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Mini-Mu-Duction as a Test for Genetic Complementation in *Escherichia coli*

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In mini-Mu-duction, segments of host DNA bracketed between two copies of an internally deleted Mu phage (a mini-Mu) can be packaged within Mu phage particles. Upon infection of a second host strain, the DNA injected by these particles can insert into the chromosomal DNA in a reaction catalyzed by the phage A gene product (transposase), which is independent of homologous recombination. This results in a partially diploid host strain in which the duplicated host DNA is bracketed by two copies of the mini-Mu phage (Faelen et al., Mol. Gen. Genet. **176**:191–197, 1979). The frequency of mini-Mu-duction reported previously was low (10^{-8} to 10^{-9} per recipient cell) thus limiting its use to rather stable mutational lesions. I have increased the frequency of mini-Mu-duction 10- to 100-fold by use of a helper phage lacking the *kil* gene and by UV irradiation of the phage stocks. I have also shown that mini-Mu-duction is a reliable complementation assay in *rec*$^+$ as well as *recA* recipient strains. This genetic complementation test does not require prior gene localization and (due to the extended host range of phage Mu) should be applicable to many enterobacterial species.

The F′ merodiploid genetic complementation system commonly used in *Escherichia coli* and related bacteria can be clumsy and tedious (17, 18). The main disadvantage is that a mutational lesion must be located on the genetic map before the proper F′ factors can be chosen. To test complementation between two mutant alleles, one allele must be transferred to the F′ by homologous recombination (replacing the wild-type allele) (18). A further difficulty is that F′ factors carrying some segments of the *E. coli* chromosome are either not available or are very unstable (17). Molecular cloning (in vivo or in vitro) provides a second complementation assay but is laborious and usually involves gene localization. A third method, abortive transduction, is applicable only to rather special alleles and does not permit biochemical analysis of the partial diploid cells.

Faelen et al. (7) and Lefebvre and Toussaint (16) reported the phenomenon of mini-Mu-duction (see below) which had the potential of providing a complementation test that does not require gene localization and resulted in the formation of stable partial diploid cells. Mini-Mu-duction is essentially a transposition of host DNA mediated by two flanking copies of mini-Mu phage. Mini-Mu phages are internally deleted derivatives of phage Mu (25). These phages transpose and replicate in the presence of helper Mu phage and become packaged into Mu capsids within a variety of DNA structures (Fig. 1). Particles from such mixed lysates are able to transduce host genes into *recA* recipient strains of *E. coli*, a process called mini-Mu-duction (7, 16), which results in partially diploid strains. However, mini-Mu-duction has not been adopted by bacterial geneticists due to the low frequency of partial diploid formation. Faelen et al. (7) and Lefebvre and Toussaint (16) reported frequencies of 10^{-8} to 10^{-9} mini-Mu-ductants formed per recipient cell, and thus only those recipient strains carrying unusually stable mutational lesions were suitable recipients. I have increased the frequency of mini-Mu-duction by about 10- to 100-fold and have found that mini-Mu-duction provides a rapid and generally applicable genetic complementation test.

Mini-Mu-duction is thought to involve transduction of a recipient cell by phage particles containing two similarly oriented copies of the deleted phage bracketing the transduced bacterial DNA (Fig. 1, structure IV). Transduction is believed to occur by transposition of the entire structure (mini-Mu:chromosomal DNA:mini-Mu) into the bacterial DNA. This event requires the A gene product (transposase) of Mu and thus does not rely on homologous recombination (7). The mini-Mu-duction structures are thought to result from the headful packaging mechanism of phage Mu which begins encapsidization from the left (*c*) end of an integrated copy of the mini-Mu (7, 16, 25). Such particles, therefore, carry a segment of bacterial DNA bracketed by two copies of the mini-Mu phage. Since phage Mu transposes into DNA sequences essentially at random (25), mini-Mu-duction structures carrying any given segment of host DNA should be recoverable.

The low frequency of mini-Mu-duction reported previously (7, 16) was primarily due to the necessity to avoid infection of the recipient cells with two phage particles, one carrying a mini-Mu-duction structure and the other a helper phage chromosome. Phage Mu lysogenizes quite infrequently (0.1 to 1%) (25), and thus most such doubly infected cells would be lysed. Expression of the *kil* gene of the helper phage (most mini-Mu phages lack *kil*) could also kill the recipient cell in the absence of lytic development (12, 25). A secondary problem is the killing of those cells originally receiving only a mini-Mu-duction particle via subsequent infection from a burst of helper phage during colony formation on the selective plate. This secondary killing would occur by titration of the Mu repressor produced by the mini-Mu phage by superinfecting helper phages (13).

I have found that a high ratio of mini-Mu-duction particles to recipient bacteria can be used if (i) the phage lysate is irradiated with UV light and (ii) the helper phage lacks the *kil* gene (Table 1). UV treatment (at 254 nm) inactivated the replication of >99% of the helper phage particles with little or no effect on the frequency of mini-Mu-duction, whereas the *kil* helper phage prevented killing of the host cells by UV-inactivated helper phages.

In the mini-Mu-duction system I used, the donor strains carried both a *kil* mini-Mu and a *kil* helper phage (Table 1). Both phages also had a temperature-sensitive lesion (*c*ts62) in the repressor gene and an antibiotic resistance determinant

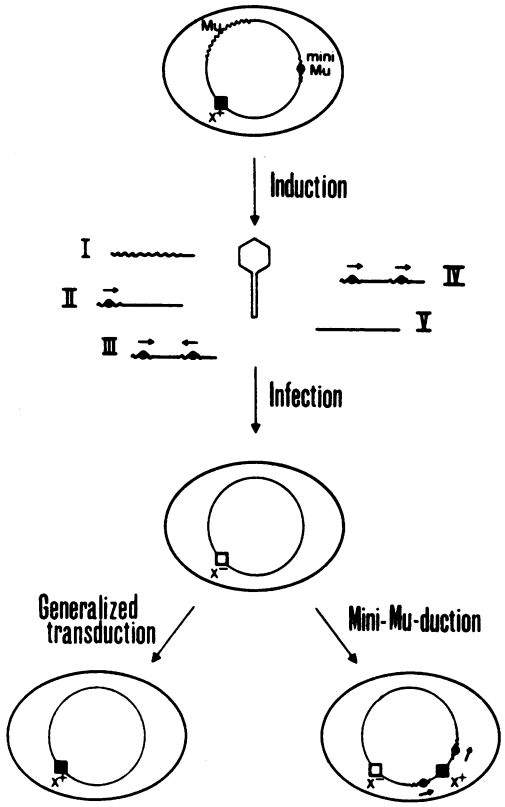

FIG. 1. DNA structures encapsidated in lysates of double lysogens of Mu and mini-Mu and the process of mini-Mu-duction. Induction of a strain carrying the X^+ chromosomal marker and also lysogenic for Mu and mini-Mu results in lysates in which 90% of the viral particles contain phage Mu DNA (wavy line) (type I). The remaining particles contain a variety of mini-Mu DNA-containing structures in addition to a few particles (type V) that contain only host DNA. The dominant mini-Mu structure (type II) contains one copy of the mini-phage with 50 to 100 base pairs of host DNA at the left (*c*) end and 39-L (L is the length of the mini-Mu phage) kilobases of host DNA at the other (*S*) end (type II) formed as a result of "headful" packaging beginning at the *c* end. Two structures, each containing two copies of the mini-phage in either the opposite orientation (type III) or in the same orientation (type IV) are also believed present (20). In both structures, the mini-phages bracket a 10- to 18-kilobase segment of host DNA (6, 20). The type IV particles are believed primarily responsible for the *recA*-independent mini-Mu-duction. All structures except type I should be capable of *recA*-dependent generalized transduction. However, types II and V are thought to give the majority of such recombinants. Structures of types I, II and III have been observed by electron microscopy (6, 20), where the presence of the other structures is inferred from genetic data (6, 25). Figure modified from Desmet et al. (6).

derived from a transposable element. The helper phage carried a segment of Tn*5* which encodes resistance to aminoglycoside antibiotics such as neomycin (Neo^r), whereas the mini-Mu phage carried ampicillin resistance (Amp^r) due to the presence of a segment of Tn*3*. Neither of the resistance determinants was capable of independent transposition due to deletions of portions of the transposable elements (16, 22; M. Howe, personal communication). The helper phage was introduced into a donor strain by infection and plating for Neo^r recombinants, whereas the mini-Mu phage was introduced on an F′ factor by conjugation followed by plating for Amp^r. The two phages could be introduced in either order but I have usually first introduced the mini-Mu phage followed by infection of the mating mixture with the helper phage (the Neo^r determinant allowed the isolation of the rare lysogens formed in the presence of the repressor encoded by the mini-Mu phage). The double lysogens were then induced by shift to 42°C (after growth at 30°C), and the lysates were irradiated with UV light and then used to infect various recipient host strains.

Both the *kil* helper phage and the UV irradiation are required for recovery of high levels of mini-Mu-ductants, the former being the more important parameter (Table 2). It should be noted that the *kil* deletion phage used also lacks the *gam* gene (22; M. Howe, personal communication) and

TABLE 1. Bacterial strains

Strain	Relevant genotype	Source or reference[a]
MC4100	*araD139* Δ(*lacIOPZYA*)*U169*	M. Casabadan
CY296	Δ(*trpA-E*)*229* of MC4100	[b]
CY305	F128::Mu18A-1[c] of CT152	See text
CY346	*zad*::Tn*10* (Mu Neo^r)[d] of W1485	See text
CY365	F128::Mu18A-1 of CY346	See text
CY368	CY365 (except helper is Mu cts62 *mom*3452)[e]	See text
C600	*thrB1 leuB6*	CGSC
X478	*proC32 purE42 metE70 lysA23 leu-6 trpE68 lacZ36 mtl-1 ara-14*	CGSC
A2	*trpA2*	
B4	*trpB4*	
C6	*trpC6*	C. Yanofsky strains
D2	*trpD2*	(27, 28)
5729E	*trpE5729*	
A229	Δ(*trpA-E*)*229*	
HfrG6MD2	Δ(*bioH-asd*)*29*[f]	CGSC
U428	*asd-1*	CGSC
AB645	*malA1*	CGSC
SJ16	*zad*::Tn*10 panD2*	Laboratory strain (5)
W1485	Prototrophic	CGSC
F::Mu18A-1/MXR	F128::Mu18A-1/MXR(Mu cts62)	A. Toussaint (16)
MXR	*recA56*	A. Toussaint (16)
GT28	*thrC1010*	
GT140	*thrC1081*	J. Gardner (8, 24)
M4131	*thrB4131*	
CT152	*gal* (Mu Neo^r)	M. Howe

[a] The CGSC allele numbers are those of the Coli Genetic Stock Center, Yale University, New Haven, Conn. The allele numbers of the strains not available from the Stock Center are those of the laboratory cited.

[b] Strain CY296 was constructed by P1 transduction of the Δ(*trp-tonB*) deletion from strain A229 into strain MC4100 with selection for *tonB* (18).

[c] Mu18A-1 is an internally deleted (ΔB-R) Mu phage 10.8 kilobase pairs in length which retains the *c*ts62 and *A* genes and has a portion of Tn*3* inserted in the G loop (20). It is carried on the F(*lac pro*) episome F128 (17).

[d] The Mu Neo^r phage is Mu *c*ts62 pf 7701::Tn*5* Δ445-3 and was the gift of M. Howe. The formation of the Tn*5* inserted phage involved deletion of the phage *kil* and *gam* genes and a portion of the Tn*5* element (M. Howe, personal communication; 22). The Δ445-3 deletion removed the phage *mom* gene.

[e] The helper phage was from a lysate of strain RH3936 (6) and was used in place of the Mu Neo^r helper.

[f] The deletion carried by strain HfrG6MD2 is DE29 of the Coli Genetic Stock Center. A 0.5-min portion of the genetic map (1) including the *bioH*, *malA*, *glpR*, *glpD*, *glgABC*, and *asd* loci was deleted.

TABLE 2. Effect of UV irradiation and *kil* mutation on mini-Mu-duction of *thrB*

Helper[a] phage	*kil*	UV[b]	Generalized transductants[c]	Mini-Mu-ductants
Mu *cts*62	+	−	420	2
Mu *cts*62	+	+	1,120	181
Mu *cts*62 Neo^r	−	−	1,704	238
Mu *cts*62 Neo^r	−	+	8,780	770

[a] The recipient was strain C600, and *thr*$^+$ was the selected marker (similar results were obtained for the *leuB* marker). The donors were strains CY368 and CY365. Both helper phages carried lesions in the nonessential *mom* gene. The phage stocks were prepared by growing the double lysogens at 30°C in the LB medium (pH 8) of Bade et al. (2) to 5×10^7 to 5×10^8 cells per ml. The cultures were then shifted to 42 to 43°C until cell lysis. The *kil*$^+$ stocks lysed about 1 h after shift, whereas the *kil* phage stocks required 1.5 to 2.5 h for lysis (probably due to deletion of the *gam* gene [12]). Shift of cultures at higher cell density to high temperature resulted in a decreased frequency of mini-Mu-duction presumably due to preferential packaging of the helper phage. The lysates commonly gave (per milliliter) about 1×10^{10} to 2×10^{10} plaque formers, 5×10^7 Neo^r recombinants, and 5×10^5 Amp^r recombinants. The lysates were sterilized with chloroform and stabilized by two cycles of centrifugation at 20,000 × *g* for 10 min. A few strains used in another work (4a) were found to give better phage production if the cultures were shifted to 37°C after 25 min at 42°C.

[b] The lysates were irradiated at 254 nm as thin films of liquid for 1 to 2 min at 2 J/m^2 per s. The irradiations were done with thorough mixing, and the irradiated lysates were stored at 4°C in the dark over chloroform. After irradiation, the plaque-forming and Neo^r-transducing ability declined 100-fold with a < twofold decrease in Amp^r transducing ability.

[c] For transductions, 2 ml (larger volumes can be used) of a phage stock (aerated to remove $CHCl_3$) was added to 2 ml of a saturated (about 2.5×10^9 cells per ml) recipient culture. $CaCl_2$ (10 or 15 mM) was added, and phage absorption was done for 30 min at 30°C. The cells were then pelleted, washed with medium E (26) to remove $CaCl_2$, and plated on a selective medium. The selective medium contained citrate to chelate the Ca^{2+} required for Mu absorption but this was not strictly required. After incubation at 30 or 37°C, the resulting colonies were replica plated onto the same medium containing 0.1 mg of ampicillin per ml. Comparison of the master and replica plates identified the generalized transductants (Amp^s) and mini-Mu-ductants (Amp^r). It was found that plating first for the recombinant phenotype and subsequently scoring Amp^r gave more consistent results than plating simultaneously for both Amp^r and the recombinant phenotype (presumably due to varying lags in phenotypic expression of Amp^r). Independent controls in which either the cells or the phage were omitted or in which the nonlysogenic parent of the donor strain (if mutant) was the recipient were run in parallel.

perhaps others of the poorly characterized phage functions (12, 25) that lie between the B and C genes of Mu. No *kil* point mutations have been isolated (12; M. Howe, personal communication), and thus, although *kil* is the most likely gene function determining the recovery of the mini-Mu-ductants, this cannot be tested directly.

The frequency of mini-Mu-duction was also increased by use of *recA*$^+$ recipient strains. For unknown reasons, the transposition of mini-Mu phages is aided by the presence of a functional *recA* gene (4, 25), whereas transposition of Mu shows no dependence on *recA* function (25). Lefevbre and Toussaint (16) reported that mini-Mu-ductants could be distinguished from the generalized transductants formed by homologous recombination in a *rec*$^+$ host by use of a mini-Mu phage (Mu18A-1) carrying an ampicillin resistance (Amp^r) determinant since of the recombinant colonies, only mini-Mu-ductants would retain the phage Amp^r marker. However, the previous workers (16) did not demonstrate that all Amp^r recombinants found in *rec*$^+$ strains were the result of mini-Mu-duction. It therefore seemed possible that some Amp^r recombinants could be formed by homologous recombination and thus give a background of "false" mini-Mu-ductants. The source of these putative Amp^r generalized transductants would be homologous recombination of the recipient chromosome with two segments of bacterial DNA that bracket a copy of mini-Mu. This could result in the recipient chromosome simultaneously acquiring both a functional copy of the selected genetic marker and an Amp^r determinant. Indeed, the DNA structures found in particles of types II and IV (Fig. 1) seemed likely substrates for such a recombination event. (Particles of type I seem unlikely to participate since the 50 to 100 base pairs of host DNA at the left end of the packaged DNA [9] seems too short for efficient crossover [11].)

To determine whether false mini-Mu-ductants were formed, I assayed generalized transduction and mini-Mu-duction, using a recipient strain carrying a deletion of the selected marker (Table 3). Deletion of the selected marker and neighboring sequences (Table 1) from the recipient chromosome decreased the frequency of generalized transduction >100-fold but had no effect on the frequency of mini-Mu-duction (Table 3). Since the formation of the putative false mini-Mu-ductants should show the same dependence on homologous recombination as does generalized transduction, I conclude that all Amp^r recombinants were formed by true mini-Mu-duction. It should be noted that use of the large chromosomal deletion was essential to this demonstration since mutants deficient in homologous recombination were not applicable. The transposition frequency of mini-Mu-phages is depressed in *recA* strains (4, 6, 25), and *recBC* strains are only marginally defective as transduction recipients (19, 23).

It should also be noted that the possibility of acquisition of the selected host sequence by generalized transduction coupled with insertion of a copy of mini-Mu by transposition is vanishingly small (10^{-11} to 10^{-12} per recipient cell) due to (i) the low level (5 to 10%) of particles carrying either mini-Mu or host DNA (4, 20) and (ii) the low frequencies (Tables 2, 3, and 4) of both mini-Mu transposition and generalized transduction (each 10^{-5} to 10^{-6} of the recipient cells receiv-

TABLE 3. Effect of homologous recombination on formation of Amp^r recombinants

Donor[a]	Recipient	Marker selected	Generalized transductants[b]	Mini-Mu-ductants[c]
CY365	HfrG6MD2 [Δ(*asd-malA*)]	*asd*$^+$	3	277
CY365	U482 (*asd-1*)	*asd*$^+$	1,152	254
CY365	HfrG6MD2 [Δ(*asd-malA*)]	*mal*$^+$	0	82
CY365	AB645 (*malA1*)	*mal*$^+$	125	74

[a] Similar results were obtained with two independent phage stocks. Tet^r-generalized transductants (700 to 1,000) and mini-Mu-ductants (50 to 200) were formed at similar frequencies in all four crosses. The transductions were done as in Table 2. In the crosses with the HfrG6MD2 recipient, 26 and 2% of the mini-Mu-ductants selected for Amp$^+$ were *glp*$^+$ and *mal*$^+$, respectively.

[b] Generalized transductants are Amp^s.

[c] Mini-Mu-ductants are Amp^r.

TABLE 4. Mini-Mu-duction of various genes[a]

Recipient marker[b]	Map location (min)	Colonies formed per 5×10^9 cells	
		Generalized transductants	Mini-Mu-ductants
Strain CY365 Donor			
ara-14	1	10^4	300
leu-6	2	710	110
zcb::Tn*10*	3	830	320
lacZ36	8	160	40
proC32	9	780	420
purE42	12	520	280
trpE68	28	380	146
lysA23	61	447	123
xyl-5	80	520	330
mtl-1	81	240	60
metE70	86	410	94
trpB4 donor			
trpA2	28	1,000	560
trpB4		0	0
trpC6		100	10
trpD2		118	101
trpE5729		300	57
trpE5729 donor			
trpB4	28	400	125
trpD2		240	110
trpE5729		0	0
thrC1083 donor			
thrB4131	0	225	90
thrB1		101	7
thrC1010		107	0

[a] The double lysogens were formed by introduction of an F::Mu18A-1 by mating with a suitable F′ strain (e.g., F::Mu18A-1/MXR) at an F′ to recipient ratio of about 0.1 at 30 to 33.5°C for 0.5 to 1 h. The mating mixture was then infected (multiplicity of about 1) with a helper phage lysate, and after phage absorption (0.5 h at 30°C), the infected cells were plated at 30°C on medium containing both ampicillin and neomycin (0.1 mg/ml each). The resulting colonies were checked for the recipient strain markers (to ensure that a rare Mu Neo^r lysogen of the conjugational donor strain had not been isolated). Donor phage stocks were obtained by heat induction (Table 2).

[b] Strain X478 was the recipient strain in the crosses with the prototrophic *zcb*::Tn*10* donor strain CY365. The donor and recipients in the other crosses were strains carrying only that auxotrophic marker (Table 1), except strain C600 which carried both *leuB6* and *thrB1*. In addition to the markers given above, complementation with a wild-type donor was observed with *his*, *gal*, *purF*, *panB*, *panC*, *fabA*, and *fabB* loci (the latter two mutations are temperature sensitive at 42°C). I also observed complementation between *fabA* and *fabB* and between *lacZ* and *lacY*.

ing either mini-Mu or host DNA). These calculations are borne out by the fact that an event of similar frequency, the formation of mini-Mu-ductants or generalized transductants that also contain a copy of the helper phage, has been found to occur at a frequency of $<10^{-5}$ of that of mini-Mu-duction (data not shown).

A complementation test between two *rec*$^+$ *trp* strains illustrates the manipulations of the test. First, one strain was made lysogenic for both the mini-phage, Mu18A-1, and the Mu *kil* helper phage. The double lysogenic strain was grown at 30°C and shifted to 42°C to induce phage replication. The resulting lysate was irradiated with UV and used to infect the recipient strain. These infected cells were then plated on medium lacking tryptophan. The resulting *trp*$^+$ colonies were a mixture of (i) *trp*$^+$ revertants, (ii) generalized *trp*$^+$ transductants, and (iii) *trp*$^+$ mini-Mu-ductants (formed only if the *trp* mutants complement). These colonies were replica plated onto an ampicillin-containing medium lacking tryptophan. Only the mini-Mu-ductants grew on this medium since the revertants and generalized transductants lacked a phage genome and were Amp^s (see above).

When the standard controls (sterility of phage stocks, reversion of selected markers) used in generalized transduction were run in parallel with the transduction, interpretation was straightforward. The presence of 50 to 1,000 Amp^r *trp* colonies indicated that the two *trp* lesions were in different genes, whereas the lack of Amp^r *trp*$^+$ colonies denoted lesions in the same gene. The presence of generalized transductants was a good internal control for a potent donor phage stock. The frequency of generalized transduction by phage Mu was increased 10- to 100-fold when the donor carried a mini-Mu phage (7, 16) (presumably via packaging of structure II), and thus a high frequency of generalized transduction was good evidence that mini-Mu-containing particles were present in the lysate. Close linkage of two mutant alleles should give fewer generalized transductants. However, I found that markers cotransduced >95% by phage P1 readily formed generalized transductants under these conditions. Such markers appear essentially unlinked for two reasons. First, phage Mu encapsidates only 39 kilobase pairs of DNA (25), and thus the frequency of encapsidation of host DNA carrying both genetic loci is considerably less than with phage P1 (13). Second, UV irradiation of transducing phage stocks increases the frequency of recombination by 10- to 50-fold (10). The presence of mini-Mu in the lysates can also be ascertained by selecting only for Amp^r. The number of Amp^r recombinants was 10^2 to 10^3 greater than the number of mini-Mu-ductants formed for a given marker. It was found that the plaque-forming titer of the helper phage was not a reliable index of the presence of mini-Mu-duction structures because under conditions of poor phage growth (e.g., high cell density), the helper phage seemed to be preferentially replicated or packaged (data not shown).

Mini-Mu-duction and generalized transduction frequencies are given in Table 4 for a number of genetic markers. The ratio of mini-Mu-ductants to generalized transductants varied from 0.1 to 0.5, and markers located in the 0- to 3-min region of the *E. coli* genetic map were preferentially transduced by both mechanisms (as previously noted for both mini-Mu-duction [7, 16] and generalized transduction by Mu [2, 7, 13]). However, for all markers examined (see also the legend to Table 4), significant numbers of mini-Mu-ductants were readily obtained. Different lesions within the same gene (*thrC*) failed to complement, and the frequency of generalized transduction remained high even when closely linked markers (the genes of the *trp* or the *thr* operons) were tested.

A definitive test of complementation was provided by the marked instability of the mini-Mu-ductants observed in the absence of selective pressure. At 37°C (at which Mu *cts* repressor is partially inactive), colony formation on a nonselective medium resulted in 10 to 50% of the colonies having regained the original mutant phenotype, thus demonstrating complementation. Segregation on nonselective media has been observed for each of the carbohydrate utilization markers in Table 4 plus *thr*, *trp*, and *his* (10 to 40 independent colonies were examined for each marker). The instability of the mini-Mu-ductants seemed due to two *recA*$^+$-

dependent processes: (i) homologous recombination between the two mini-Mu phages bracketing the transduced DNA (25) and (ii) deletion of the transduced segment as the result of mini-Mu-engendered imprecise excision (16) (which is greatly stimulated in *recA*$^+$ strains [15]). It should be noted that all segregants remained Amp^r and Mu immune. If needed, the mini-Mu-duction structure could be stabilized to about 1% segregation by introduction of a Mu c^+ repressor (plasmid pJB4JI [3] was used) or by inactivation of the *recA* gene (I used a *recA* strain lysogenic for λ*recA*$^+$ and cured the prophage). The mini-Mu-duction structures can also be inserted into a specific (rather than random) chromosomal location by selection simultaneously for repair of the mutant phenotype and inactivation of a positively selectable gene. I have inserted *trp*- and *his*-carrying mini-Mu-duction structures into the *araCBA* (18) and *bgl* (21) loci (data not shown), whereas Faelen et al. (7) have detected insertion into other genes.

The use of mini-Mu-duction as a complementation test is not restricted to *E. coli*. The wide host range of Mu and its derivatives should allow the application of this technique to a wide range of gram-negative bacteria (16, 25). It should be noted that this technique may not be applicable to temperature-sensitive mutants in which the host mutation prevents temperature induction and growth of the mini-Mu lysate. However, phage D108, a close relative of Mu, has been reported (unlike Mu) to be induced by UV light (14).

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