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Molecular cloning of DNA fragments produced by restriction endonucleases *Sal*I and *Bam*I

(DNA joining/plasmid/insertional inactivation of genes/*Drosophila melanogaster*)

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ABSTRACT **The highly specific restriction endonucleases *Sal*I and *Bam*I produce DNA fragments with complementary, cohesive termini that can be covalently joined by DNA ligase. The *Escherichia coli* kanamycin resistance factor pML21 has one *Sal*I site, at which DNA can be inserted without interfering with the expression of drug resistance or replication of the plasmid. A more convenient cloning vehicle can be made with the tetracycline resistance factor pSC101, since insertion of DNA either at its single site for *Sal*I or at that for *Bam*I inactivates plasmid-specified drug resistance but not replication. To take advantage of this insertional inactivation, pSC101 was joined to a ColE1-ampicillin resistance plasmid having no *Sal*I site, and to a ColE1-kanamycin resistance plasmid having no *Bam*I site. Chimeras formed with the resulting hybrid vehicles can be identified simply by replica plating. These three vehicles, which all replicate under relaxed control, have been used to clone and amplify *Drosophila melanogaster* DNA fragments.**

Molecular cloning is a powerful tool for the purification and amplification of DNA fragments. Two basic steps are involved in this technique. First, the fragment to be cloned is joined, *in vitro*, to an autonomously replicating vehicle molecule, such as a drug resistance plasmid (1, 2), colicinogenic factor E1 (3), or bacteriophage λ genome (4–6). Second, the chimeric DNA molecules are introduced into *Escherichia coli* by transformation or transfection, then cloned by single colony isolation or plaquing. This general procedure has been applied to a variety of DNA molecules (reviewed in ref. 7) including drug resistance factor DNA fragments (1, 8), the *E. coli* tryptophan operon (3), *Xenopus laevis* DNA coding for ribosomal RNA (9), *Drosophila melanogaster* DNA fragments (10–12), and sea urchin histone genes (13).

A convenient method for the *in vitro* recombination step has been to use restriction endonuclease *Eco*RI (14), which generates DNA fragments with complementary, cohesive termini (15, 16). These fragments can be annealed to one another at low temperature, then covalently joined by the action of DNA ligase (16, 17). It would be advantageous to extend this technique for use with other endonucleases, especially those which cleave DNA less frequently than *Eco*RI.

Recently, we have investigated the specificity and the nature of the termini produced by restriction endonuclease *Sal*I, isolated from sporulating *Streptomyces albus* G. (D. H. Hamer, G. C. Foster, J. R. Hutton, J. U. Upcroft, and C. A. Thomas, Jr., manuscript in preparation); the existence and specificity of this enzyme were first indicated to us by R. Roberts. This enzyme cleaves various DNAs about once every 8 kb (kilobases), as compared to about once every 4 kb for *Eco*RI (16, 18). The resulting fragments have cohesive termini, and can be joined to one another in head-to-tail, head-to-head, and probably tail-to-tail orientation. Another highly specific restriction endonuclease that produces cohesive termini is *Bam*I, from *Bacillus amyloliquefaciens* H (G. Wilson and F. Young, personal communication). We have shown that it cleaves *D. melanogaster* DNA about once every 6 kb. This report describes the construction and verification of plasmid vehicles that allow one to clone and amplify potentially any DNA fragment produced by *Sal*I or *Bam*I, and to identify the bacteria harboring chimeras by replica plating.

Abbreviations: *Eco*RI, *Sal*I, and *Bam*I, restriction endonucleases from *Escherichia coli*, *Streptomyces albus* G., and *Bacillus amyloliquefaciens* H, respectively; Tc, tetracycline; Km, kanamycin; Ap, ampicillin; ^{R}, resistance; ^{S}, sensitivity; ImmE1$^+$, immune to colicin E1; colicin E1$^+$, able to produce colicin E1; RepSC101, replication function(s) of pSC101; kb, 1000 base pairs of double-stranded or 1000 bases of single-stranded DNA. Designation of hybrid or chimeric plasmids: a plasmid comprised of a DNA fragment *X* joined to a DNA fragment *Y* at a site for restriction endonuclease *R* is designated as *X/R-Y/R*.

MATERIALS AND METHODS

E. coli strains HMS49 thymine$^-$ and HMS51 thymine$^-$ *polA1* were obtained from C. C. Richardson. Strain GM4 is HB101 B restriction- and modification-negative ($r_B^-m_B^-$) proline$^-$ galactose$^-$ streptomycin^R *recA*$^-$ (19); our isolate, obtained from J. Morrow, is also lactose$^-$ arabinose$^-$ arginine$^-$. Plasmid pSF2124 was isolated and kindly provided to us by M. So, R. Gill, and S. Falkow. Plasmid pML21, constructed by V. Hershfield, D. Helinski, and H. Boyer, was obtained from K. Backman, and pSC101 was obtained from J. Morrow. pDm113 is a pML21/*Eco*RI-*D. melanogaster*/*Eco*RI chimera that has one *Bam*I site in the 6.5 kb fragment of *D. melanogaster* DNA. *E. coli* was transformed with covalently closed circular DNA or ligase reaction mixtures as described by Cohen *et al.* (20). Immunity to colicin E1 (ImmE1$^+$) and the ability to produce colicin E1 (colicin E1$^+$) were determined by the overlay procedure (21). Drug concentrations used were 25 μg/ml for tetracycline (Tc) and kanamycin (Km) and 35 μg/ml for ampicillin (Ap). All strains carrying *D. melanogaster* DNA were handled under "low risk" conditions as defined in the "Summary Statement of the Asilomar Conference on Recombinant DNA Molecules" (22).

Restriction endonucleases *Eco*RI (14, 18), *Bam*I (G. Wilson and F. Young, personal communication), and *Sal*I (D. H. Hamer, G. C. Foster, J. R. Hutton, J. U. Upcroft, and C. A. Thomas, Jr., manuscript in preparation, and R. Roberts, personal communication) were purified by conventional methods. Phage T_4-induced DNA ligase was prepared (23) and given to us by C. C. Richardson. An enzyme unit is defined in ref. 23. *Eco*RI and *Bam*I endonuclease reaction mixtures contained 50 mM Tris·HCl (pH 7.8), 10 mM $MgCl_2$, and 0.2 mM EDTA, whereas *Sal*I reaction mixtures contained 8 mM Tris·HCl (pH 7.6), 6 mM $MgCl_2$, 0.2 mM EDTA, 150 mM NaCl, and 50 μg/ml of bovine serum albumin. For ligation, endonuclease cleavage reaction mixtures were adjusted to 3–10 pmol of DNA fragment per ml in 40 mM Tris·HCl (pH 7.8), 8 mM $MgCl_2$, 0.1 mM EDTA, and less than 70 mM NaCl. After incubation at 68° for 5 min, then at 0° for 2–12 hr, ATP was added to 0.067

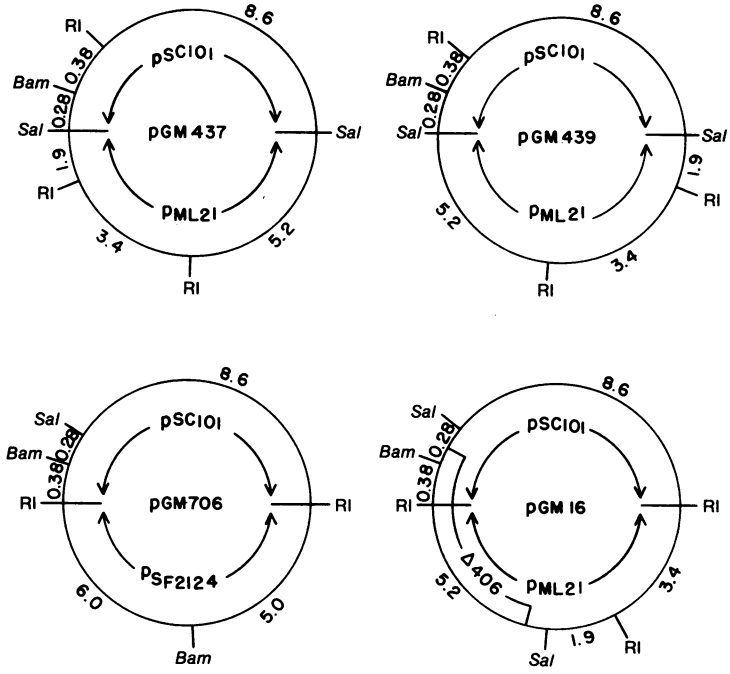

FIG. 1. Restriction endonuclease cleavage maps of hybrid plasmids. The length of pSC101 (9.2 kb) is taken from Wensink *et al.* (10). The lengths of pSF2124 (11.0 kb) and pML21 (10.5 kb) were estimated from their mobilities relative to covalently closed circular pSC101 and a pSC101-ColE1 hybrid on 0.9% agarose gels. Distances between restriction sites, indicated in kb, were determined by electrophoresis of the appropriate digests through 0.9% agarose gels with λ/*Eco*RI fragments (30) as markers, or through 3.2% agarose gels using λ/*Hae*III (18, 31) and simian virus 40/*Hae*III (32) fragments as markers. The orientation of pSC101 relative to pML21 in pGM437 and pGM439 was determined by cleavage with *Eco*RI; pGM437 gave three fragments with lengths of 13.6 kb (68% of the DNA), 3.4 kb (20%), and 2.5 kb (12%), whereas pGM439 gave three fragments with lengths of 10.5 kb (57%), 5.9 kb (32%), and 3.4 kb (11%). The orientation of pSC101 relative to pSF2124 in pGM706 was shown by *Bam*I cleavage, which yielded two fragments with lengths of 13.9 kb (71%) and 6.4 kb (29%). Cleavage of pGM16 with *Sal*I gave two fragments with lengths of 13.9 kb (73%) and 6.0 kb (27%). The site and orientation of insertion shown in the figure was confirmed by the isolation (described in the *text*) of a *Bam*I-resistant, 15.5 kb deletion plasmid, pGM16Δ405. *Eco*RI cleavage of this plasmid gave two fragments with lengths of 12.2 kb (75%) and 3.4 kb (25%), whereas *Sal*I cleavage gave two fragments with lengths of 13.5 kb (86%) and 1.9 kb (14%).

mM and ligase to 0.1–0.4 units/pmol of DNA fragment, and the mixture was held at 12.7° for 16–24 hr.

For the preparation of DNA, cells harboring plasmids replicating under stringent control (see below) were grown in Penassay broth plus antibiotics to 2 to 4 × 10^9/ml. Cells harboring plasmids replicating under relaxed control were grown to 2 to 4 × 10^8/ml, treated with 250 μg/ml of chloramphenicol to stop the synthesis of chromosomal but not of plasmid DNA, and incubated an additional 8–16 hr. Covalently closed circular plasmid DNA was prepared by Brij lysis followed by centrifugation in a cesium chloride–ethidium bromide density gradient (24). The preparation of *D. melanogaster* embryo DNA and λ DNA and procedures for analytical and preparative agarose gel electrophoresis have been described (18).

RESULTS

Construction of pSC101/*Sal*I-pML21/*Sal*I hybrid plasmids

We found two *E. coli* plasmids, pSC101 and pML21, that had a single site for *Sal*I. pSC101 (1, 25) is a 9.2 kb tetracycline resistance plasmid that replicates under stringent control; i.e., it is present at 1–2 copies per chromosome in logarithmically growing cells, and its replication requires new protein synthesis but not the product of the *polA* gene, DNA polymerase I (26, 27). pML21 is a 10.5 kb plasmid consisting of a DNA fragment specifying kanamycin resistance joined to a mini-ColE1 fragment (1, 3). The hybrid retains immunity to colicin E1, though it does not produce colicin, and it replicates under relaxed control; i.e., it is present at 20–30 copies per chromosome in normally growing cells, and its replication requires DNA polymerase I but not new protein synthesis (3, 28).

To determine the effect of inserting new DNA at the *Sal*I site of pSC101 and of pML21, the two plasmids were mixed, cleaved with *Sal*I, treated with DNA ligase, then introduced into strains HMS49 and HMS51 *polA1* by transformation. Selection for pSC101/*Sal*I-pML21/*Sal*I hybrids was based on the following logic. Insertion into pSC101, depending on location, might inactivate tetracycline resistance (Tc^R) or replication (RepSC101), but not both. The *Sal*I site on pML21 is located within the kanamycin resistance fragment (Fig. 1), so insertion into this plasmid might inactivate kanamycin resistance (Km^R), but not immunity to colicin E1 (ImmE1) or replication. Therefore, a hybrid could have just one of six different phenotypes: Tc^R Km^R ImmE1$^+$ RepSC101, Tc^R Km^R ImmE1$^+$, Km^R ImmE1$^+$ RepSC101, Tc^R ImmE1$^+$ RepSC101, Tc^R ImmE1$^+$, or ImmE1$^+$ RepSC101.

Transformation of HMS51 *polA1* with a ligated mixture of pSC101/*Sal*I plus pML21/*Sal*I, but not with a control mixture of untreated DNAs, did give rise to kanamycin-resistant clones (Table 1). Two such clones, designated HMS51(pGM437) and HMS51(pGM439), were kept. Both retained the *polA1* mutation, as indicated by their sensitivity to methylmethanesulfonate and ultraviolet light, and both were immune to colicin E1 but sensitive to tetracycline. Plasmid DNA isolated from these clones transformed both *polA*$^+$ and *polA1* cells to kanamycin resistance and immunity to colicin E1 with high efficiency, but transformation to tetracycline resistance was not observed (Table 1). Thus, both clones were considered to harbor Km^R ImmE1$^+$ RepSC101 hybrids.

Table 1. Transformation of HMS49 and HMS51 by *polA1* pSC101, pML21, and chimeric plasmids

DNA	Transformants/μg of DNA					
	HMS49			HMS51 *polA1*		
	Tc^R	Km^R	Tc^R Km^R	Tc^R	Km^R	Tc^R Km^R
pSC101 + pML21	5.5×10^2	2.5×10^4	1.7×10^1[a]	5.0×10^2	<2	<2
pSC101/*SalI* + pML21/*SalI* + ligase	2.3×10^2	1.1×10^4[c]	8[b]	3.0×10^2[d]	2.0×10^1[e]	<2
pGM437	<2	1.2×10^4[f]	<2	<2	3.0×10^4[f]	<2
pGM439	<2	3.7×10^4[f]	<2	<2	1.2×10^5[f]	<2

Each 0.3 ml transformation contained 4 to 8×10^8 competent cells and 2–4 μg of DNA. In lines 1 and 2, the weight ratio of pSC101 to pML21 DNA was 0.06, and the number of transformants was calculated on the basis of the total input DNA.

[a] Three out of three clones harbored both pSC101 and pML21.

[b] Five out of five clones harbored both pSC101 and pML21.

[c] Six out of 140 clones were immune to colicin E1; each of these was also kanamycin-resistant.

[d] Zero out of 82 clones were immune to colicin E1.

[e] Twelve out of 12 clones were immune to colicin E1, and sensitive to tetracycline, ultraviolet light, and methylmethanesulfonate.

[f] Three out of three clones were immune to colicin E1.

Plasmid DNA prepared from these clones by centrifugation in an ethidium bromide–cesium chloride density gradient was examined by restriction endonuclease cleavage and agarose gel electrophoresis (Fig. 2, gels, 4, 5, 12, and 13). Both hybrid plasmids had a length of approximately 20 kb, as determined by their mobilities relative to appropriate markers, and both gave two equimolar fragments with lengths identical to those of pSC101 and pML21 after treatment with *SalI*. However, digestion with *Eco*RI showed that whereas both hybrids contain one copy each of the pSC101 and the pML21 fragment, they differ in the relative orientation of these fragments (Fig. 1). In one hybrid, both *SalI* joints are head-to-tail, whereas in the other, one is head-to-head and the other is tail-to-tail.

Construction of a pSC101/*Eco*RI-pSF2124/*Eco*RI hybrid plasmid vehicle for cloning DNA fragments produced by *SalI*

These results indicated that insertion of DNA at the *SalI* site of pSC101 inactivates its expression of tetracycline resistance. We therefore sought to construct a hybrid cloning vehicle by joining pSC101 to a DNA fragment having an additional drug resistance marker but no additional *SalI* site. Insertion at the single *SalI* site of such a hybrid would inactivate resistance to tetracycline, but not to the second drug, so that chimeras could be identified simply by replica plating. In addition, we wished the hybrid vehicle to carry the relaxed ColE1 replicon, facilitating the preparation of large quantities of plasmid DNA.

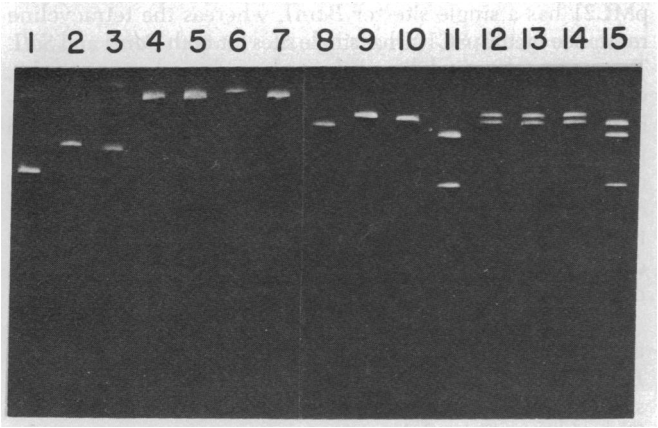

FIG. 2. Analysis of hybrid plasmids by restriction endonuclease cleavage and agarose gel electrophoresis. 1–7, Covalently closed circular DNAs electrophoresed through 0.9% agarose gels: 1, pSC101; 2, pSF2124; 3, pML21; 4, pGM437; 5, pGM439; 6, pGM706; 7, pGM16. 8–15, Restriction endonuclease-cleaved DNAs electrophoresed through 1.2% agarose gels: 8, pSC101/*Eco*RI; 9, pSF2124/*Eco*RI; 10, pML21/*SalI*; 11, pML21/*Eco*RI; 12, pGM437/*SalI*; 13, pGM439/*SalI*; 14, pGM706/*Eco*RI; 15, pGM16/*Eco*RI.

We constructed such a hybrid by using pSF2124, a ColE1 factor carrying ampicillin resistance. Insertion at the single *Eco*RI site of this plasmid interferes with colicin production but not with colicin immunity or ampicillin resistance (ref. 29, and M. So, R. Gill, and S. Falkow, personal communication). Insertion at the single *Eco*RI site of pSC101 does not interfere with its replication or with the expression of its tetracycline resistance gene (1, 8). Therefore, a mixture of these two DNAs was cleaved with *Eco*RI, treated with DNA ligase, and introduced into *E. coli* C600. A Tc^R Ap^R $ImmE1^+$ colicin $E1^-$ clone was picked and designated C600 (pGM706). Plasmid DNA from this clone had a length of about 20 kb, as expected for a plasmid having one copy each of the 9.2 kb pSC101 fragment and the 11.0 kb pSF2124 fragment (Fig. 2, gel 6). Treatment with *Eco*RI gave two equimolar fragments with the same lengths as pSF2124 and pSC101 (Fig. 2, gel 14), whereas treatment with *SalI* gave a single fragment with a length of 20.2 kb (Fig. 3, gel 7). Cleavage with *BamI* (not shown) demonstrated that the pSF2124 and pSC101 fragments are oriented relative to one another as shown in Fig. 1.

Construction of a pSC101/*Eco*RI-pML21/*Eco*RI hybrid plasmid vehicle for cloning DNA fragments produced by *BamI*

Plasmid pSC101 also has a single *BamI* site, located 0.28 kb from the *SalI* site. It seemed likely that insertion at this site, just as at the *SalI* site, would inactivate the expression of tetracycline resistance. To test this idea, pSC101 was cleaved with *BamI* and ligated to a pML21/*Eco*RI-*D. melanogaster*/*Eco*RI chimera, pDm113, which was cleaved with *BamI* at a single site within the *D. melanogaster* DNA. Transformation of HMS51 *polA1* with this mixture, but not with a control mixture of untreated DNAs, gave rise to kanamycin-resistant, colicin-immune, tetracycline-sensitive clones, as would be expected if insertion at the *BamI* site of pSC101 had inactivated its expression of drug resistance but not its replication.

To construct a vehicle for the cloning of DNA fragments produced by *BamI*, pSC101 was joined to pML21, which has no *BamI* site. Plasmid pML21, which contains two *Eco*RI sites, was partially digested with *Eco*RI, and full length linear molecules were isolated on an agarose gel. These were ligated to

*Eco*RI-cleaved pSC101, and the reaction mixture was used to transform GM4. A Tc^R Km^R ImmE1$^+$ colicin E1$^-$ clone was picked and designated GM4 (pGM16). Intact plasmid DNA from this strain had a length of about 20 kb (Fig. 2, gel 7). Cleavage with *Eco*RI (Fig. 2, gel 15) gave three equimolar fragments with the same lengths as pSC101 and the kanamycin-resistance and mini-ColE1 fragments of pML21, whereas cleavage with *Bam*I (Fig. 3, gel 10) gave a single fragment with a length of 19.7 kb. Of the four possible configurations for the hybrid, that shown in Fig. 1 was shown to be correct by *Sal*I cleavage and by studies of a deleted plasmid (described in the legend to Fig. 1).

Cloning of *D. melanogaster* DNA fragments

The utility of pML21 and the two pSC101 hybrid plasmids as molecular vehicles has been verified by using them to clone *D. melanogaster* DNA fragments.

The effects of joining pSC101 to pML21 at their *Sal*I sites, described above, suggested that foreign DNA could be inserted at the *Sal*I site of pML21 without altering its expression of kanamycin resistance or its replication. Accordingly, a 40:1 weight:weight mixture of *D. melanogaster* embryo DNA and pML21 DNA was digested with *Sal*I, treated with ligase, and transformed into GM4. Of 339 Km^R clones analyzed, 14 had covalently closed circular DNA with a length at least 20% greater than pML21, as determined by agarose gel electrophoresis of total cell Sarkosyl–Pronase lysates. Plasmid DNA from four of the chimeric isolates was examined by cleavage with *Sal*I (Fig. 3, gels 3–6). Each yielded two equimolar fragments; one had the length of pML21, the other had a length between 3.5 and 8.4 kb, and the sum of the fragment lengths was equal to the length of the intact DNA. *Eco*RI digests of these chimeras (not shown) gave two to four equimolar fragments, one of which had the same mobility as the mini-colE1 fragment of the vehicle. These data demonstrate that each of the chimeras consists of a single *Sal*I fragment of *D. melanogaster* DNA joined to a single copy of pML21, and that the *D. melanogaster* DNA is, as expected, inserted within the kanamycin resistance fragment of the vehicle. These results were confirmed by electron microscopic heteroduplex mapping of several of the plasmid chimeras.

In the previous experiment, clones harboring plasmid chimeras were identified by direct examination of their DNA. The utility of the pSC101/*Eco*RI-pSF2124/*Eco*RI hybrid, pGM706, as a vehicle for recognizing chimeras by replica plating was tested as follows. *D. melanogaster* and pGM706 DNAs were digested with *Sal*I, mixed, treated with ligase, and introduced into competent GM4. As a control, the two endonuclease-cleaved DNAs were treated separately with ligase, then mixed just before use in transformation. Zero out of 200 control Ap^R transformants and seven out of 195 experimental Ap^R transformants were sensitive to tetracycline. Plasmid DNA isolated from two of the Ap^R Tc^S clones was analyzed by *Sal*I cleavage (Fig. 3, gels 8 and 9). Each gave one fragment with the length of pGM706 plus one or two new fragments ranging in length from 6.4 to 16 kb.

D. melanogaster DNA fragments produced by *Bam*I were cloned using the pSC101/*Eco*RI-pML21/*Eco*RI hybrid, pGM16, as the vehicle. *Bam*I-digested *D. melanogaster* and pGM16 DNAs were treated with ligase, either separately or together, and introduced into competent GM4. Transformants were selected on kanamycin and replicated onto tetracycline plates. Zero out of 64 control transformants and 27 out of 62 experimental transformants were tetracycline-sensitive. *Bam*I cleavage patterns of plasmid DNA extracted from three of the

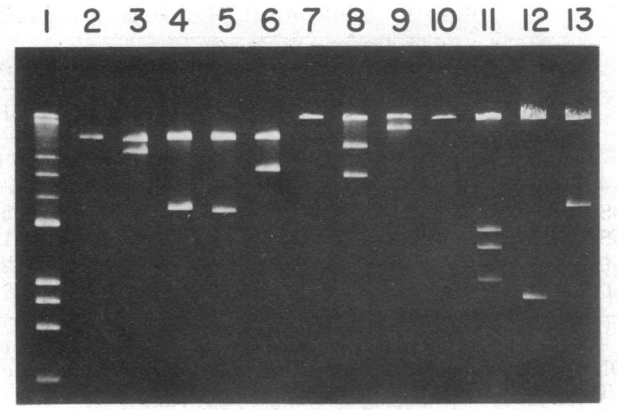

FIG. 3. Analysis of *D. melanogaster* plasmid chimeras by restriction endonuclease cleavage and 1.2% agarose gel electrophoresis. 1, λvir/*Eco*RI (ref. 30) plus λCI857S7plac5/*Hae*III (refs. 18 and 31) marker fragments. 2, pML21/*Sal*I. 3–6, pML21/*Sal*I-*D. melanogaster*/*Sal*I chimeras cleaved by *Sal*I. 7, pGM706/*Sal*I; 8–9, pGM706/*Sal*I-*D. melanogaster*/*Sal*I chimeras cleaved by *Sal*I; 10, pGM16/*Bam*I. 11–13, pGM16/*Bam*I-*D. melanogaster*/*Bam*I chimeras cleaved by *Bam*I.

Km^R Tc^S clones are shown in Fig. 3 (gels 14–16). Each of these chimeras gave one fragment with the length of pGM16 plus one to three new fragments ranging from 1.8 to 7.5 kb. However, plasmid DNA from one of the Km^R Tc^S clones isolated in this experiment was found to be shorter than the vehicle and resistant to *Bam*I. An analysis of this plasmid (pGM16Δ405) by *Eco*RI and *Sal*I cleavage (described in the legend to Fig. 1) suggested that it had suffered a 4.2 kb deletion with one endpoint between the *Sal*I and *Bam*I sites on pSC101, and the other endpoint in the kanamycin resistance fragment of pML21.

DISCUSSION

Our attention was drawn to the restriction endonucleases *Sal*I and *Bam*I because they cut *Drosophila melanogaster* DNA into significantly longer fragments than does *Eco*RI. Both enzymes produce staggered cuts, suggesting that it would be possible to clone the fragments in *E. coli* if appropriate plasmid vehicles having a single cleavage site could be found or constructed. A limited survey revealed that the kanamycin resistance factor pML21 has a single site for *Bam*I, whereas the tetracycline resistance factor pSC101 has single sites for both *Bam*I and *Sal*I. To examine the suitability of these plasmids as cloning vehicles, we joined pSC101 to pML21 using *Sal*I, or to the pML21 derivative pDM113, using *Bam*I. Both of the resulting hybrid plasmids specified resistance to kanamycin but not to tetracycline, and they retained the *cis*-acting replication function(s) of pSC101. These properties indicated that insertion at either the *Sal*I or *Bam*I site on pSC101 inactivates its expression of drug resistance, whereas insertion at the BamI site on pML21 does not.

To take advantage of the insertional inactivation of tetracycline resistance in pSC101, this plasmid was joined at its *Eco*RI site to an ampicillin resistance plasmid that had no *Sal*I site, or to a kanamycin resistance plasmid that had no *Bam*I site. These hybrid plasmids have two advantages as cloning vehicles. First, insertion of new DNA inactivates the expression of resistance to tetracycline, but not to ampicillin or kanamycin, so that chimeras can be identified simply by replica plating. This is particularly useful when the DNA to be cloned is available only in small quantities, or when the efficiency of ligation is low. Second, the vehicles replicate under relaxed control, so that

appreciable quantities of the cloned DNA can readily be prepared (about 2 mg/liter of culture).

The efficacy of pML21 and the two hybrid plasmids as molecular vehicles was demonstrated by cloning *D. melanogaster* DNA fragments. In each case, the formation of presumptive chimeras depended upon treating the vehicle and *D. melanogaster* DNAs together with ligase. With several of the cloned fragments, *in situ* hybridization to polytene chromosomes and reassociation with embryo DNA shows that they do, indeed, consist of *D. melanogaster* DNA, and that each is homologous to a distinct chromosomal locus or set of loci (J. R. Hutton, D. H. Hamer, and C. A. Thomas, Jr., unpublished experiments). It is not known, however, whether the cloned fragments are accurate representations of chromosomal sequences, or if they have undergone rearrangement in *E. coli*.

The vehicles described here make it possible to clone selectively and to amplify potentially any DNA fragment produced by *Sal*I or *Bam*I. Since these nucleases cleave DNA at different sequences, two adjacent fragments produced by one nuclease will be overlapped by a single fragment produced by the second. This fact should, in principle, allow one to reconstruct the order of these fragments in the chromosome. About 50,000 chimeras would be needed to map the entire *D. melanogaster* genome.

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