JOURNAL OF BACTERIOLOGY, Oct. 1986, p. 357–364
0021-9193/86/100357-08$02.00/0

Vol. 168, No. 1

Mini-Mu Bacteriophage with Plasmid Replicons for In Vivo Cloning and *lac* Gene Fusing

EDUARDO A. GROISMAN AND MALCOLM J. CASADABAN*

Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, Illinois 60637

Received 28 April 1986/Accepted 24 June 1986

New mini-Mu transposons with plasmid replicons were constructed with additional features for in vivo DNA cloning and *lac* gene fusing in *Escherichia coli*. These mini-Mu replicons can be used to clone DNA by growing them with a complementing Mu bacteriophage and by using the resulting lysate to transduce Mu-lysogenic cells. These mini-Mu phage have selectable genes for resistance to kanamycin, chloramphenicol, and spectinomycin-streptomycin, and replicons from the high-copy-number plasmids pMB1 and P15A and the low-copy, broad-host-range plasmid pSa. The most efficient of these elements can be used to clone genes 100 times more frequently than with the previously described mini-Mu replicon Mu dII4042, such that complete gene banks can be made with as little as 1 μl of a lysate containing 10^6 helper phage. The 39-kilobase-pair Mu headful DNA packaging mechanism limits the size of the clones formed. The smallest of the mini-Mu elements is only 7.9 kilobase pairs long, allowing the cloning of DNA fragments of up to 31.1 kilobase pairs, and the largest of them is 21.7 kilobase pairs, requiring that clones carry insertions of less than 17.3 kilobase pairs. Elements have been constructed to form both transcriptional and translational types of *lac* gene fusions to promoters present in the cloned fragment. Two of these elements also contain the origin-of-transfer sequence *oriT* from the plasmid RK2, so that clones obtained with these mini-Mu bacteriophage can be efficiently mobilized by conjugation.

The cloning of DNA sequences is an important step in many biological studies. Usually, cloning is done by isolating DNA, digesting it with restriction endonucleases, ligating it to an appropriately cut vector, and introducing it into a cell by transformation or transduction. As an alternative, transposable elements can carry out recombination reactions to translocate DNA onto plasmid or bacteriophage vectors to form gene clones (15, 17, 34–36). The transposon bacteriophage Mu is a temperate phage that can infect *Escherichia coli* K-12 as well as other members of the family *Enterobacteriaceae* (for a recent review, see reference 34). Mu is especially suitable for in vivo cloning because it transposes hundreds of times as it replicates when derepressed for its lytic functions. Derepression can be achieved synchronously with temperature-sensitive alleles of the Mu repressor.

We recently developed a more efficient in vivo cloning system by incorporating a plasmid replicon inside a mini-Mu element (17). Transposition of this mini-Mu replicon can occur on both sides of a particular gene to form a structure that can be encapsidated by the efficient Mu headful packaging mechanism (34). This structure can be introduced by phage infection into a Mu-lysogenic cell where recombination can occur between the Mu sequences to generate plasmid clones. This mini-Mu replicon element, Mu dII4042, can also form hybrid protein *lac* gene fusions which are useful in studies of gene structure and expression (9–11, 30).

Here we describe the construction of a set of mini-Mu elements with different selectable drug resistance markers, plasmid replicons, *lac* fusing elements, and the *oriT* origin-of-transfer region from plasmid RK2 (18). Two compatible high-copy replicons and a low-copy, broad-host-range replicon were incorporated into the new constructs. In addition, segments of the *lac* operon were incorporated to form both the hybrid protein type of fusions, as present in the original mini-Mu replicon phage (17), and the transcriptional type of fusions.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phages. Descriptions and genotypes are shown in Table 1.

Media. LB, YT, MacConkey indicator, and minimal M63 media have been described previously (27). Antibiotics used were as follows (in micrograms per milliliter): ampicillin, 25; chloramphenicol, 25; kanamycin, 40; spectinomycin, 20 for chromosome-encoded resistance and 50 for plasmid-encoded resistance; streptomycin, 10 for plasmid-encoded resistance; and tetracycline, 10.

DNA biochemistry. Restriction endonucleases and T4 ligase were purchased from New England BioLabs, Inc., Boehringer Mannheim Biochemicals, P-L Biochemicals, Inc., or Amersham Corp., and used according to their specifications. Rapid small-scale isolation of plasmid DNA was carried out by the method of Holmes and Quigley (21). Large-scale isolation of plasmid DNA was done by the procedure described by Kupersztoch and Helinski (24). Other protocols were taken from Maniatis et al. (25).

Preparation and use of Mu lysates. Lysates of mini-Mu replicon phage were prepared by thermoinduction of strains containing the mini-Mu element as a plasmid as well as a Mu *c*ts62 insertion in the chromosome to complement the defective mini-Mu element for the morphogenic functions. Typically, an overnight culture grown at 30°C in LB broth with a drug whose resistance is encoded by the mini-Mu replicon plasmid was diluted 1:100 in 15 ml of LB broth without drugs in a 125-ml Erlenmeyer flask to allow good aeration and was grown at 30°C with shaking for approximately 3 h to mid-exponential phase. The cultures were then shifted to 42°C and incubated with shaking until lysis occurred or for 2.5 h. Chloroform (1% of the volume), $MgSO_4$ to a final concentration of 2 mM, and $CaCl_2$ to a final concentration of 0.2 mM were added to the lysates. Cell particles were removed by

* Corresponding author.

TABLE 1. Strains, phage, and plasmids[a]

Strain, phage, or plasmid	Description of genotype	Source or reference
Strains		
C600 Mu *cts*	*thr leuB6 thi supE44 lacY1 fhuA21* Mu cts	2
M8820	F⁻ *araD139* Δ(*araCOIBA leu*)*7697* Δ(*proAB argF lacIPOZYA*)XIII *rpsL*	8
M8820 Mu *cts*	M8820 with Mu cts	12
M8820S Mu *cts*	M8820 Mu cts with *rpsE rpsL*⁺	12
MC1040-2	F⁻ *araD169 araB*::Mu cts Δ*lacX74 galU galK rpsL*	12
QSR17	C600 with Δ*trpE5*	J. Shapiro
XPh43 Mu *cts*	F⁻ Δ(*argF lacIPOZYA*)*U169 trp* Δ(*brnQ phoA proC phoB phoR*)*24* Mu cts	6
Phage		
Mu *cts*	Mu cts62	M. Howe
Mu dII4042	Mu cts62 *A*⁺ *B*⁺Cm^r rep$_{P15A}$ *lac*(*'ZYA*)*931*	17
Mu d5005	Mu cts62 *A*⁺ *B*⁺Km^r rep$_{pMB1}$	Fig. 2
Mu dII5085	Mu cts62 Δ*AB* Cm^r rep$_{P15A}$ *lac*(*'ZYA*)*931*	Fig. 2
Mu dI5086	Mu cts62 *A*⁺*B*⁺ Km^r rep$_{PMB1}$ *trp-lac*(*'BAZYA*)*W205*	Fig. 2
Mu dII5117	Mu cts62 *A*⁺ *B*⁺ Km^r Sp^r/Sm^r rep$_{pSA}$ *lac*(*'ZYA*)*931*	Fig. 2
Mu dII5118	Mu cts62 *A*⁺ *B*⁺ Km^r Sp^r/Sm^r rep$_{pSa}$ *lac*(*'ZYA*)*931*	This study
Mu dI5155	Mu cts62 *A*⁺ *B*⁺ Km^r rep$_{pMB1}$ RK2 *oriT trp-lac*(*'BAZYA*)*W205*	Fig. 2
Mu dI5166	Mu cts62 *A*⁺ *B*⁺ Cm^r rep$_{pMB1}$ RK2 *oriT trp-lac*(*'BAZYA*)*W205*	Fig. 2
Plasmids		
pBC0	pUC9 with *Hae*II *lac* fragment removed (2.3 kb)[b]	17
pBC4042	pBC0::Mu dII4042 (19.0 kb)	17
pEG5005	pBC0::Mu d5005 (10.2 kb)	Fig. 1
pEG5086	pBC0::Mu dI5086 (17.2 kb)	Fig. 1
pEG5155	pBC0::Mu dI5155 (17.8 kb)	Fig. 1
pEG5166	pBC0::Mu dI5166 (18.0 kb)	Fig. 1
pEG5117	pSC101::Mu dII5117 (31.0 kb)	Fig. 1
pEG5118	pSC101::Mu dII5118 (31.0 kb)	This study
pMC871	Km^r rep$_{P15A}$ W205 *trp-lac* (10.1 kb)	10
pMC3612	Km^r rep$_{pMB1}$ (3.6 kb)	M.Ditto, J.Kans, and M. Casadaban[c]
pMC6364	pUC9::Cm^r cloned as *Hin*dIII-*Bam*HI fragment (3.7 kb)	M. Ditto, J. Kans, and M. Casadaban[d]
pPO1678	pSC101::Mu dII1678 (31.7 kb)	12
pRK24	Tc^r Ap^r *trpE*⁺ derivative of RK2 (61.6 kb)	26
pSC101	Tc^r (9.3 kb)	5, 13
pUB5578	Km^r Sp^r-Sm^r rep$_{pSa}$ (9.0 kb)	38
PUC8	Ap^r *lac'IPOZ* rep$_{pMB1}$ (2.6 kb)	37
pUC9	Ap^r *lac'IPOZ'* rep$_{pMB1}$ (2.6 kb)	37
pTJS53	pUC8::RK2 *oriT* (3.4 kb)	D. Helinski

[a] Ap, Ampicillin; Cm, chloramphenicol; Km, kanamycin; Sp, spectinomycin; Sm, streptomycin; Tc, tetracycline; r, resistance; *A*⁺*B*⁺, Mu transposition-replication genes; rep$_{P15A}$, replicon from plasmid P15A; rep$_{pMB1}$, replicon from plasmid pMB1; rep$_{pSa}$, replicon from plasmid pSa. *E. coli* gene designations are summarized by Bachmann (3). In the text, Mu cts following a strain name indicates a lysogen. *ara* alleles are from *E. coli* B. Mutants of *argF* are not arginine requiring because *E. coli* K-12 has an isozyme gene *argI*.

[b] Sizes refer to the complete plasmid. Mini-Mu replicon sizes are given in Fig. 2 and Table 3.

[c] Plasmid pMC3612 is a derivative of pBR322 containing the Aph (3') aminoglycoside phosphotransferase gene from Tn*5* as follows: *Eco*RI-*Hin*dIII (base pairs 0 to 29) on the standard pBR322 sequence (31); the *Hin*dIII-*Pst*I fragment from Tn*5* (1197 to 2653) containing the kanamycin gene (4); the 21-base-pair *Pst*I-*Eco*RI linker segment of the pUC7 plasmid (37); the 10-base-pair *Eco*RI-*Bam*HI linker segment of pUC8 (37); the 76-base-pair *Sau*3A fragment from pBR322 between coordinates 3137 and 3213 (31); the 20-base-pair *Bam*HI-*Hin*dIII fragment from pUC8 (37); and base pairs 29 to 3131 from pBR322 but with a deletion from 1431 to 2521 from pBR327.

[d] Plasmid pMC6364 consists of a 1,043-base-pair segment of transposon Tn*9* (1, 29), from the *Pvu*II site in the IS*1* sequence to the *Taq*I site at the end of the coding region of the chloramphenicol resistance gene, adapted and inserted between the *Hin*dIII and the *Bam*HI sites of pUC9 (37).

centrifugation, and the resulting lysates were titrated (27) and used within 1 week of preparation since Mu lysates can be unstable.

Infections were carried out by mixing 100 μl of an overnight culture of recipient cells grown in LB broth with 100 μl of lysate. Absorption was carried out at 30°C for 30 min without shaking. Two milliliters of LB broth was added, and the mixture was incubated at 30°C with shaking for approximately 75 min to allow for gene expression. Cells were washed with 10 mM NaCl before plating.

Bacterial genetic techniques. Transformation with plasmid DNA was carried out as described by Cohen et al. (14). Matings were done by crosspatching freshly streaked colonies onto a rich-medium plate such as YT, incubating them at 30°C for approximately 2 h, and then restreaking them onto selective media.

RESULTS

The mini-Mu replicon elements described here were constructed from the mini-Mu-containing plasmids pBC4042 (17) and pPO1678 (12). Different genetic elements were inserted by digesting these plasmids at restriction enzyme sites present inside the mini-Mu elements but absent in the vector sequences. Plasmid pBC4042 has the mini-Mu element Mu dII4042 present in the pBC0 plasmid, which was

derived from the pUC9 plasmid by removing the *Hae*II fragment that contains the *lac* and polylinker sequences. This plasmid is very convenient for the constructions described below because the pBC0 segment is missing the recognition sequences for most of the commonly used restriction enzymes. Mu dII4042 has a chloramphenicol resistance marker, the P15A high-copy-number replicon, and a segment of the *lac* operon for making hybrid protein gene fusions (17).

Mu d5005, a smaller mini-Mu replicon with a kanamycin resistance marker and a ColE1-type replicon. The Tn*5* kanamycin resistance gene and the pMB1 ColE1-type replicon from the pBR322 derivative pMC3612 (Table 1) were incorporated into the mini-Mu phage in plasmid pBC4042 (17). pMC3612 plasmid DNA was digested with *Bam*HI and *Sal*I and ligated to *Bam*HI- and *Xho*I-digested pBC4042 plasmid DNA. *Xho*I and *Sal*I generate the same sticky ends, which can be ligated together. The ligation mixture was used to transform strain C600 Mu *c*ts. A Mu-lysogenic strain was used to repress expression of the Mu genes present on the plasmid DNA. Kanamycin- and ampicillin-resistant transformants were selected and scored for chloramphenicol sensitivity. Plasmid DNA from 10 such transformants was prepared and examined by restriction enzyme digestion and gel analysis. Since the parent plasmid pMC3612 has three *Bam*HI sites and three *Sal*I sites, several different structures could be generated. One transformant containing the contiguous piece of DNA from pMC3612 between the *Bam*HI and the *Sal*I sites (Fig. 1) was named pEG5005. The mini-Mu element formed was named Mu d5005 and is shown in detail (Fig. 2). This 7.9-kilobase-pair (kb) mini-Mu element has a different drug resistance marker and a replicon that is compatible with the one in the previously described Mu dII4042 (see below). It does not have the *lac* DNA segment present in the parental Mu dII4042. Its small size allows the cloning of larger pieces of DNA.

Mu dI5086, a mini-Mu replicon to make transcriptional *lac* operon gene fusions. The promoterless *lac* operon segment from plasmid pMC871 was incorporated into Mu d5005 to make a mini-Mu element capable of forming transcriptional *lac* fusions to cloned promoters. pEG5005 and pMC871 DNAs were digested with *Bam*HI and *Sal*I, and the resulting fragments were ligated and used to transform the Δ*lac*, Mu-lysogenic strain MC1040-2. Kanamycin- and ampicillin-resistant transformants that expressed the *lac* genes at a level different from that of either parent, as judged by their color on lactose MacConkey indicator medium, were chosen as candidates. Plasmid DNA was prepared and analyzed by using restriction endonucleases and gel electrophoresis. One plasmid was designated pEG5086 (Fig. 1) with the mini-Mu replicon Mu dI5086 (Fig. 2).

Mu dI5155, a mini-Mu replicon with an *oriT* site for conjugal mobilization. The origin of transfer, *oriT*, of the broad-host-range plasmid RK2 is the only *cis*-acting DNA element needed for conjugal transfer (18). A 769-base-pair *Sma*I-*Sal*I fragment from plasmid pTJS53 containing *oriT* was purified and incorporated into *Sma*I- plus *Sal*I-digested pEG5086 DNA. Clones were selected as kanamycin- and ampicillin-resistant transformants of MC1040-2. Plasmid DNA was prepared and examined by restriction enzyme analysis. One of them was called pEG5155 and is shown in Fig. 1. It contained the mini-Mu phage Mu dI5155 (Fig. 2). The presence of the *oriT* segment was tested by demonstrating that pEG5155 could be mobilized in a triparental mating. For this, QSR17 cells containing the pRK24 plasmid, a self-transmissible, kanamycin-sensitive derivative of plasmid RK2, were mated with MC1040-2 cells containing pEG5155 and at the same time with M8820S Mu *c*ts cells as the third parent. The exconjugants were selected as kanamycin- and spectinomycin-resistant colonies.

Mu d5166, a mini-Mu replicon with resistance to chloramphenicol and *oriT*. The Tn*9*-derived chloramphenicol resistance gene on plasmid pMC6364 (Table 1) was incorporated into the *oriT* mini-Mu phage Mu dI5155 on the pEG5155 plasmid. pEG5155 DNA was digested with *Sma*I and *Bgl*II and ligated to *Pvu*II- plus *Bam*HI-cut pMC6364 (Fig. 1). The ligation mixture was used to transform strain MC1040-2 to chloramphenicol and ampicillin resistance. Plasmid DNA from kanamycin-sensitive colonies was examined. One was called pEG5166 with the mini-Mu replicon Mu dI5166 (Fig. 2).

Mu dII5117, a mini-Mu with a low-copy-number, broad-host-range replicon. The 9-kb IncW, pSa-derived plasmid pUB5578 was incorporated into the mini-Mu element Mu dII1678. This plasmid has genes for kanamycin and spectinomycin-streptomycin resistance (32, 38). Plasmid pUB5578 was linearized at the unique *Bgl*II site and ligated to *Bgl*II-cut pPO1678 DNA. Clones were selected as kanamycin- and tetracycline-resistant transformants of strain MC1040-2 and scored for ampicillin sensitivity and spectinomycin resistance. pUB5578 DNA was found inserted in opposite orientations in plasmids pEG5117 (Fig. 1) and pEG5118 with Mu dII5117 (Fig. 2) and Mu dII5118, respectively.

Mu dII5085, a mini-Mu replicon with deletions of the transposition genes. A mini-Mu replicon derivative missing the *A* and *B* transposition genes was made by removing the fragment between the *Pst*I site in the *A* gene (located 1,674 base pairs from the Mu left end) and the *Pst*I site in the IS50L (located 5.05 kb from the mini-Mu Mu dII4042 left end). Plasmid pEG5085 (Fig. 1) has the mini-Mu element Mu dII5085 (Fig. 2).

Use of different mini-Mu replicons. The different mini-Mu replicons were tested for their ability to clone genes and generate *lac* fusions. They were also compared with one another for their efficiency in generating gene banks and cloning a particular gene. Mini-Mu replicon-containing plasmids were introduced into the same genetic background by transforming strain MC1040-2 and selecting for their drug resistance markers. Transformants were purified and used to prepare Mu lysates as described above. Transductants of strain XPh43 Mu *c*ts were selected for both drug resistance and *proC*$^+$ (Table 2). Drug-resistant transductants were obtained at frequencies from 2.5×10^{-7}/PFU to 6.7×10^{-4}/PFU of the helper phage. *proC*$^+$ transductants were obtained at frequencies from 3.2×10^{-9} to 3.3×10^{-6} per PFU of the helper phage with the exception of the mini-Mu replicon Mu dII5085 (see below).

The mini-Mu phages described in this paper have three different compatible replicons. This means that DNA segments cloned with compatible mini-Mu replicons can be maintained in the same cell. We took advantage of this property to sequentially clone two different operons in the same strain genetic background. First, the original mini-Mu replicon Mu dII4042 with a P15A-derived replicon was used to clone the *proAB*$^+$ genes into strain M8820 Mu *c*ts. Then Mu d5005 with the compatible pMB1-derived replicon was used to clone the *leu*$^+$ genes by infecting strain M8820 Mu *c*ts/Mu dII4042 *proAB*$^+$ M8820 Mu *c*ts without a plasmid. Clones were obtained with the same frequency regardless of the presence of Mu dII4042 plasmid clones in the recipient strain. This experiment was done in reverse order of genes

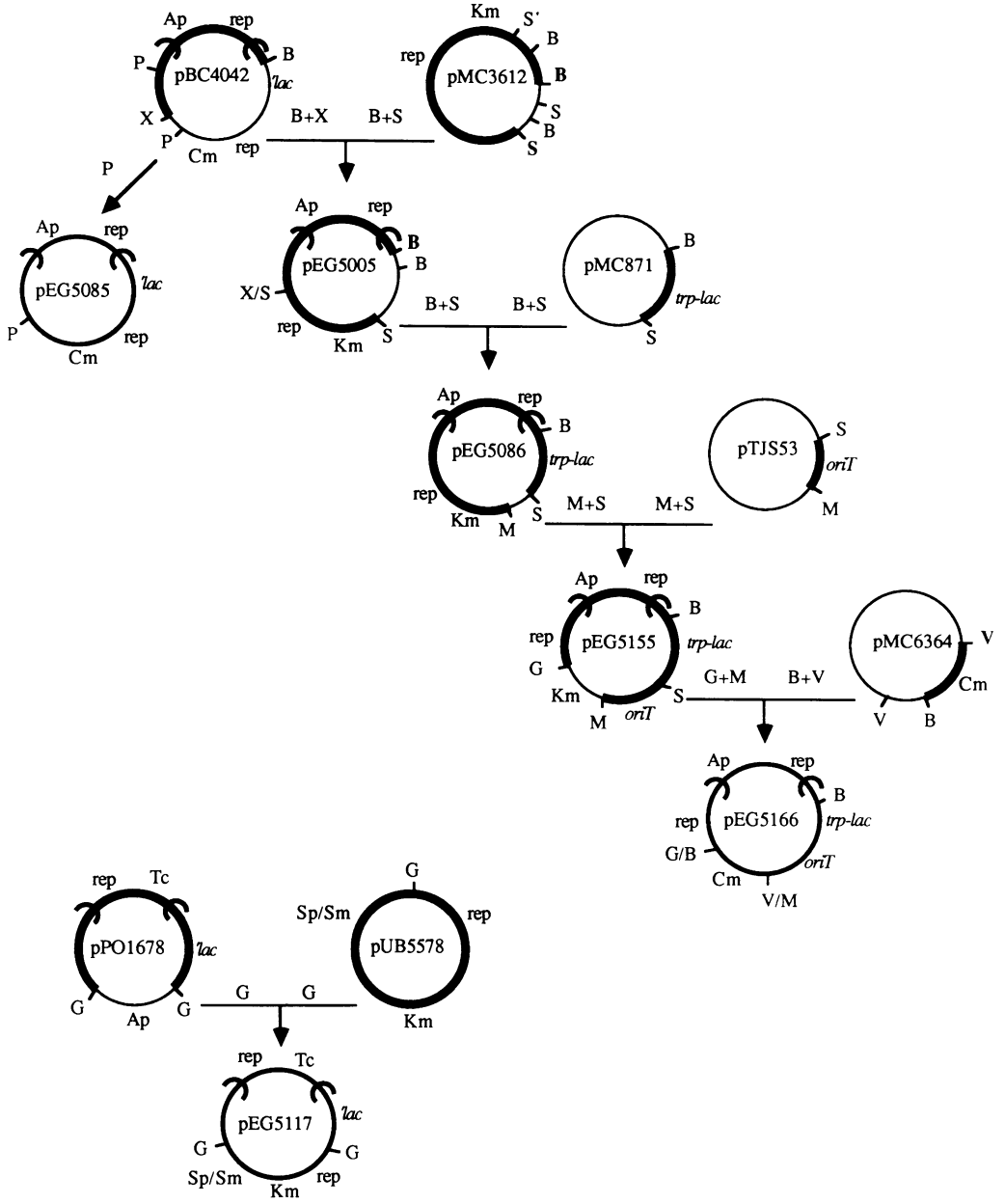

FIG. 1. Construction of plasmids harboring mini-Mu replicons Mu d5005, Mu dI5086, Mu dI5155, Mu dI5166, Mu dII5117, and Mu dII5085. Cleavage recognition sites for restriction enzymes are designated as follows: B, *Bam*HI; G, *Bgl*II; M, *Sma*I; P, *Pst*I; S, *Sal*I; V, *Pvu*II; X, *Xho*I. The particular *Bam*HI and *Sal*I sites on plasmid pMC3612 used to make pEG5005 and the particular *Pvu*II site in pMC6364 used to make pEG5166 are shown in boldface type. Cleavage recognition sites destroyed during cloning are indicated by a slash separating the sites (e.g., X/S). All occurrences of these restriction sites are not shown, but for each plasmid every occurrence of each individual restriction site is given except for V, *Pvu*II. Ends of the mini-Mu phage are shown in brackets. Thick lines correspond to DNA segments used to make the plasmid immediately below in the figure except for the construction of pEG5085. Detailed genetic and physical maps of the mini-Mu elements are shown in Fig. 2. The sequence from the pUC9 (31, 37)-derived plasmid pBC0 (17), and from pSC101 (5) have been published.

proAB$^+$ and *leu*$^+$ with the same results. The stability of the Mu dII4042 and Mu d5005 in M8820 Mu *c*ts was the same regardless of the presence of the other mini-Mu replicon clones in the same cell.

The mini-Mu replicon-generated clones have different stabilities. Clones obtained with the mini-Mu elements containing the P15A- and the pMB1-type replicons (Table 3) proved to be unstable. Spontaneous curing of the plasmid in the absence of selection occurred at a frequency of 50% of the cells in a colony, with the simultaneous loss of the ability to grow in the presence of the appropriate drug and loss of prototrophy. The plasmid clones obtained with the more stable pSa-derived mini-Mu replicon Mu dII5117 were lost at a frequency of approximately 12%.

The frequency of Lac$^+$ transductants was 9.3% for the operon type of fusions and 0.6% for the hybrid protein type of fusions. Lysates prepared from strains containing the mini-Mu replicons that can generate operon fusions (Mu

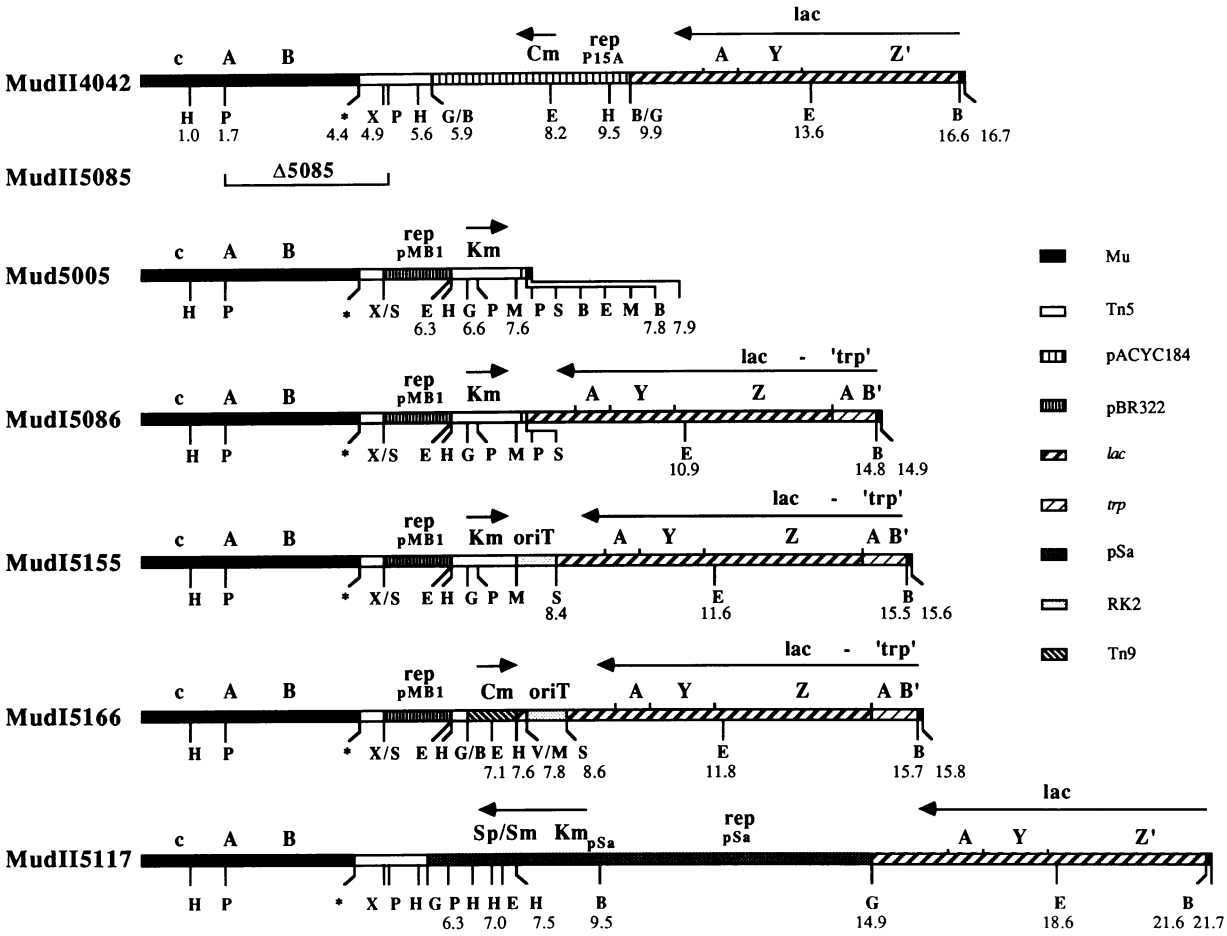

FIG. 2. Genetic and physical maps of the different mini-Mu replicon bacteriophage available for in vivo DNA cloning and *lac* gene fusing. Cleavage recognition sites for restriction enzymes are as in Fig. 1: E, *Eco*RI; H, *Hin*dIII. An asterisk indicates the joint between Mu and Tn*5* DNA (12). Positions are given in kilobases from the left side of the mini-Mu replicon but omitted if they are the same as in the mini-Mu element immediately above it. All occurrences of each restriction site shown are given except for V, *Pvu*II. Note that the substrate profiles of the gene products of the kanamycin resistance genes from Tn*5* (4) and pSa (32) are different. The DNA sequences of some of the segments present in the different mini-Mu elements have been published: Mu (16, 19, 22, 28, 33), Tn*5* (4), Sp/Sm (32), Tn*9* (1, 29), pBR322 (31), *oriT* (18), *lac* (7, 20, 23), and *trp* (39).

dI5086, Mu dI5155, and Mu dI5166) were used to infect the Δ*lac* strain XPh43 Mu *cts*. We found that 9.3% of the antibiotic-resistant colonies were dark red, 7.3% were white, and the rest were light red as indicated by their phenotype on lactose MacConkey indicator plates. The light red color in the majority of the transductants may be due to the presence of promoterlike sequences in the *trp* DNA in these mini-Mu replicons. The frequency of Lac$^+$ transductants, 9.3%, was higher than the 1% found for mini-Mu-generated fusions in the chromosome (7, 12), possibly because fusions in high copy numbers may result in the expression of a Lac$^+$ phenotype as indicated by lactose MacConkey indicator

TABLE 2. Cloning with the different mini-Mu replicons[a]

Mini-Mu	PFU/ml	Drug^r/PFU	ProC$^+$/Drug^r	Lac$^+$/Drug^r
Mu dII4042	1.8×10^9	4.5×10^{-4}	2.5×10^{-4}	6.0×10^{-3}
Mu d5005	5.4×10^9	2.0×10^{-4}	7.3×10^{-3}	NA[b]
Mu dI5086	3.5×10^9	2.6×10^{-4}	7.1×10^{-3}	9.3×10^{-2}
Mu dI5155	2.4×10^9	6.7×10^{-4}	5.0×10^{-3}	9.3×10^{-2}
Mu dI5166	7.0×10^9	1.7×10^{-5}	1.5×10^{-2}	9.3×10^{-2}
Mu dII5117	1.7×10^{10}	8.2×10^{-7}	3.9×10^{-3}	6.0×10^{-3}
Mu dII5085	1.1×10^9	2.5×10^{-7}	$<3.6 \times 10^{-1}$[c]	1.1×10^{-3}

[a] Phage lysates were prepared, plaque titrated, and used to infect strain XPh43 Mu *cts* as described in the text. Drug^r, Drug resistance. Kanamycin-chloramphenicol- or spectinomycin-resistant transductants were selected in lactose MacConkey medium containing the appropriate antibiotic. ProC$^+$ clones were selected in minimal M63 medium supplemented with tryptophan and the appropriate antibiotic.

[b] NA, Not applicable.

[c] No ProC$^+$ transductants were detected among the 27 chloramphenicol-resistant colonies obtained.

TABLE 3. Properties of mini-Mu replicons[a]

Mini-Mu	Drug resistance[b]	Replicon	Stability[c] (%)	Lac fusing segment	Other	Size (kb)
Mu dII4042	Cm	P15A	50	Translational		16.7
Mu d5005	Km	pMB1	50	None		7.9
Mu dI5086	Km	pMB1	50	Transcriptional		14.9
Mu dI5155	Km	pMB1	50	Transcriptional	RK2 *oriT*	15.6
Mu dI5166	Cm	pMB1	50	Transcriptional	RK2 *oriT*	15.8
Mu dII5117	Km, Sp-Sm	pSa	12	Translational		21.7
Mu dII5085	Cm	P15A	50	Translational	ΔMu *A,B*	13.3

[a] All mini-Mu replicons have the left-most Mu 4.37-kb sequence including the Mu *c* repressor gene with the *c*ts62 temperature-sensitive allele and the Mu *A* and *B* transposition-replication genes, except for Mu dII5085, as indicated. Thus, they can confer immunity to the cell and can act as transposons even in the absence of a complementing Mu prophage.

[b] Cm, Chloramphenicol; Km, kanamycin; Sp, spectinomycin; Sm, streptomycin.

[c] Plasmid stability was measured by streaking mini-Mu replicon-generated clone-containing colonies in nonselective media and then picking individual colonies to test whether they could still confer antibiotic resistance and relieve the particular auxotrophic requirement of the cell.

plates, even in the presence of very weak transcriptional signals. The white antibiotic-resistant colonies contained defective mini-Mu elements that were unable to retranspose and yield new plasmid clones. Plasmid DNA prepared from them was analyzed physically, and it was found that the mini-Mu replicons contained deletions that remove the Mu right end and various amounts of *trp-lac* DNA. These structures could be explained as mini-Mu transposition events inside other mini-Mus, with recombination in the recipient to resolve the structure.

We also found that higher transduction frequencies could be obtained with the original mini-Mu element Mu dII4042 when it was present as part of a plasmid clone linked to the *E. coli proC* gene, as in plasmid pEG109 (17), when compared with the original construct pBC4042, which has Mu dII4042 linked to the pBC0 plasmid.

The mini-Mu replicon Mu dII5085 is deleted for the Mu transposition genes but can be complemented by a helper Mu prophage for both the transposition and morphogenic functions. Clones formed with this element can be selected in non-Mu-lysogenic cells. They are genetically stable in cells that do not have the Mu *A* and *B* transposition-replication genes. Mu dII5085 was tested for its ability to transpose and generate plasmid clones. Clones were isolated, as indicated by the loss of the ampicillin resistance marker in some of the transductants and the presence of Lac^+ colonies, but no clones of the specific genes selected were obtained. The frequency of transduction was too low to generate a complete gene bank in the experiment performed with only 0.1 ml of lysate. This could be due to several factors, including inefficient *trans* complementation of the mini-Mu Mu dII5085 present as a high-copy-number plasmid by the chromosomal helper phage.

DISCUSSION

We expanded and improved our recently described in vivo cloning system by constructing and testing a new set of mini-Mu replicon bacteriophage with additional features (Table 3). Some of these contain the pMB1-type origin of replication and show a marked increase in the frequency with which clones are obtained when compared with the original vector Mu dII4042. This finding may be due to the presence of a different replicon or some other change in composition. Several offer the alternative of different drug resistance markers, including kanamycin resistance, from the transposable element Tn*5* that has been successfully used in a wide variety of gram-negative bacterial species. The new vectors are so efficient that, in the best case, as with Mu d5005, gene banks can be constructed with as little as 1 μl of lysate containing 10^6 helper phage. In addition, we demonstrated that compatible mini-Mu replicons can be used in sequence to clone two different operons in the same recipient genetic background. Moreover, the high frequency at which clones are obtained suggests that it may be possible to directly select clones for traits that require two unlinked genetic segments.

The Mu headful packaging mechanism encapsidates approximately 39 kb. The size of the DNA cloned is limited by the amount of DNA that can be packaged inside the Mu head minus the size of the mini-Mu replicon. In the case of Mu d5005, plasmid clones of up to 31.1 kb can be isolated. On the other hand, the mini-Mu Mu dII5117 allows the cloning of up to 17.3 kb, although the amount of DNA may actually be less; in this way, one would have the gene of interest in a small piece of DNA. Restriction enzyme mapping of a few independent clones obtained with the mini-Mu replicon in a single experiment allows the localization of a gene by looking at the minimum size of overlapping fragments that confer the phenotype of interest. We have used this approach to map the location of the *proC* genes of *Shigella flexneri*, *Salmonella typhimurium*, *E. coli*, and *Citrobacter freundii* (Groisman and Casadaban, unpublished data). The clones obtained do not have repeated Mu sequences and so have a stable structure in both *recA* and $recA^+$ backgrounds, which is not the case of fragments isolated by in vivo cloning methods that use transposons without replicons to flank a cloned gene (15, 35, 36).

Some of the mini-Mu replicons described contain a segment of the *lac* operon, so that transcription initiated in the cloned DNA segments may proceed across the 117 base pairs of the Mu right end into the *lac* operon and result in the expression of β-galactosidase activity. These fusions may allow the easy localization of promoters, regulatory elements, and transcription termination signals, help to establish the direction of transcription of a cloned gene, and indicate features of the structure of the fused gene (30).

The clones generated with these mini-Mu replicons are present in plasmids, which makes it possible to form fusions to essential genes since there is a wild-type copy of all the essential genes in the chromosome. Their plasmid location also makes it easier to isolate their DNA and to transfer them to different genetic backgrounds. Furthermore, the mini-Mu elements Mu dI5155 and Mu dI5166 have an *oriT* sequence which allows them to be conjugally mobilized to other strain backgrounds by a helper plasmid.

The presence of different selectable drug resistance markers, plasmid origins of replication, and the *oriT* origin of transfer of the broad-host-range plasmid RK2 should allow the easy introduction of these elements into different bacterial species that are not sensitive to Mu or phage P1. We have recently introduced different mini-Mu replicons along with a helper Mu cts62 phage into Mu-resistant strains of *C. freundii* and *Proteus mirabilis* by conjugation and prepared lysates which were used to isolate clones of genes from these species into *E. coli* K-12 (Groisman and Casadaban, unpublished data).

The low-copy-number replicon in Mu dII5117 may be useful for cloning genes that are lethal when present in many copies per cell. In addition, the low-copy replicon may be helpful for gene expression studies, using fusions to the *lac* operon, in which distorted results may be found when the fusion is present in multiple copies per cell.

ACKNOWLEDGMENTS

We thank James Shapiro, David Figurski, Donald Helinski, Mary Ditto, and Jonathan Kans for strains and plasmids. We thank Jonathan Kans for suggestions throughout the course of this work.

M.C. was supported by Public Health Service grant AI00468 from the National Institutes of Health. This work was supported by Public Health Service grant GM29067 from the National Institutes of Health.

LITERATURE CITED

1. **Alton, N. K., and D. Vapnek.** 1979. Nucleotide sequence analysis of the chloramphenicol resistance transposon Tn9. Nature (London) **282**:864–869.
2. **Appleyard, R. K.** 1954. Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *Escherichia coli* K12. Genetics **39**:440–452.
3. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
4. **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.
5. **Bernardi, A., and F. Bernardi.** 1984. Complete sequence of pSC101. Nucleic Acids Res. **12**:9415–9426.
6. **Brickman, E., and J. Beckwith.** 1975. Analysis of the regulation of *Escherichia coli* alkaline phosphatase synthesis using deletions and φ80 transducing phages. J. Mol. Biol. **96**:307–316.
7. **Buchel, D. E., B. Gronenborn, and B. Muller-Hill.** 1980. Sequence of the lactose permease gene. Nature (London) **283**: 541–545.
8. **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. USA **72**:809–813.
9. **Casadaban, M. J., and J. Chou.** 1984. *In vivo* formation of gene fusions encoding hybrid β-galactosidase proteins in one step with a transposable Mu-*lac* transducing phage. Proc. Natl. Acad. Sci. USA **81**:535–539.
10. **Casadaban, M. J., 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 deletion and cloning of translational initiation signals. J. Bacteriol. **143**:971–980.
11. **Casadaban, M. J., and S. 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. USA **76**:4530–4533.
12. **Castilho, B. A., P. Olfson, and M. J. Casadaban.** 1984. Plasmid insertion mutagenesis and *lac* gene fusion with mini-Mu bacteriophage transposons. J. Bacteriol. **158**:488–495.
13. **Cohen, S. N., and A. C. Y. Chang.** 1977. Revised interpretation of the origin of the pSC101 plasmid. J. Bacteriol. **132**:734–737.
14. **Cohen, S. N., A. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *E. coli* by R-factor DNA. Proc. Natl. Acad. Sci. USA **69**: 2110–2114.
15. **Faelen, M., and A. Toussaint.** 1976. Bacteriophage Mu-1: a tool to transpose and to localize bacterial genes. J. Mol. Biol. **104**:525–539.
16. **Groenen, M. A. M., E. Timmers, and P. van de Putte.** 1985. DNA sequences at the ends of the genome of bacteriophage Mu essential for transposition. Proc. Natl. Acad. Sci. USA **82**: 2087–2091.
17. **Groisman, E. A., B. A. Castilho, and M. J. Casadaban.** 1984. *In vivo* DNA cloning and adjacent gene fusing with a mini-Mu-*lac* bacteriophage containing a plasmid replicon. Proc. Natl. Acad. Sci. USA **81**:1480–1483.
18. **Guiney, D., and E. Yakobson.** 1983. Location and nucleotide sequence of the transfer origin of the broad-host-range plasmid RK2. Proc. Natl. Acad. Sci. USA **80**:3595–3598.
19. **Harshey, R. M., E. D. Getzoff, D. L. Baldwin, J. L. Miller, and G. Chaconas.** 1985. Primary structure of the phage Mu transposase: homology to Mu repressor. Proc. Natl. Acad. Sci. USA **82**:7676–7680.
20. **Hediger, M. A., D. F. Johnson, D. P. Nierlich, and I. Zabin.** 1985. DNA sequence of the lactose operon: the *lacA* gene and the transcriptional termination region. Proc. Natl. Acad. Sci. USA **82**:6414–6418.
21. **Holmes, D. S., and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114**: 193–197.
22. **Kahmann, R., and D. Kamp.** 1979. Nucleotide sequences of the attachment sites of bacteriophage Mu DNA. Nature (London) **280**:247–250.
23. **Kalnins, A., K. Otto, and B. Muller-Hill.** 1983. Sequence of the *lacZ* gene of *Escherichia coli*. EMBO J. **2**:593–597.
24. **Kupersztoch, Y., and D. Helinski.** 1973. A catenated DNA molecule as an intermediate in the replication of the resistance transfer factor R6K in *Escherichia coli*. Biochem. Biophys. Res. Commun. **54**:1451–1459.
25. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Meyer, R. J., D. Figurski, and D. R. Helinski.** 1977. Properties of the plasmid RK2 as a cloning vehicle, p. 559–566. *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.
27. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Miller, J. L., S. K. Anderson, D. J. Frujita, G. Chaconas, D. L. Baldwin, and R. M. Harshey.** 1984. The nucleotide sequence of the B gene of bacteriophage Mu. Nucleic Acids Res. **12**: 8627–8638.
29. **Ohtsubo, H., and E. Ohtsubo.** 1978. Nucleotide sequence of an insertion element, IS1. Proc. Natl. Acad. Sci. USA **75**:615–619.
30. **Silhavy, T. J., and J. R. Beckwith.** 1985. Uses of *lac* fusions for the study of biological problems. Microbiol. Rev. **49**:398–418.
31. **Sutcliff, J. G.** 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. **43**:77–90.
32. **Tait, R. C., H. Rempel, R. L. Rodriguez, and C. I. Kado.** 1985. The aminoglycoside-resistance operon of the plasmid pSa: nucleotide sequence of the streptomycin-spectinomycin resistance gene. Gene **36**:97–104.
33. **Toussaint, A., M. Faelen, L. Desmet, and B. Allet.** 1983. The products of the gene A of the related phages Mu and D108 differ in their specificities. Mol. Gen. Genet. **190**:70–79.
34. **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.
35. **van Gijsegem, F., and A. Toussaint.** 1982. Chromosome transfer and R-prime formation by an RP4::mini-Mu derivative in *E. coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Proteus mirabilis*. Plasmid **7**:30–44.
36. **van Gijsegem, F., and A. Toussaint.** 1983. In vivo cloning of

Erwinia carotovora genes involved in the catabolism of hexuronates. J. Bacteriol. **154:**1227–1235.

37. **Viera, J., and J. Messing.** 1982. The pUC plasmids, and M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:**259–268.
38. **Ward, J. M., and J. Grinsted.** 1982. Physical and genetic analysis of the Inc-W group plasmids R388, Sa, and R7K. Plasmid **7:**239–250.
39. **Yanofsky, C., T. Platt, I. P. Crawford, B. P. Nichols, G. E. Christie, H. Horowitz, M. VanCleemput, and A. M. Wu.** 1981. The complete nucleotide sequence of the tryptophan operon of *E. coli*. Nucleic Acids Res. **9:**6647–6668.