152
Mu dI1	Mu *cts62*::IS*121* d(Ap^r *trp*′B⁺A′-ΔW209-*lac*′*ZYA*) a defective *lac* transcription fusion phage	13; MAL103 (Fig. 1)
Mu dII301	Mu *cts62*::IS*121* d(Ap^r *lac*′*ZYA*) a defective *lac* transcription-*lacZ* translation fusion phage	14a; MAL315 (Fig. 1)
Mu dI7701-1	Mu dI1 with Km^r of Mu pf7701	BAC1
Mu dII7701-301	Mu dII301 with Km^r of Mu pf7701	PO3 (Fig.2)
Mu dI1678	Mu dI7701-1 with Δ(Km^r) *Bgl*II	POI1678
Mu dII1678	Mu dII7701-301 with Δ(Km^r) *Bgl*II	pPO1678 (Fig. 2)
Mu dI1681	Mu dI7701-1 with Δ(Ap^r) *Bam*HI	POI1681
Mu dII1681	Mu dI7701-301 with Δ(Ap^r) *Bam*HI	pPO1681 (Fig. 2)
Mu dI1734	Mu dI1681 with Δ(Mu *A B*) *Hin*dIII	POI1734
Mu dII1734	Mu dII1681 with Δ(Mu *A B*) *Hin*dIII	pPO1734 (Fig. 2)
Mu dII4041	Mu dII7701-301 with Δ(Ap^r *lac*) *Bam*HI	pPO4041 (Fig. 2)
Plasmids		
pBR322	Tc^r Ap^r replicon ColE1	52
pSC101	Tc^r replicon pSC101	21
pPO1669	pSC101::Mu dII7701-301 (Ap^r Km^r *lac*)	PO401 with pSC101 X MC1040
pPO1678	pSC101::MudII1678 (Ap^r *lac*)	pPO1669 deletion (Fig. 2)
pPO1681	pSC101::Mu dII1681 (Km^r *lac*)	pPO1669 deletion (Fig. 2)
pPO1734	pSC101::Mu dII1734 (Km^r *lac*)	pPO1669 deletion (Fig. 2)
pPO4041	pSC101::Mu dII4041 (Km^r)	pPO1669 deletion (Fig. 2)

[a] Deletions of *argF* are not Arg⁻ because *E. coli* K-12 has an isozyme gene *argI*.

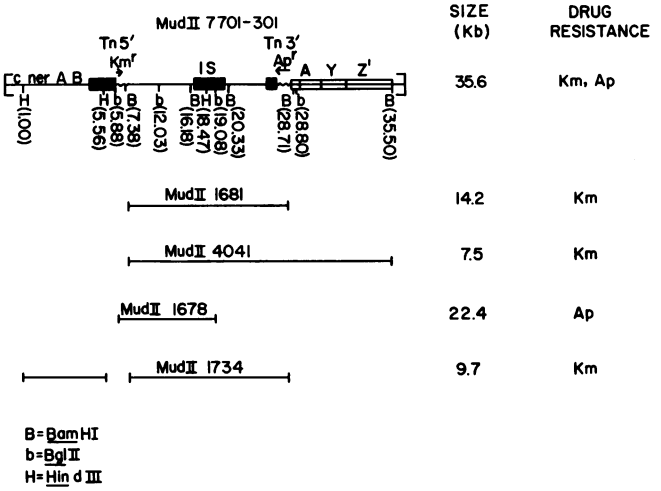

FIG. 2. Mini Mu-*lac*'s. Shown is the genetic and restriction map of Mu dII7701-301 on the pPO1669 plasmid which is an insertion of this phage into pSC101 (Tc^r). The IS*121* insertion from Mu dII (Fig. 1) was retained as determined by its characteristic *Hin*dIII and *Bgl*II sites. This restriction map is correlated with the maps and known sequences of Mu (1, 35, 46), Mu dI1 (16, 45), Mu dII (14a), Mu pf7701 (C. Thompson and M. Howe, unpublished data), Tn*5* (Km^r) (3, 49), Tn*3* (Ap^r) (30), and *lac* (7, 15, 17, 34) and is consistent with the phenotypes of the deletions isolated. Underlined are the DNA fragments deleted by restriction digestion, ligation, and transformation to select the clones pPO1678, pPO1681, pPO1734, and pPO4041. The resulting plasmids, deletions, and Mu d phage are designated with the same numbers.

tants, 99% were Tc^s and approximately 1% were Lac$^+$ on lactose MacConkey media, implying that the Mu dIIs were no longer connected to the Tc^r pSC101 plasmid sequences and apparently inserted into different locations in the chromosome. The Mu dII1734 lysate, however, yielded 100-fold fewer Km^r transductants, but the same 10^9 PFU of the complementing Mu *c*ts prophage per ml. Over 90% of the Mu dII1734 Km^r transductants were also Tc^r, implying that the original pSC101 plasmid with the Mu dII1734 insertion was frequently being transduced without transposition of the Mu dII1734 to the chromosome, as would be expected for a Mu A$^-$ B$^-$ phage.

To propagate the mini-Mu dII phage without the pSC101 plasmid, Mu dII1678 and Mu dII1681 lysogens of strain M8820 were associated with a complementing Mu *c*ts prophage by mating them with the Hfr Mu *c*ts strain D7053.3 with selection for Leu$^+$, Km^r or Ap^r, and Str^r exconjugants. Selection for Leu$^+$ ensured that the Mu *c*ts insertion in the adjacent *ara* genes of D7053.3 was crossed into the *ara-leu* deletion strain M8820. This formed strains POII1678 and POII1681. A similar strain, BAC101, was made for Mu d4041 in a different way, by infecting strain M8820TRMucts with the Mu dII4041 lysate and selecting for Km^r lysogens for which the Mu dII4041 had inserted into the chromosome in violation of the Mu *c*ts immunity. (It was noticed that this violation occurred more frequently when infection was carried out at the partially inducing temperature of 37°C rather than at repressing temperatures below 30°C.)

A similar strain with Mu dII1734 was made in another way to ensure that the Mu dII1734 was in the chromosome. Mu dII1734 on pPO1734 was introduced into the Hfr strain D7053.3 by transformation, partially induced for transposition by growth at 37°C, and mated with the F$^-$ Mu *c*ts lysogen strain MC1040, with selection for Km^r and Str^r. Since Hfr strains only transfer DNA from the chromosome, and not from the pSC101 plasmid, it was expected that the Km^r Mu dII1734 would be conjugally transferred only if it had transposed to the chromosome. Indeed, all the exconjugants tested (as POII1734) were Tc^s and had no detectable plasmid DNA in extracts run on cesium chloride-ethidium bromide gradients.

The frequency of Mu transduction and *lac* fusion formation was checked for all these strains which no longer had the pSC101 plasmid. All except for POII1734, with the Mu A$^-$ B$^-$ *Hin*dIII deletion phage Mu dII1734, gave the same titers as the original Mu dII301 lysogen strain MAL315: 2×10^9 to 6×10^9 plaques per ml and 5×10^5 to 50×10^5 Km^r or Ap^r transductants per ml, with approximately 1% of the transductants being Lac$^+$ for the type II phage. The 1734 phage gave the same plaque titers, but only 10^4 Km^r transductants per ml, and only 10^{-4} of these were Lac$^+$. The mechanism by which the 1734 phage gives rise to any lysogens at all is discussed below.

Construction of mini-Mu dI phage by recombination with Mini-Mu dII. The 1678, 1681, and 1734 deletion derivatives of Mu dII7701-301 shown in Fig. 2 were transferred to the Mu dI1 transcription fusion phage by homologous recombination by Mu and *lac* homology. Strain MAL103, containing the Ap^r Mu dI1 and the complementing Mu *c*ts prophages, was infected at 30°C with lysates of the Km^r Mu dII1681 and Mu dII1734 phage from strains POII1681 and POII1734, with selection for Km^r. Recombinants were scored by their loss of the Ap^r marker, and the strains were named POI1681 and POI1734. Similarly, Mu dI1678 (Ap^r) recombinants were selected in a second step by infecting the Mu dI1681 (Km^r) recombinant strain POI1681 with a Mu dII1678 lysate with selection for Ap^r and scoring for Km^s. In these crosses, approximately half of the selected colonies were recombinants, with the others presumably being new insertions in violation of immunity, as was seen in the earlier cross between Mu pf7701 (Km^r) and POL3 (Mu dII301, Ap^r).

Alternative strains MALII1681 and MALII1734 and Mu dII1681 and Mu dII1734, and a complementing Mu *c*ts, were made by recombination with the Ap^r Mu dII301 lysogen MAL315 with selection for Km^r and scoring for Ap^s. This established a set of mini-Mu dI and Mu dII lysogens which were isogenic with the original Mu dI1 and Mu dII301 lysogenic strains MAL103 and MAL315, which are useful for careful comparisons. These strains also gave slightly higher phage titers than the POII strains.

Mini-Mu insertions into plasmids. Lysogeny with bacteriophage Mu results in Mu insertions in different sites in the chromosome or in a plasmid present in a cell. To select Mu insertions located exclusively in a small genetic region, we devised the mini-Mu plasmid transduction scheme outlined in Fig. 3. This procedure can be carried out with small high-copy-number plasmids whose DNA can be easily isolated and examined for its physical structure by gel analysis. Alternative localization procedures involve phage (54) or plasmid transfer (19, 25, 37, 41, 55).

To test this scheme for selecting insertions into plasmids, we first chose the pSC101 intermediate-copy-number plasmid which we knew would be viable with a Mu insertion. This plasmid was introduced, by DNA transformation with selection for Tc^r, into strains POI1678, POI1681, POII1678, and POII1681, which have Ap^r and Km^r mini-Mu dI and Mu dII prophage along with a complementing Mu *c*ts prophage. All these strains yielded phage lysates which gave the expected 2×10^9 to 5×10^9 plaques per ml and 5×10^5 to 50×10^5 Ap^r or Km^r transductants per ml in nonimmune M8820

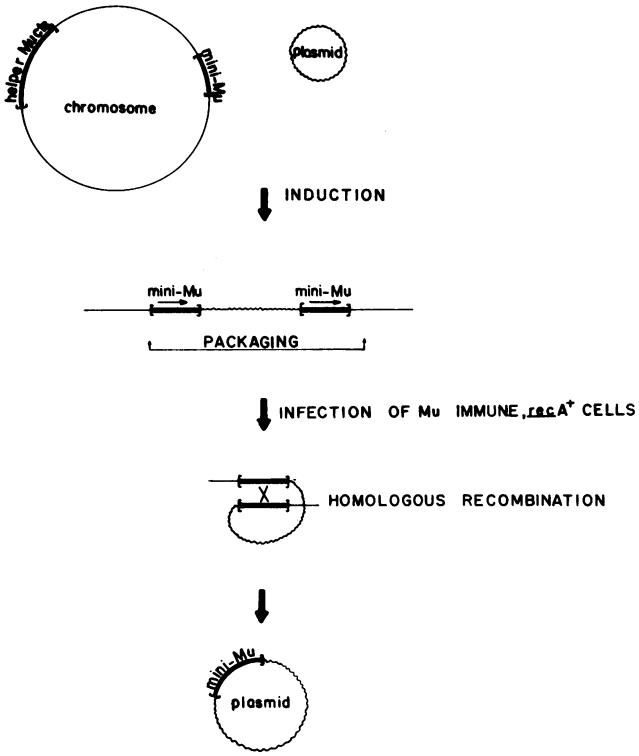

FIG. 3. Scheme for selecting mini-Mu insertions into plasmids. A plasmid is introduced, as by DNA transformation, into a cell containing both a mini-Mu-defective prophage and a complementing Mu *c*ts prophage (thick lines), usually in the chromosome (thin lines). A culture is grown and heat-induced for Mu lytic growth. The mini-Mu and Mu *c*ts prophage both replicate and transpose hundreds of times in each cell and occasionally insert into a plasmid sequence by a cointegrate transposition event as shown. Mu DNA sequences are then packaged into Mu phage heads from the Mu left end to include a headful of approximately 38 kb of DNA (10). If packaging starts from a mini-Mu sequence on the left side of a mini-Mu plasmid cointegrate structure and if the size of the mini-Mu plus the size of the plasmid is less than the 38 kb, then duplicated copies of mini-Mu sequences will be packaged. Upon infection of a new cell with such a phage particle, homologous recombination can occur between the duplicated Mu sequences to form a plasmid with an insertion. The recipient cell must be capable of homologous recombination (*recA*$^+$). More of these plasmid mini-Mu insertions are obtained if the recipient cell is Mu immune (contains the Mu repressor, as in a Mu lysogen) to repress expression of the incoming Mu genes. To prevent formation of multimeric forms of the plasmid in the original cell by homologous recombination, the donor cell can be made *recA*. Insertions of mini-Mu into multimer forms of a plasmid are unstable since recombination can occur between duplicated plasmid sequences with the loss of the insertion.

cells, and 10^3 to 10^4 Tc^r transductants per ml with Mu-immune M8820Muc$^+$ and M8820Mucts cells. Fewer Tc^r transductants, 10/ml, were found if nonimmune M8820 cells were used. The Tc^r transductants usually (>90%) had the Ap^r or Km^r marker and contained plasmids with the expected restriction maps for pSC101::Mu d plasmids (Fig. 2). Approximately 25% of the type I and 5% of the type II Tc^r transductants were Lac$^+$, and approximately half of these became Lac$^-$ on lactose MacConkey medium without tetracycline, implying that fusions were being formed with tetracycline-inducible plasmid promoters (53). Apparently, these insertions did not inactivate any essential parts of genes for proteins needed for tetracycline resistance.

More detailed studies were next done with the higher-copy-number pBR322 plasmid. This plasmid has been entirely sequenced (52) and encodes Ap^r (from the transposon Tn*3*) and Tc^r (from pSC101, but no longer tetracycline-inducible) determinants. The pBR322 plasmid was introduced, by DNA transformation, into strains POI1681, POII1681, and POII1734 which have mini-Mu Km^r prophage. The Ap^r mini-Mu phage were not used because they have homology with the Ap^r marker on pBR322. For all three transformant strains, Ap^r, Tc^r, and Km^r, transductants were selected after infecting Mu lysogens of M8820. It was noticed that more plasmid marker (Ap^r and Tc^r) transductants were obtained with Mu *c*$^+$ lysogen recipients than with Mu *c*ts recipients when adsorption was carried out at 30°C and that the Mu *c*ts lysogen transductants obtained often made small colonies. However, when adsorption was done at a lower temperature of 22°C, almost the same number of transductants were obtained, and these all formed normal-sized colonies. This implied that the Mu *c*ts62 repressor was perhaps not fully functional at 30°C upon initial introduction of a Mu prophage on a high-copy-number plasmid.

It was also observed that the Tc^r and Ap^r transductants were often very unstable for the Km^r marker and gave rise to Km^s descendants. Examination of plasmid DNA by gel analysis indicated that many Mu insertions were in multimeric forms of pBR322 such that homologous recombination between the two copies of the pBR322 sequences could loop out the Mu insertion and leave an original pBR322 plasmid. This effect could also be inferred from the low frequency of Ap^r Tc^s and Tc^r Ap^s transductants which would be due to insertion into the Ap or Tc genes and which would be masked by the multimer forms.

To minimize the effect of plasmid multimers, we made the donor cells *recA*$^-$ by generalized transduction with phage P1CM (48) with selection for a Tc^r Tn*10* insertion in a closely linked *srl* gene (22) to form the strains in Table 1 with names ending in "TR". Into these strains, monomeric pBR322 plasmid DNA (isolated after electrophoresis on an agarose gel) was introduced by transformation with selection for Ap^r. Lysates were prepared and used to transduce both nonimmune and immune Mu *c*ts and Mu *c*$^+$ lysogens of M8820 to Ap^r, Tc^r, or Km^r or all three. Adsorption was initiated at room temperature to minimize problems with the *c*ts62 allele.

The frequency of transduction for the Ap^r plasmid marker and the Km^r phage marker is shown in Table 2. These transductants stably maintained their markers upon further cell growth. Km^r transduction occurred more frequently in nonimmune cells which do not repress the incoming phage transposition functions necessary for integration into the chromosome. Ap^r transduction occurred more frequently in Mu-immune cells, which is consistent with the plasmid transduction process described in Fig. 3. By this process, transposition is not required in the recipient cell, and the preexisting lysogenic state removes the need to establish repression, thereby resulting in a higher frequency of transduction of plasmids with mini-Mu insertions. This plasmid transduction process also requires *recA*$^+$ homologous-recombination-proficient recipient cells. When a *recA*$^-$ recipient cell was used, the frequency of Ap^r transduction was reduced 100-fold.

The frequencies at which Mu dI and Mu dII1681 insertions in pBR322 formed *lac* gene fusions or inactivated the Ap^r or Tc^r determinants or both are given in Table 3, with M8820Muc$^+$ as the recipient strain. Individual Km^r transductants were picked from lactose MacConkey agar media

TABLE 2. Mini-Mu duction of pBR322

Mini-Mu's in donors with monomeric pBR322	Selection	Recipient strains (transductants/ml)		
		M8820	M8820Mucts	M8820Muc$^+$
Mu dI1681	Km^r	1×10^5	7×10^3	1×10^3
	Ap^r	3×10^1	4×10^3	2×10^3
	Km^r Ap^r	4×10^1	4×10^3	1×10^3
Mu dII1681	Km^r	1×10^7	7×10^5	2×10^5
	Ap^r	1×10^2	4×10^5	4×10^5
	Km^r Ap^r	3×10^0	2×10^5	1×10^5

and checked for Ap^r and Tc^r. The 3% of the Km^r transductants examined which were both Ap^s and Tc^s are not included in these data since for all cases examined these did not contain any plasmid DNA and presumably were due to rare Mu transposition or recombination in the Mu-immune cells.

We noted that the frequency of Mu d insertions in the Ap^r or Tc^r determinants (Table 3) was approximately sixfold lower than would be expected from the size of these regions on pBR322 (52). The frequency of insertions that are Lac$^+$ in the Ap and Tc resistance determinants is 69 and 41%, respectively, for Mu dI1681, and 12 and 7%, respectively, for Mu dII1681, in comparison to an expected almost 50% (two possible orientations) for Mu dI transcription fusions and 16.7% (two orientations and three phases) for Mu dII in-frame translation fusions. These variations from expectations may be due to nonrandomness of Mu insertion (see below) or to lethality of some insertions. The Ap^r determinant codes for a periplasmic protein β-lactamase and the Tc^r determinant codes for membrane-bound proteins, and hybrid protein β-galactosidase gene fusions to these types of genes have previously been reported (28) to often be lethal.

Plasmid DNAs from several insertions of each type shown in Table 3 were isolated and examined by gel analysis after digestion with several restriction enzymes. All were found to have appropriate structures for Mu d insertions into pBR322. No detectable deletions or rearrangements were found, except that for occasional DNA preparations, some faint restriction digestion-generated bands could be seen on gels which may have been due to Mu-induced rearrangements in a small fraction of the plasmids. The positions and orientations of mini-Mu insertions in pBR322 were mapped and found to be distributed around the plasmid, although not necessarily at random (see below). Insertions that formed *lac* fusions were mapped in the Ap^r and Tc^r determinants, in the *rop* gene (18), and in unknown genetic regions (51, 52) at 1.4 and 3.15 kb from the *Eco*RI site (in the clockwise direction). Insertions of the 1734 phage were also examined and found to have structures expected for simple insertions.

Mu d insertions into pBR322 were also shown not to have any detectable defects for Mu growth or transduction. Mu *c*ts lysogens containing mini-Mu insertions in pBR322 were induced and the lysates used to transduce M8820 cells. The Km^r transductants obtained had new insertions, as judged by the various levels of *lac* expression observed on lactose MacConkey medium and by the loss of the plasmid marker in most cases. However, the titers of Mu d (Km^r) transduction and of the complementing Mu *c*ts phage were reduced approximately 10- and 10^3-fold, respectively, as compared with the original phage lysates. This we suspect is due to interference by the high number of copies of the defective Mu d phage on the pBR322 plasmid which could competitively inhibit the growth of the unit copy complementing Mu *c*ts phage in the chromosome. When Mu d insertions in pBR322 were moved to the chromosome (as Km^r transductants of M8820) and induced (after the introduction of a Mu *c*ts-complementing prophage from a cross with D7053.3), normal transduction frequencies and phage titers were observed.

DISCUSSION

We described the construction of small Mu-*lac* gene fusion elements that can be used in vivo to form either transcriptional or transcriptional-translational *lac* gene fusions. Fusions were formed with the ampicillin (β-lactamase) and tetracycline resistance determinants and the *rop* gene on pBR322 and with tetracycline-inducible genes on pSC101. The latter fusions were selected by their regulation of *lac* expression and not by an insertion-inactivation phenotype such as sensitivity to tetracycline.

Insertions of these mini-Mu elements can be localized to sequences on particular plasmids, including the high-copy-number plasmids commonly used for DNA cloning. Insertions have been obtained in plasmids with several types of replicons and copy numbers, including pSC101 and ColE1 type plasmids (such as pBR322) as described here, and F factor- and p15a- (as pACYC184) (20) derived plasmids. No type of plasmid was found that could not tolerate insertions of these mini-Mu elements. The only known limitation to the plasmid insertion procedure described here (Fig. 3) is the size of the plasmid, since bacteriophage Mu can package only approximately 38 kb of DNA (10). For larger plasmids, other procedures, such as conjugation (19, 25, 37, 41, 55), can be used to select insertions specifically in the plasmid.

As few as 116 base pairs from the Mu right end and 1,006 base pairs from the left end were retained in these mini-phages (Fig. 2), which are still competent for intracellular transposition and growth as a phage when complemented by a wild-type Mu phage. Most of the DNA sequences on these phage have been determined previously (3, 7, 33, 34, 46, 49). These mini-Mu phage are deleted for most of the Mu genes, including the *lys* (lysis) and *kil* (host cell killing) genes which may affect cell viability when present in a high-copy-number plasmid. Insertions of these elements are stable in ColE1-derived plasmids, in contrast to some but not all reports for full-length Mu insertions. Inselburg (32) and others (as discussed in reference 50) have reported the failure to obtain Mu insertions in ColE1-derived plasmids, whereas Maynard-Smith et al. (40) and Liebart et al. (38) have reported the isolation of such insertions with Mu *c*$^+$ and Mu *c*ts. The first of the positive reports (40) have presented only three insertions, two of which have deletions of plasmid sequences near the insertions, and the other one (38) does not characterize the insertions.

lac gene fusions formed by Mu-*lac* transposition can be genetically unstable in the presence of the Mu transposition

TABLE 3. Frequency of lac$^+$, Ap^s, and Tc^s insertions in pBR322

Phage	Phenotype	% of inserts with following phenotypes:		
		Ap^r Tc^r	Ap^r Tc^s	Ap^s Tc^r
Mu dI1681	Lac$^+$	43.0	1.7	2.7
	Lac$^-$	49.0	2.4	1.2
Mu dII1681	Lac$^+$	5.1	0.5	0.3
	Lac$^-$	85.3	6.7	2.1

genes since the Mu-*lac* can transpose to new sites or make transposition-generated terminal deletions. This can particularly interfere with selections for mutants that have altered gene expression. To circumvent this problem, fusions can be made with a transposition-defective phage such as the Mu d1734 phage described here. Alternatively, fusions made with a transposition-proficient phage can be stabilized by removing the transposition genes or a Mu end by mutation or by recombination with a defective phage such as Mu d1734 or λ (*lac* Mu) (13, 36). Mu insertions in plasmids can also be stabilized by removing appropriate restriction fragments.

The scheme outlined in Fig. 3 can be used to isolate insertions of the transposition gene-deleted phage Mu d1734, since there is complementation in the donor cell and the transposase gene is not needed in the recipient cell. We have also found that Mu d1734 insertions into the chromosome can be formed after infection with a lysate, even though no Mu transposition genes were expected to be present in the recipient. This transposition was not due to transient complementation by a coinfecting Mu *cts* phage, since lysogens were obtained linearly with dilutions of the phage lysate. Recent preliminary experiments (P. Olfson, S. Suh, B. Castilho, and M. Casadaban, unpublished data) indicate that this transposition does depend on a Mu A gene being carried into the recipient cell, since the number of transductants is greatly reduced when the 1734 phage is grown with a Mu A amber helper phage in an amber-suppressing cell and the resulting lysate is used to infect a nonsuppressing cell. Perhaps the Mu transposition genes are occasionally carried on the flanking DNA packaged from the chromosome when there is a nearby insertion of a helper Mu prophage in the donor cell.

The process depicted in Fig. 3 for localization of mini-Mu insertions into plasmids can be used to obtain hundreds of insertions in a small-scale experiment which should be enough to saturate all possible Mu insertion sites in a small plasmid. This brings up the question of just how random Mu insertion sites are. Most insertions that have been studied in a defined region, such as in a particular gene, have been shown to be in different sites, although a few, as determined by recombination tests, may have occurred in precisely the same location (11, 24, 26, 47). Precise sequence mapping of these insertions have not been reported. Insertions of other transposons in limited regions have been shown upon careful analysis to have hot-spot sites with various specificities (4, 5, 27, 29, 56, 57). In further experiments (unpublished data), we used the procedure outlined in Fig. 3 to prepare pools of mini-Mu insertions in a shortened version of the pBR322 plasmid and found that many insertions do indeed occur in a few specific hot-spot regions, with particular sites within each region being used at different frequencies.

Mu insertions in plasmids can be used as a convenient source of Mu DNA for further Mu genetic or biochemical analysis (19). For example, Mu dII4041 insertions in pBR322-derived plasmids have been used to isolate deletions which define the functional ends of bacteriophage Mu. So far, the essential sequences on the right end of Mu have been localized to begin between 93 and 68 base pairs from that end (unpublished data).

Mu insertion plasmid DNA can also be used to conveniently introduce Mu into other species. Mu dII1681 insertions in pBR322 have been used as a source of DNA to transform the gram-negative bacterium *Agrobacterium tumefaciens*, in which Mu-*lac* has been found to transpose and form *lac* fusions (F. Richaud and M. Casadaban, unpublished data). More conventional methods for Mu transfer to other gram-negative bacterial species have been carried out with Mu dII1681 insertions into permissive conjugative plasmids for transfer between *Escherichia coli* and *A. tumefaciens* (F. Richaud and M. Casadaban, unpublished data) and *Pseudomonas aeruginosa* (J. Shapiro, personal communication). Mu dII1681 has also been introduced into the lower eucaryotic yeast *Saccharomyces cerevisiae* with insertions in *E. coli-S. cerevisiae* shuttle vector plasmids (6), but no evidence for transposition was found by using the formation of β-galactosidase gene fusions as an assay for transposition (M. Ditto and M. Casadaban, unpublished data).

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LITERATURE CITED

1. **Allet, B., F. Blattner, M. Howe, M. Magazin, D. Moore, K. O'Day, D. Schultz, and J. Schumm.** 1977. Genetic and physical map of bacteriophage Mu, p. 745–746. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
2. **Bassford, P., J. Beckwith, M. Berman, E. Brickman, M. Casadaban, L. Guarante, I. Saint-Girons, A. Sarthy, M. Schwartz, and T. Silhavy.** 1978. Genetic fusions of the *lac* operon: a new approach to the study of biological processes, p. 245–261. *In* J. H. Miller and W. S. Reznikoff (ed.), The operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. **Beck, E., G. Lugwig, E. A. Auerswald, B. Reiss, and H. Schaller.** 1982. Nucleotide sequence and exact localization of the neomycin phosphotransferase gene from transposon Tn5. Gene **19**:327–336.
4. **Berg, D. E., A. Weiss, and L. Crossland.** 1980. Polarity of Tn*5* insertion mutations in *Escherichia coli*. J. Bacteriol. **142**:439–446.
5. **Bossi, L., and M. S. Campi.** 1981. DNA sequences at the sites of three insertions of the transposable element Tn5 in the histidine operon of *Salmonella*. Mol. Gen. Genet. **183**:406–408.
6. **Botstein, D., C. Falco, S. Stewart, M. Brennan, S. Scherer, D. Stinchcomb, K. Struhl, and R. Davis.** 1979. Sterile host yeasts: a eukaryotic system of biological containment for recombinant DNA experiments. Gene **8**:17–24.
7. **Buchel, D. B., B. Gronenborn, and B. Muller-Hill.** 1980. Sequence of the lactose permease gene. Nature (London) **283**:541–545.
8. **Bukhari, A. I.** 1976. Bacteriophage Mu as a transposition element. Annu. Rev. Genet. **10**:389–411.
9. **Bukhari, A. I., and E. Ljungquist.** 1977. Bacteriophage Mu: methods for cultivation and use, p. 749–756. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **Bukhari, A. I., and A. L. Taylor.** 1975. Influence of insertions on packaging of host sequences covalently linked to bacteriophage Mu DNA. Proc. Natl. Acad. Sci. U.S.A. **72**:4399–4403.
11. **Bukhari, A. I., and D. Zipser.** 1972. Random insertion of Mu-1 DNA within a single gene. Nature (London) New Biol. **236**:240–243.
12. **Casadaban, M.** 1975. Fusion of the *Escherichia coli lac* genes to the *ara* promoter: a general technique using bacteriophage Mu-1 insertions. Proc. Natl. Acad. Sci. U.S.A. **72**:809–813.
13. **Casadaban, M.** 1976. Transposition and fusion of the *lac* genes to selected promoters in *E. coli* using bacteriophage lambda and Mu. J. Mol. Biol. **104**:541–555.

14. **Casadaban, M.** 1976. Regulation of the regulatory gene for the arabinose pathway, *ara*C. J. Mol. Biol. **104**:557–566.
14a. **Casadaban, M., and J. Chou.** 1984. *In vivo* formation of hybrid β-galactosidase gene fusions in one step with a new transposable Mu-*lac* transducing phage. Proc. Natl. Acad. Sci. U.S.A. **81**:535–539.
15. **Casadaban, M., J. Chou, and S. N. Cohen.** 1980. In vitro gene fusions that join an enzymatically active β-galactosidase segment to amino-terminal fragments of exogenous proteins: *Escherichia coli* plasmid vectors for the detection and cloning of translational initiation signals. J. Bacteriol. **143**:971–980.
16. **Casadaban, M., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: in vivo probe for transcriptional control sequences. Proc. Natl. Acad. Sci. U.S.A. **76**:4530–4533.
17. **Casadaban, M., and S. N. Cohen.** 1980. Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. J. Mol. Biol. **138**:179–207.
18. **Cesareni, G., M. A. Muesing, and B. Polisky.** 1982. Control of ColE1 DNA replication: the *rop* gene product negatively affects transcription from the replicon primer promoter. Proc. Natl. Acad. Sci. U.S.A. **79**:6313–6317.
19. **Chaconas, G., F. J. deBruijn, M. Casadaban, J. R. Lupski, T. J. Kwok, R. M. Harshey, M. S. DuBow, and A. I. Bukhari.** 1981. *In vitro* and *in vivo* manipulations of bacteriophage Mu DNA: cloning of Mu ends and construction of mini-Mu's carrying selectable markers. Gene **13**:37–46.
20. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**:1141–1156.
21. **Cohen, S. N., and A. C. Y. Chang.** 1977. Revised interpretation of the origin of the pSC101 plasmid. J. Bacteriol. **132**:734–737.
22. **Csonka, L. N., and A. J. Clark.** 1979. Deletions generated by the transposon Tn10 in the *srl recA* region of the *Escherichia coli* K-12 chromosome. Genetics **93**:321–343.
23. **Csonka, L. N., M. M. Howe, J. L. Ingraham, L. S. Pierson III, and C. L. Turnbough, Jr.** 1981. Infection of *Salmonella typhimurium* with coliphage Mu d1(Ap^r *lac*): construction of *pyr*:*lac* gene fusions. J. Bacteriol. **145**:299–305.
24. **Daniell, E., R. Roberts, and J. Abelson.** 1972. Mutation in the lactose operon caused by bacteriophage Mu. J. Mol. Biol. **69**:1–8.
25. **Dixon, R., R. R. Eady, G. Espin, S. Hill, M. Iaccarino, D. Kahn, and M. Merrick.** 1980. Analysis of regulation of *Klebsiella pneumoniae* nitrogen fixation (*nif*) gene cluster with gene fusions. Nature (London) **286**:128–132.
26. **Emr, S. D., and T. Silhavy.** 1980. Mutations affecting localization of an *Escherichia coli* outer membrane protein, the bacteriophage λ receptor. J. Mol. Biol. **141**:63–90.
27. **Galas, D. J., M. P. Calos, and J. H. Miller.** 1980. Sequence analysis of Tn9 insertions in the *lac*Z gene. J. Mol. Biol. **144**:19–41.
28. **Hall, M. N., and T. J. Silhavy.** 1981. Genetic analysis of the major outer membrane proteins of *Escherichia coli*. Annu. Rev. Genet. **15**:91–142.
29. **Halling, S. M., and N. Kleckner.** 1981. A symmetrical six basepair target site sequence determines Tn10 insertion specificity. Cell **28**:155–163.
30. **Heffron, F., B. J. McCarthy, H. Ohtsubo, and E. Ohtsubo.** 1979. DNA sequence analysis of the transposon Tn3: three genes and three sites involved in transposition of Tn3. Cell **18**:1153–1163.
31. **Howe, M. M., and E. G. Bade.** 1975. Molecular biology of bacteriophage Mu. Science **190**:624–632.
32. **Inselburg, J.** 1974. Isolation and characterization of mutants of colicin plasmids E1 and E2 after Mu bacteriophage infection. J. Bacteriol. **119**:469–477.
33. **Kahmann, R., and D. Kamp.** 1979. Nucleotide sequences of the attachment sites of bacteriophage Mu DNA. Nature (London) **280**:247–250.
34. **Kalmins, A., K. Otto, U. Rüther, and B. Müller-Hill.** 1983. Sequence of the *lacZ* gene of *Escherichia coli*. EMBO J. **2**:593–597.
35. **Kania, J., and B. Müller-Hill.** 1977. Construction, isolation and implications of repressor-β-galactosidase: β-galactosidase hybrid molecules. Eur. J. Biochem. **79**:381–383.
36. **Komeda, Y., and T. Iino.** 1979. Regulation of expression of the flagellin gene (*hag*) in *Escherichia coli* K-12: analysis of *hag-lac* gene fusions. J. Bacteriol. **139**:721–729.
37. **Lee, J.-H., L. Heffernan, and G. Wilcox.** 1980. Isolation of *ara-lac* gene fusions in *Salmonella typhimurium* LT2 by using transducing bacteriophage Mu *d*(Ap^r *lac*). J. Bacteriol. **143**:1325–1331.
38. **Liebart, J. C., P. Ghelardini, and L. Paolozzi.** 1982. Conservative integration of bacteriophage Mu DNA into pBR322 plasmid. Proc. Natl. Acad. Sci. U.S.A. **79**:4362–4366.
39. **Maniatis, T., E. Fritsch, and J. Sambrook.** 1982. Molecular cloning. Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.
40. **Maynard-Smith, S., D. Leach, A. Coelho, J. Carey, and N. Symonds.** 1980. The isolation and characteristics of plasmids derived from the insertion of Mup Ap1 into pML2: their behavior during transposition. Plasmid **4**:34–50.
41. **Mendoza, D., D. Clark, and J. E. Cronan, Jr.** 1981. One-step gene amplification by Mu-mediated transposition of *E. coli* genes to a multicopy plasmid. Gene **15**:27–32.
42. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
43. **Müller-Hill, B., G. Heidecker, and J. Kania.** 1976. Repressor-galactosidase chimaeras, p. 167–179. *In* R. Markham and R. W. Horne (ed.), Structure-function relationships of proteins. North-Holland Publishing Co., New York.
44. **Müller-Hill, B., and J. Kania.** *Lac* repressor can be fused to β-galactosidase. 1974. Nature (London) **249**:561–562.
45. **O'Connor, M. B., and M. H. Malamy.** 1983. A new insertion sequence, IS*121*, is found on the Mu dI1(Ap *lac*) bacteriophage and the *Escherichia coli* K-12 chromosome. J. Bacteriol. **156**:669–679.
46. **Priess, H., D. Kamp, R. Kahmann, B. Brauer, and H. Delius.** 1982. Nucleotide sequence of the immunity region of bacteriophage Mu. Mol. Gen. Genet. **186**:315–321.
47. **Raibaud, O., M. Roa, C. Braun-Breton, and M. Schwartz.** 1979. Structure of the *mal*B region in *Escherichia coli* K-12. I. Genetic map of the *malK-lam*B operon. Mol. Gen. Genet. **174**:241–248.
48. **Rosner, J.** 1972. Formation, induction and curing of bacteriophage P1 lysogens. Virology **48**:679–689.
49. **Rothstein, S. J., R. A. Jorgensen, K. Postle, and W. S. Reznikoff.** 1980. The inverted repeats of Tn5 are functionally different. Cell **19**:795–805.
50. **Schumann, W., and E. G. Bade.** 1979. *In vitro* constructed plasmids containing both ends of bacteriophage Mu DNA express phage functions. Mol. Gen. Genet. **169**:97–105.
51. **Stüber, D., and H. Bujard.** 1981. Organization of transcriptional signals in plasmids pBR322 and pACYC184. Proc. Natl. Acad. Sci. U S.A. **78**:167–171.
52. **Sutcliffe, J. G.** 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. **43**:77–90.
53. **Tait, R. C., and H. W. Boyer.** 1978. On the nature of the tetracycline resistance controlled by the plasmid pSC101. Cell **13**:73–81.
54. **Toussaint, A., M. Faelen, and A. Resibois.** 1981. Chromosomal arrangements induced by mini-Mu and mini-D108. Mini-review and new data. Gene **14**:115–119.
55. **Toussaint, A., and A. Resibois.** 1983. Phage Mu: transposition as a life-style, p. 105–158. *In* J. Shapiro (ed.), Mobile genetic elements. Academic Press, Inc., New York.
56. **Tu, C.-P. D., and S. N. Cohen.** 1980. Translocation specificity of the Tn3 element: characterization of sites of multiple insertions. Cell **19**:151–160.
57. **Weisberg, R. A., and A. Landy.** 1983. Site-specific recombination in phage lambda, p. 211–250. *In* R. W. Hendrix, J. W. Roberts, F. W. Stahl, and R. A. Weisberg (ed.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.