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Phasmid Vectors for Identification of Genes by Complementation of *Escherichia coli* Mutants

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A bacteriophage lambda cloning vector was designed to facilitate the isolation of genes from procaryotic organisms by complementation of *Escherichia coli* mutants. This vector, λSE4, was constructed by attaching a very-low-copy-number replication system (from the plasmid NR1) and a spectinomycin resistance gene to the left arm of λ1059 (Karn et al., Proc. Natl. Acad. Sci. U.S.A. 77:5172–5176, 1980). This phasmid cloning vector is capable of growing lytically as a phage in a nonimmune host or lysogenically as a phasmid in an immune host. This phasmid utilizes the Spi⁻ selection for insertions of DNA into the vector and has the ability to accept 2- to 19-kilobase *Sau*3A1, *Bam*HI, *Bgl*II, *Bcl*I, or *Xho*II fragments; recombinants lysogenize immune hosts as single-copy-number selectable plasmids at 100% frequency. An *E. coli* library was constructed by using the initial vector λSE4, and clones of a number of representative genes were identified. A typical clone, λ*ant*⁺, was shown to be readily mutagenized by a mini-Tn*10* transposon. A general method for transferring cloned DNA segments onto bacteriophage λ was developed. The method involves the use of in vivo recombination with a selection and was used to construct two derivatives of λSE4. Possible uses of these vectors and of the method for transferring cloned DNA onto phage lambda are discussed.

More than 1,000 *Escherichia coli* genes have been identified by mutation (1), and more are continually being reported. Complementation of these *E. coli* mutants provides an extremely powerful way of identifying *E. coli* genes as well as analogous genes from other organisms, particularly procaryotes.

Over the past decade, a number of plasmid and bacteriophage λ recombinant DNA vectors have been developed for the identification and isolation of DNA fragments of interest. Genes that can be identified by complementation of *E. coli* mutations are usually isolated from plasmid or cosmid libraries. However, many such libraries suffer from limitations such as (i) incompleteness due to the lethality or instability of certain genes and sequences when present in high copy number, or (ii) altered phenotypes observed when genes are present at high copy number.

A variety of vectors based on bacteriophage lambda are available which circumvent the problems associated with high-copy-number plasmids. However, many of these have been optimized for the detection of eucaryotic genes by hybridization or other techniques (2, 3, 12). Certain of these have lost the *att* site and cannot lysogenize *E. coli* by normal *int*-mediated recombination and so are generally useful only for identification of genes for which a selection exists. Other lambda vectors lacking the *int* or *c*I genes are only able to lysogenize *E. coli* if coinfected with a helper phage; the difficulty of discerning double lysogens from single lysogens makes it difficult to isolate genes that must be identified by screening. A few lambda vectors, such as λNM1151 (14), and several λgt vectors (15, 20) are available that can lysogenize nonlysogenic strains of *E. coli*. However, most of these have few convenient restriction sites, can only accept relatively small inserts, and often lack a selection for insertion of fragments into the vector.

In this paper we describe the construction of a family of phasmid cloning vectors based on bacteriophage lambda which have been designed for the purpose of cloning genes that can be expressed in *E. coli* by complementation of *E. coli* mutants. These vectors have a selection for the insertion of recombinant DNA and a selection for lysogenization, and two of them are capable of lysogenizing lambda immune hosts at high frequency and replicating as a very-low-copy-number plasmid. They are capable of accepting 2- to 19-kilobase (kb) fragments generated by partial digestion with *Sau*3A1 so that relatively random libraries can be constructed easily. Their relatively large capacity for inserted DNA and their easy selection for lysogenization make them extremely useful for the isolation of genes that must be identified by screening. The methods we have used to construct two of these vectors are convenient and general and should facilitate the construction of other specialized vectors in the future. In addition, they provide a facile, general means of transferring cloned genes onto phage lambda.

MATERIALS AND METHODS

Bacterial strains, bacteriophage, and plasmids. The bacteria, phage, and plasmids used in this study are described in Table 1.

Construction of λSE4. λSE4 was constructed as outlined in Fig. 1. A 1-μg sample of *Bam*HI-cleaved λ1059 DNA was ligated to 1 μg of *Bam*HI-*Bgl*II-digested pDPT427 DNA in a volume of 20 μl. This ligation mixture was packaged in vitro (8) and diluted to a volume of 0.5 ml. Dilutions of these packaged phage were each absorbed to 0.1 ml of a logarithmically growing culture of M5158 cells (10^8 cells per ml) for 30 min at 30°C without shaking. These mixtures were then incubated at 30°C for an additional 45 min with shaking to ensure expression of the drug resistance genes. Cells were then plated on LB plates containing spectinomycin (50 μg/ml) and ampicillin (50 μg/ml) and incubated for 24 h at 30°C. Ap^r Sp^r transductants were tested for temperature sensitivity and Cm^s (the *Bam*HI-*Bgl*II fragment of pDPT427 which lacks the *ori* contains the Cm^r gene). Colonies which

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TABLE 1. Bacterial strains, bacteriophage, and plasmids

Strain	Relevant markers	Source or reference
E. coli		
Q359	P2 lysogen, *hsdR hsdM*$^+$	R. Maurer, 10
RB132	Ap^r Tc^r Tn*10*Δ*4HH104*(pNK127)	R. Maurer, 6
M5158	λ *c*I857 *N7 N53* Δ*H1 kil*$^+$	E. Signer, 7
AB1157	*proA2 his-4*	18
JC2926	*recA13*	18
GW1000	Δ*lacU169*	11
GW5307	(*ant'-lacZ*$^+$) λ lysogen	19
Bacteriophage		
λ1059	Spi$^+$ Ap^r	10
λSε4*ant*$^+$	*ant* Sp^r	19
Plasmid		
pSE101	Km^r, pSC101 replication system	5
pDPT427	Sp^r Cm^r, NR1 replication system	16
pKB444	Ap^r Tc^r	K. Backman

were Ap^r Sp^r Cm^s and were partially temperature sensitive at 42°C were chosen for further study. These clones were tested for the ability to release phage on thermal induction at 42°C. All clones tested released phage. Furthermore, the phage released were able to transduce M5158 cells to Ap^r Sp^r at the same frequency at which they were able to form plaques on nonlysogens. Phasmid DNA was prepared from these strains as described below and then subjected to restriction endonuclease cleavage analysis with *Bam*HI. A phasmid which contained the internal 16-kb *Bam*HI fragment of λ1059 unchanged but which contained a larger left arm was identified and designated λSE4.

Construction of an *E. coli* genomic library in λSE4. Fragments (13 to 17 kb) generated by partial digestion of W3110 DNA with *Sau*3A1 were isolated from low-melting-point agarose gels (Bethesda Research Laboratories, Gaithersburg, Md.). A 1-μg sample of this DNA was ligated to 1 μg of *Bam*HI-cleaved λSE4 DNA in a volume of 10 μl. This ligation mixture was then packaged into phage in vitro. The resulting recombinant phage were plated and amplified on strain Q359 (10), a P2 lysogen, on LB plates. Approximately 8×10^4 independent Spi$^-$ clones were isolated in this experiment; this was 20% of the total number of packaged phage. When growing λ1059 or any of the λSE vectors described in this paper to prepare DNA for making a library, we found that growth of the phage on a *recA* host helped minimize spontaneous deletions that lead to a Spi$^-$ phenotype. Furthermore, since parental phage containing the *gam* and *red* genes maintain a selective growth advantage over recombinant Spi$^-$ phage, amplification of these libraries should be performed with either strain Q359 or a *recB recC sbcB* strain.

Construction of plasmids. All plasmid DNAs used for cloning were purified by CsCl-ethidium bromide buoyant density centrifugation. Ligations were typically performed at 10 μg of both vector and insert DNAs per ml with T4 DNA ligase (New England BioLabs, Inc., Beverly, Mass.) at 4°C for 10 h. DNA fragments from partial digests were purified from 1% (wt/vol) low-melting-point agarose gels. Phasmid DNAs from λSE4, λSE5, and λSE6 for restriction analysis were prepared from lysogens as described by Holmes and Quigley (9). Transformations were performed as described by Cohen and Chang (4).

pSE103 was constructed by ligating *Bgl*II-cleaved λ*c*I857 DNA with *Bam*HI-cleaved pSE101 (5). This ligation mixture was restriction selected by cleaving again with *Bam*HI and transforming into AB1157 cells. Transformants were selected on plates containing kanamycin (50 μg/ml) and seeded with 10^8 λ*c*I *h*80. Plasmid DNA prepared from transformants selected in this manner was analyzed by restriction endonuclease cleavage analysis. Strains harboring plasmids containing the correct insert were tested for their sensitivity to λ at 30 and 42°C and for their sensitivity to λ*vir* at 30°C. All plasmids tested showed λ immunity at 30°C and sensitivity at 42°C and were sensitive to λ*vir* at 30°C. One plasmid was chosen and designated pSE103. This plasmid carries the λ *c*I857 gene. Its replication system is that of the plasmid pSC101, and it is compatible with the pDPT427-derived replication carried by λSE4 and λSE6 as well as with the replication systems of such cloning vectors as pBR322 and pACYC184.

pSE104 (Fig. 2) was constructed by cloning *Eco*RI-*Kpn*I-cleaved λSE4 DNA into *Eco*RI-*Kpn*I-cleaved pKB444. pKB444 is a pBR322 derivative which has had its *Pvu*II site converted into a *Kpn*I site (K. Backman, unpublished results). The λSE4 *Eco*RI-*Kpn*I fragment of interest contained the gene encoding spectinomycin resistance, and so Ap^r Sp^r transformants were selected in strain GW1000. Restriction endonuclease analysis of plasmid DNAs allowed us to identify several clones containing the 11-kb *Eco*RI-*Kpn*I fragment of interest. One clone was chosen and designated pSE104.

pSE106 (Fig. 2) was constructed by ligating *Hin*dIII-*Bam*HI-cleaved pSE104 DNA to *Hin*dIII-*Bam*HI-cleaved pSE101 DNA. This ligation mixture was transformed into GW1000 cells, and Km^r Ap^r clones were selected. These transformants were screened for Sp^s, and Km^r Ap^r Sp^s clones were chosen for further study. Plasmid DNA was isolated, and restriction analysis was used to identify several plasmids which had the *Hin*dIII-*Bam*HI fragment of pSE101 containing the *npt* gene replacing the *Hin*dIII-*Bam*HI fragment of pSE104 that contained the gene encoding resistance to spectinomycin. A plasmid containing the proper fragments was chosen and designated pSE106.

pSE105 (Fig. 2) was constructed by ligating *Hin*dIII-*Sal*I-cleaved pSE104 with *Hin*dIII-*Sal*I-cleaved pSE101 (5). This ligation mixture was transformed into GW1000 cells, and Sp^r Km^r Ap^r transformants were selected. Restriction endonuclease cleavage analysis was used to identify several plasmids in which the *Hin*dIII-*Sal*I fragment from pSE101 containing the *npt* gene had replaced the small *Hin*dIII-*Sal*I fragment of pSE104, thereby destroying the low-copy-number origin of replication. A representative clone was chosen and designated pSE105.

Construction of λSE6. A plate stock of λSE4 was grown on the strain GW1000(pSE106), and a titer of 10^9 PFU/ml was obtained. Dilutions of these phage were infected into M5158 cells, and Km^r transductants were selected at 30°C. Km^r transductants arose at a frequency of 9×10^{-3} per Sp^r transductant. Lysogens were tested for Ap^r Sp^r. Km^r Ap^r Sp^s lysogens were identified and grown to a density of 10^8 cells per ml at 30°C and then shifted to 42°C for 2 h with shaking to induce the phage. These phage were then used to transduce M5158 cells to Km^r Ap^r again. All phage tested transduced M5158 cells to Km^r Ap^r at a titer approximately equal to their plaque-forming titer. Plasmid DNA was isolated from these transductants and subjected to restriction endonuclease cleavage analysis. All plasmids analyzed had the *Hin*dIII-*Bam*HI fragment containing the *npt* gene replacing the *Hin*dIII-*Bam*HI fragment containing the gene encoding spectinomycin resistance, just as in the parental plasmid pSE106. A representative phasmid was chosen and designated λSE6.

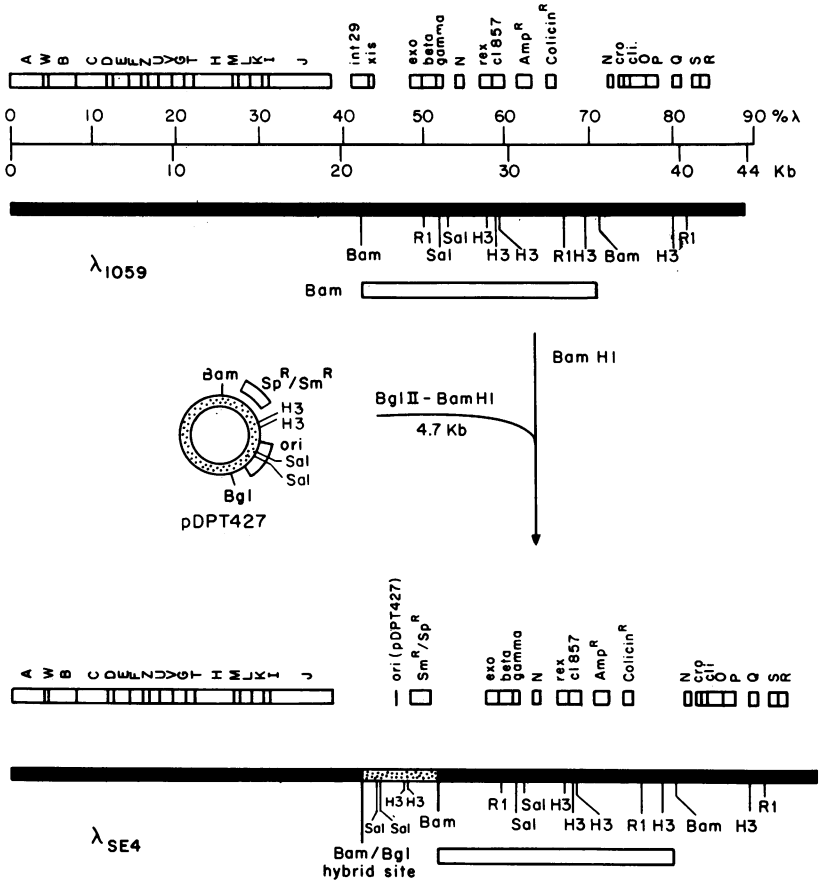

FIG. 1. Construction of λSE4. *Bam*HI-cleaved λ1059 was ligated with *Bam*HI-*Bgl*II-digested pDPT427. The 4.7-kb *Bgl*II-*Bam*HI fragment of pDPT427 carries the replication system of pDPT427 and a gene encoding spectinomycin resistance. λSE4 has had this 4.7-kb fragment (shown as a stippled region) joined to the left arm of λ1059 in the orientation which increases the size of the 19.4-kb left arm to 24.1 kb when the phage DNA is digested with *Bam*HI. The 16-kb *Bam*HI fragment of dispensable DNA (shown as an open bar) is retained. The 4.7-kb *Bgl*II-*Bam*HI fragment lacks restriction sites for *Eco*RI, *Hpa*I, *Xho*I, *Xba*I, and *Sac*I. The *Bgl*II-*Bam*HI junction created in this fusion interrupts the coding region of the *int* gene. The maps of λ1059 and pDPT427 are redrawn from those of Karn et al. (10) and Sninsky et al. (16), respectively.

Construction of λSE5. A plate stock of λSE4 was grown on the strain GW1000(pSE105), and a titer of 10^9 PFU/ml was obtained. Dilutions of these phage were infected into M5158 cells, and Km^r Sp^r Ap^r transductants were selected at 30°C. Individual lysogens were thermally induced, and phage thus obtained were shown to be able to once again transduce M5158 cells to Km^r Ap^r Sp^r at a high frequency. These phage were also shown to have retained the Spi^+ phenotype because they failed to form plaques on strain Q359. Plasmid DNA was prepared from these strains and analyzed by restriction endonuclease cleavage. All phasmids tested had the *Hin*dIII-*Bam*HI fragment containing the *npt* gene adjacent to the *Hin*dIII-*Bam*HI fragment containing the gene encoding spectinomycin resistance. The restriction map of this region was completely analogous to the restriction map of pSE105, indicating that the double recombination event had occurred as expected. One phasmid was selected and designated λSE5.

Transposon mutagenesis. A stock of λSE4 *ant*$^+$ phage (19) was grown on strain RB132 on a plate, and a titer of 10^9 PFU/ml was obtained. Approximately 5×10^7 phage were infected into 2×10^8 logarithmically growing GW5307 [Φ(*ant'-lacZ*$^+$)] cells (which are *c*I$^+$), incubated with shaking for 45 min at 30°C, plated on LB plates containing tetracycline (20 μg/ml) and spectinomycin (50 μg/ml), and incubated at 30°C for 24 h. Tc^r Sp^r transductants were tested for their ability to complement *ant* mutations as described by Yerkes et al. (19).

RESULTS

λSE4: a low-copy-number phasmid vector encoding spectinomycin resistance. Our strategy in designing the vector λSE4 was to modify an existing lambda vector by introducing a low-copy-number plasmid replication system and a drug resistance gene. If such a phasmid were introduced into a cell containing the lambda *c*I gene, its lambda functions would be repressed and the phasmid would replicate by the low-copy-number plasmid replication system; cells containing the phasmid could be selected for by the drug resistance. On a strain lacking the λ *c*I gene, the phasmid would grow as a lytic phage. The low-copy-number replication system chosen was that of the plasmid pDPT427 which in turn was derived from the plasmid NR1 (16, 17). Plasmids using this replication system are present in cells at a copy number of approximately 1 (17). M5158 was chosen as the recipient strain in these experiments because it carries the λ *c*I857 gene (a temperature-sensitive allele of the λ *c*I gene) in its chromosome but has had most of the remaining λ sequences removed by deletion (7).

We chose λ1059 (9) as the phage we would modify to

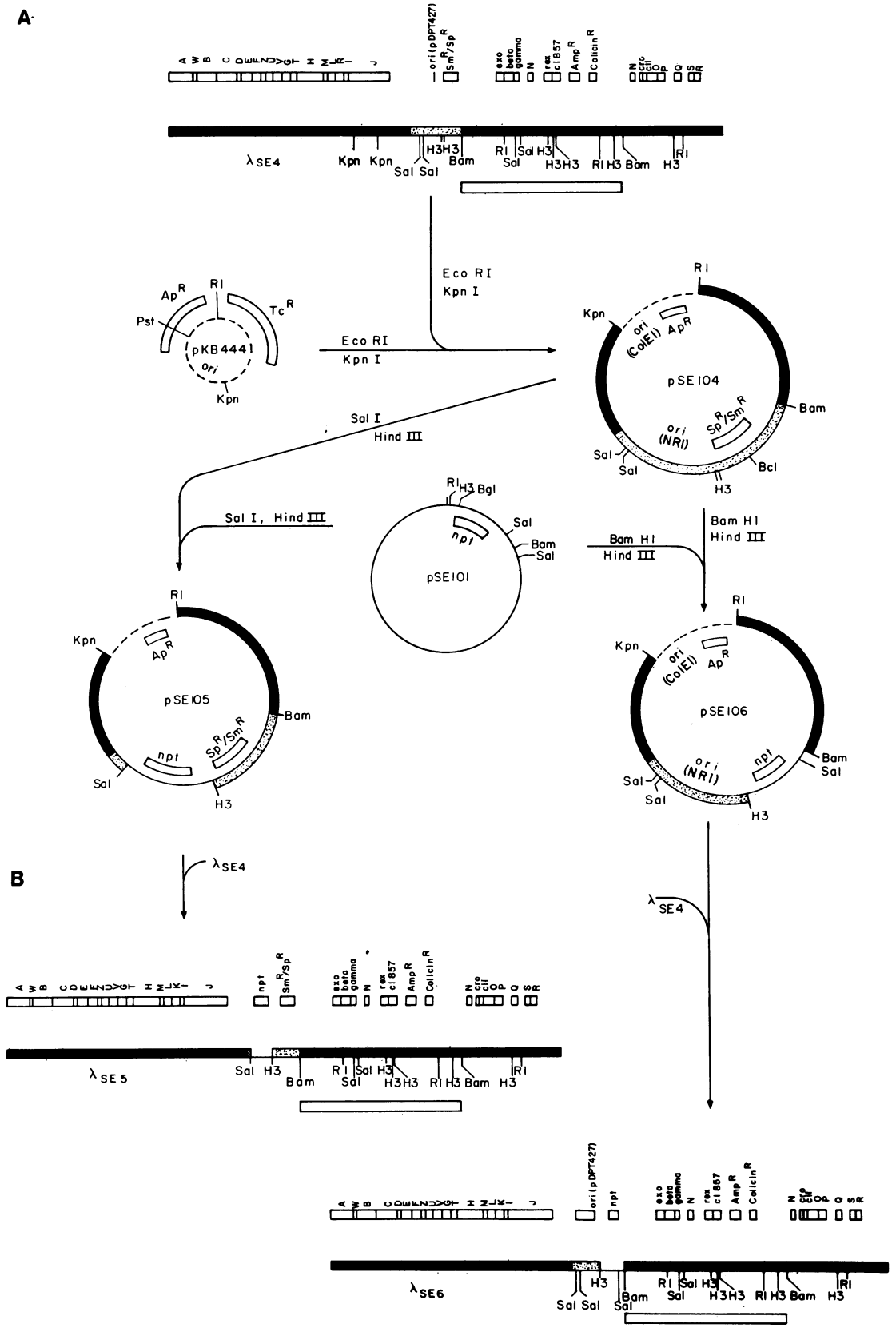

FIG. 2 (a) Construction of pSE104, pSE105, and pSE106. pSE104 was constructed by the ligation of *Eco*RI-*Kpn*I-cleaved λSE4 with *Eco*RI-*Kpn*I-cleaved pKB444. pSE105 was constructed by replacing the *Sal*I-*Hin*dIII fragment of pSE105 that carries the replication system of pDPT427 with the *Sal*I-*Hin*dIII fragment of pSE101 that carries the *npt* gene. This construction destroys the low-copy-number origin of

become our phasmid cloning vehicle because it has a number of attractive features. First, it is able to accept insertions of 6- to 24-kb fragments with *Sau*3A1 ends, so that it is easy to construct a relatively random library of chromosomal DNA. It also has the Spi$^-$ selection for recombinant phage; λ phage carrying the λ *red* and *gam* genes are unable to grow on *E. coli* strains which are P2 lysogens (the Spi$^+$ phenotype). In λ1059, the *red* and *gam* genes are located on an internal 16-kb *Bam*HI fragment which, in recombinant phage, is replaced by cloned DNA, thereby permitting growth on P2 lysogens. Also residing on the internal 16-kb *Bam*HI fragment is a gene encoding Ap^r and a high-copy-number plasmid replication system which allows the easy isolation of vector DNA as a plasmid. Clones of λ1059 which have the 16-kb *Bam*HI fragment replaced can no longer replicate as plasmids.

To construct λSE4 we joined the 4.7-kb *Bam*HI-*Bgl*II fragment of pDPT427 (16), which contains the low-copy-number plasmid replication system and the gene encoding resistance to spectinomycin, to one of the arms of λ1059 (Fig. 1). The insertion of the 4.7-kb fragment in the desired orientation increased the size of the 19.4-kb left arm of *Bam*HI-digested λ1059 to 24.1 kb but left intact the 16-kb internal *Bam*HI fragment responsible for the Spi$^+$ phenotype. This construction not only retains the Spi$^-$ selection for recombinant phage but also allows the recombinant phage to replicate as selectable low-copy-number plasmids when resident in cells containing the λ *c*I gene. In contrast, recombinant derivatives of the original phage λ1059 have lost the ability to replicate as plasmids.

Construction of an *E. coli* genomic library in λSE4. To test the ability of λSE4 to function as a Spi$^-$ cloning vehicle as well as a low-copy-number, high-frequency lysogenizing, selectable phasmid, we constructed a genomic library of *E. coli* DNA. Although λSE4 should be able to accept fragments as small as 2 kb, we chose to have our smallest fragment greater than 10 kb so that the combination of any two fragments would exceed the packaging capacity of this vector, thus ensuring single inserts. Chromosomal DNA of strain W3110 was partially digested with *Sau*3A1, and 13- to 17-kb fragments were ligated with *Bam*HI-cleaved λSE4 DNA. After in vitro packaging, the phage were plated and amplified on a P2 lysogen to select for recombinant derivatives of λSE4.

To test the molecules in this library for their ability to replicate as plasmids, we made lysogens of approximately 10^3 λSE4 derivatives in M5158 cells by infecting and selecting for Sp^r at 30°C. None of the Sp^r colonies formed were found to be Ap^r. All the Sp^r colonies were found to be temperature sensitive for growth at 42°C and released phage after the temperature shift. Derivatives of strain M5158 containing recombinant derivatives of λSE4 were in fact more temperature sensitive than were strains containing the parent, λSE4. This increased temperature sensitivity may be due to the fact that M5158 cells containing λSE4 contain large amounts of the λ *c*I857 repressor, since λSE4 carries a copy of the λ *c*I857 gene and replicates from the high-copy-number plasmid replication system on the 16-kb *Bam*HI fragment. In contrast, M5158 cells carrying recombinant derivatives of λSE4 contain relatively low amounts of the λ *c*I857 repressor, since they have only the single copy of the λ *c*I857 gene present on the chromosome. Furthermore, we isolated plasmid DNA from several clones, showing that the phasmids are actually existing as plasmids in the lysogenic state.

To confirm that the λSE4 derivatives we had isolated were in fact recombinants, we tested their ability to grow on a *recA* strain. The original vector λSE4 carries the λ *red* and *gam* genes on its 16-kb *Bam*HI fragment and can therefore form plaques on a *recA* host, whereas recombinant derivatives have lost the 16-kb *Bam*HI fragment and cannot. Several random independent clones were tested, and none could form plaques on JC2926, a *recA* strain.

Isolation of genes by complementation. One of the primary motivations for constructing λSE4 was to simplify the process of identifying genes by complementation. This was tested by isolating phasmid clones of several representative genes. For example, we first chose to isolate λSE4 derivatives which would complement the *proA2* mutation in strain AB1157. We made AB1157 cells λ immune by introducing the plasmid pSE103, a derivative of pSE101 (5) containing the λ *c*I857 gene. We infected this strain with the library and selected Sp^r transductants on LB-spectinomycin plates at 30°C. We then replica plated these colonies onto minimal glucose plates supplemented with all the strain AB1157 requirements except for proline. We obtained proline prototrophs at a frequency of 1 in 350 Sp^r colonies. Phasmids from these clones were thermally induced and used to transduce AB1157(pSE103) to Sp^r. All Sp^r colonies tested were also Pro$^+$, indicating 100% linkage between Sp^r and proline prototrophy. In a similar fashion we also cloned the locus complementing the *his-4* mutation of strain AB1157. The frequency of complementing phage and subsequent linkage data were similar to those for the cloning of the *proA* gene.

A number of other genes have been isolated by using this library of *E. coli* DNA. For example, a phasmid which complements a mutation at the *ant* (*an*aerobic electron *t*ransport) locus has been isolated (19). A phasmid which complements mutations at the *ada* locus has been identified (P. K. Lemotte and G. C. Walker, submitted for publication). Phasmids complementing mutations at the *metA*, *metE*, and *metF* loci (13), respectively, have been isolated (J. Kreuger and G. C. Walker, unpublished results). A phasmid that complements mutations at the *rpoB* locus was identified by K. Normington and S. Zimmerman (personal communication), as has one that complements *hemA* mutations (T. Macaluso, personal communication). A number of genes involved in riboflavin biosynthesis have been isolated from this library (J. Prince, personal communication). We also isolated clones of the *lacZ* locus by plating the library on the *lacZ* deletion strain GW1000 on LB plates containing isopropyl-β-D-thiogalactopyranoside (0.1 mM) and X-Gal (50 μg/ml) and screening for blue plaques. Phasmids isolated in this manner were still able to transduce immune cells to Sp^r.

A library of *Salmonella typhimurium* chromosomal DNA generated by *Sau*3A1 partial digestion has been prepared in λSE4. Phasmids bearing clones of the *mutL* gene, the *mutH*

replication. pSE106 was constructed by replacing the *Hin*dIII-*Bam*HI fragment of pSE104 which contains the gene encoding spectinomycin resistance with the *Hin*dIII-*Bam*HI fragment of pSE101 containing the *npt* gene. (b) Construction of λSE5 and λSE6. λSE5 was constructed by growing a plate stock of λSE4 on strain GW1000(pSE105) as described in the text and selecting for the desired double recombination event that crossed the modified portion of pSE105 back onto the bacteriophage. Similarly, λSE6 was constructed in an analogous fashion by growing λSE4 on strain GW1000(pSE106) and selecting for the desired double recombinant.

gene, and the *tet* gene of *mutS121*::Tn*10* insertion have been identified (P. Pang and G. C. Walker, unpublished results).

An advantage of a library in this vector is that it may be stored as lysogens and frozen at −70°C in 9% dimethyl sulfoxide. Portions may be thawed, grown, and thermally induced when needed.

Transposon mutagenesis of λSE4 clones. Once a particular clone of interest has been isolated, it is often advantageous to be able to inactivate the complementing activity conveniently. Transposon mutagenesis is a particularly useful method of mutagenesis, because there is a drug resistance selection for insertion of most transposons, the mutations created are usually null alleles and are often polar, and insertions alter the DNA restriction endonuclease cleavage map in a defined way which then defines the region of cloned DNA responsible for the complementing activity.

To mutagenize derivatives of λSE4, we used a method developed by R. Maurer (personal communication) for the mutagenesis of phage λ by a mini-Tn*10* transposon. Briefly, a λSE4 derivative of interest was grown on the strain RB132, which contains Tn*10Δ4HH104*, a Tn*10* mutant with an increased transposition frequency, and pNK217 (6), a pBR322 clone of Tn*10Δ16Δ17*, a 2.5-kb derivative of Tn*10* which is capable of transposition in the presence of Tn*10Δ4HH104*. The Tn*10Δ16Δ17* transposes onto λSE4 derivatives at a frequency of 1×10^{-5} to 3×10^{-5}. Insertion mutants can be selected by lysogenizing the phasmids passaged through strain RB132 into a strain which is λ immune and selecting for Sp^r Tc^r transductants. If the λ immune strain used contains the original mutation, insertion mutants of the λSE4 derivative can be identified directly by their failure to complement. For example, this method was used to mutagenize an *ant*$^+$ clone in λSE4 (19). The mutagenized phasmids were used to infect GW5307, an *ant* strain lysogenic for λ (19), and Tc^r Sp^r derivatives were selected. Approximately 25% of these derivatives were Ant^-. This frequency is reasonable, since the *ant*$^+$ phasmid carries a 15-kb insert of chromosomal DNA and the *ant* locus is at least 5 kb in size (19).

λSE6: a low-copy-number phasmid vector encoding neomycin-kanamycin resistance. One problem that arose during the use of λSE4 was that *E. coli* is not sensitive to spectinomycin in minimal media. Since phasmids using this low-copy-number origin of replication tend to be lost at a significant frequency in the absence of drug pressure, the inability to select for spectinomycin resistance in minimal medium may interfere with the ability to identify certain classes of genes by complementation. For these reasons, we decided to construct λSE6, a derivative of λSE4 in which the λSE4 spectinomycin resistance gene has been replaced by the *npt* gene from Tn*5*. The *npt* gene encodes resistance to neomycin and kanamycin, antibiotics effective in minimal media.

To facilitate the construction of λSE6, we used a strategy of cloning a segment of λSE4 onto a plasmid vector, carrying out the desired recombinant DNA modifications of the cloned segment while it was in the plasmid vector, and then genetically crossing the modified segment back onto the phasmid vector (Fig. 2). First, the 10.7-kb *Kpn*I-*Eco*RI fragment of λSE4 containing the plasmid origin of replication and the spectinomycin resistance gene was subcloned onto *Kpn*I-*Eco*RI-cleaved pKB444, a pBR322 derivative which has the *Pvu*II site changed into a *Kpn*I site (K. Backman, unpublished results). The Ap^r Sp^r plasmid created in this construction was named pSE104. pSE104 was then modified by replacing the *Hin*dIII-*Bam*HI fragment containing the spectinomycin resistance gene with a pSE101-derived *Hin*dIII-*Bam*HI fragment carrying the *npt* gene. The resulting plasmid was termed pSE106 (Fig. 2). Finally, a stock of λSE4 was grown on a strain containing pSE106. These phage were then infected into the immune strain M5158, and Km^r transductants were selected at 30°C. These transductants were expected to be Sp^s because a single crossover event would make the phage too large (63 kb) to package. The only event which would produce a Km^r transductant would be the double crossover event resulting in the replacement of the DNA segment encoding spectinomycin resistance by the DNA segment encoding neomycin-kanamycin resistance. The derivatives obtained were in fact Sp^s, and phage prepared from these transductants were Spi^+ in addition to being able to transduce strain M5158 to Km^r Ap^r. DNA was prepared from these transductants, and restriction analysis revealed that the expected double crossover had indeed occurred. One representative phasmid was chosen and designated λSE6 (Fig. 2).

λSE5: a lambda cloning vector carrying selectable markers. We have also used the strategy outlined for the construction of λSE6 to construct λSE5, a variant of λSE4 that carries the *npt* gene but lacks the low-copy-number plasmid origin of replication. If a recombinant derivative of λSE5 is infected into a strain containing a plasmid carrying the λ *c*I gene and Km^r is selected, lysogens will be obtained in which integration of the phage has occurred by homologous recombination between the chromosomal segment on the recombinant phage and the chromosome. By inducing such lysogens, chromosomal mutations can be crossed onto the recombinant phage at high frequency.

The construction of λSE5 is outlined in Fig. 2. The *Sal*I-*Hin*dIII fragment of pSE104 that carries the pDPT427-derived low-copy-number plasmid replication system was replaced by a pSE101-derived *Sal*I-*Hin*dIII fragment carrying the *npt* gene to give the plasmid pSE105 (Fig. 2). λSE4 was grown on a pSE105-containing strain, and these phage were used to transduce the lysogenic strain M5158 to Sp^r Ap^r Km^r. These transductants were thermally induced, and phage from these lysates were used to transduce strain M5158 to Sp^r Ap^r Km^r again. The phasmids in these lysates all transduced strain M5158 to the proper drug resistances with an efficiency equivalent to their plaque-forming ability. These phasmids were shown to retain the Spi^+ phenotype by failing to form plaques on Q359 cells. DNA was prepared from several lysogens, as described above. Restriction endonuclease cleavage analysis revealed that these phasmids had arisen by a double recombination event between λSE4 and pSE105 that had resulted in the replacement of the DNA carrying the low-copy-number plasmid replication system by DNA encoding the *npt* gene. A representative phasmid was chosen and designated λSE5. This cloning vector retains the Spi^- selection for recombinant phage and selectable drug markers but has lost the ability to replicate as a plasmid after the central *Bam*HI fragment is replaced.

DISCUSSION

In this paper we have described the construction of a family of phasmid vectors specifically designed for the identification of genes by complementation of *E. coli* mutants and a general technique for easily producing other variants of these vectors. Particular features of the vectors λSE4 and λSE6 include (i) the Spi^- selection for the insertion of recombinant DNA into the vectors; (ii) the ability to carry reasonably large (approximately 2 to 19 kb) inserts of DNA; (iii) the ability to accept DNA fragments generated by

partial *Sau*3A1 digestion so that random libraries can be generated easily; (iv) replication of the recombinant phasmids either as lytic phage in nonlysogenic hosts or as plasmids in hosts that carry the λ *c*I gene; (v) the use of a very-low-copy-number plasmid replication system by the phasmids when replicating as plasmids in a strain containing the λ *c*I gene, thus avoiding many of the problems that can arise from increased gene dosage; (vi) a drug resistance gene(s) that allows for the efficient selection and stable maintenance of lysogens in immune hosts; (vii) a 100% lysogenization frequency of recombinant phasmids when infected into immune hosts; and (viii) the ability of libraries on these plasmids to be stored or amplified as phage or as lysogens. A very large number of *E. coli* mutations are available (1), and these vectors should aid in the identification of genes from a variety of procaryotes, and possibly certain eucaryotes, that can complement such *E. coli* mutants.

We have demonstrated these features with λSE4, and we and others have isolated a number of genes from libraries prepared with this vector. Furthermore, we have shown that the recombinant phasmids can readily be mutagenized by the transposon mini-Tn*10*. Such transposon mutagenesis can facilitate the rapid identification of the region of recombinant DNA responsible for complementing the *E. coli* mutation and of the protein encoded by that gene. If transposon mutagenesis is to be used with a given library, care should be taken to construct the library with fragments no larger than 15 to 16 kb to ensure proper packaging of the mutagenized clones.

Although we have not yet done much work with λSE5, it should be useful for crossing mutations on and off the *E. coli* chromosome by integration via homologous recombination and subsequent excision. In principle, any λSE4 recombinant derivative could be converted into the corresponding λSE5 derivative by removing the plasmid replication system by in vivo recombination with pSE105 and then selecting for Km^r Sp^r transductants in a lysogenic strain. Alternatively, lysogenization of the recombinant phage could be achieved at higher frequency by coinfection of a nonlysogenic strain with a wild-type λ helper which would provide *int* functions in *trans*.

We found the strategy of crossing DNA segments on and off λ to be very helpful in the construction of these vectors, and it should be useful in the construction of other λ derivatives. For example, we have also crossed the *npt* gene and the low-copy-number replication system from pSE106 onto wild-type lambda and the *npt* gene from pSE105 onto wild-type lambda. Such a strategy could in principle be used to carry out operations such as the construction of phasmid vectors carrying a broad-host-range plasmid replication system. We estimate that any sequence up to 10 kb or less in length, once cloned onto one of the transfer plasmids we have described, could be recombined onto virtually any lambda phage, thereby creating a b-region substitution. This recombination event could be selected for by using the proper drug resistances on the plasmid. This method provides an efficient and simple means of genetically transferring any cloned gene onto phage lambda with a positive selection.

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