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Bacteriophage P1 cloning system for the isolation, amplification, and recovery of DNA fragments as large as 100 kilobase pairs

(DNA packaging/*pac* cleavage/genome mapping/gene isolation)

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ABSTRACT The development of a bacteriophage P1 cloning system capable of accepting DNA fragments as large as 100 kilobase pairs (kbp) is described. The vectors used in this system contain a P1 packaging site (*pac*) to package vector and cloned DNA into phage particles, two P1 *loxP* recombination sites to cyclize the packaged DNA once it has been injected into a strain of *Escherichia coli* containing the P1 Cre recombinase, a *kan*ʳ gene to select bacterial clones containing the cyclized DNA, a P1 plasmid replicon to stably maintain that DNA in *E. coli* at one copy per cell chromosome, and a *lac* promoter-regulated P1 lytic replicon to amplify the DNA before it is reisolated. An essential feature of the cloning system is a two-stage *in vitro* packaging reaction that packages vector DNA containing cloned inserts into phage particles that can deliver their DNA to *E. coli* with near unit efficiency. The packaging reaction can generate 10^5 clones with high molecular weight DNA inserts per μg of vector DNA. Using *Not* I fragments from *E. coli* DNA, it was shown that the system can clone 95- and 100-kbp fragments but not a 106-kbp fragment. Presumably, the combined size of the latter fragment and the vector DNA (13 kbp) exceeds the headful capacity of P1.

The recent construction of a detailed linkage map of the human genome (1) facilitates the localization of genes to regions of a chromosome corresponding in size to 1–10 megabases. Further genetic linkage is usually carried out either by genetic means, if suitable probes are available, or by Southern blot analyses using pulse-field agarose gel techniques. Finally, genes are precisely localized by cloning high molecular weight DNA inserts into yeast artificial chromosomes (YACs) in *Saccharomyces cerevisiae* (2) and by DNA "walking" and "jumping" strategies using segments of DNA cloned into cosmid vectors in *Escherichia coli* (3, 4). The major advantage of the cosmid system for cloning is that insert DNA can be packaged efficiently into phage λ particles (10^6–10^7 phage with inserts can be produced per μg of vector) that can inject their DNA into cells with near unit efficiency. However, the maximum amount of DNA that can be cloned into any one cosmid vector, 45–48 kilobase pairs (kbp), is limited by the size of the λ head. In contrast, YAC cloning vectors can accept and propagate DNA that is as large as 200–800 kbp. Disadvantages of the YAC system are (*i*) the low efficiency of transformation with vector DNA containing large inserts (≈10^3 transformants are produced per μg of vector); (*ii*) the decreasing transformation efficiency with increasing insert size (P. Heiter, personal communication); (*iii*) the need to process transformants individually prior to screening; and (*iv*) the difficulty in obtaining large amounts of insert DNA from transformed cells.

To overcome some of the shortcomings of the cosmid and yeast cloning systems and to provide an alternative system to clone large molecular weight DNAs, a bacteriophage P1 cloning system has been developed and is described here. The P1 system permits the cloning, isolation, and recovery of DNA inserts as large as 100 kbp with an efficiency that is intermediate between that of cosmids and YACs.

MATERIALS AND METHODS

Phage and Bacterial Strains. P1*cm-2* r^-m^- *c1.100 am9.16* and P1*cm-2* r^-m^- *c1.100 am10.1* are multiply mutated P1 phage that were used here to prepare extracts for *in vitro* packaging. JM109 is *recA*⁻ *hsdM*⁺ *hsdR*⁻ *lacI*^{q} (5). NS2974 is JM109 with the λ*imm*434nin5X1-cre prophage (6). The latter contains a constitutively expressed, cloned P1 *cre* recombinase gene. Strain N99 is described by Shimada *et al.* (7). NS2961 is N99 (P1*cm-2* r^-m^- *c1.100 am9.16*) and NS2962 is N99 (P1*cm-2* r^-m^- *c1.100 am10.1*).

Vectors. The construction and structure of the two P1 cloning vectors pNS358 and pNS582 are described in Fig. 1. The starting plasmid is pBS69 (8). It contains two directly repeated P1 *loxP* recombination sites flanking the pBR322 *bla* (*amp*ʳ) gene and multicopy replicon on one side, and the *S. cerevisiae LEU2* gene on the other. To construct the P1 cloning vectors the P1 packaging site (pac) (9, 10) was first inserted as a 450-bp *Xho* I/*Sal* I fragment at the unique *Xho* I site in the *amp*ʳ domain of the vector. The aminoglycoside 3′-phosphotransferase (*kan*ʳ) gene from Tn*903* (11) was then inserted as a 1.3-kbp *Acc* I fragment into the unique *Cla* I site in the *LEU2* gene. The P1 plasmid maintenance region containing plasmid replicon and partition systems (12) was then inserted as a 7.2-kbp *Kpn* I fragment at the unique *Kpn* I site. To complete the construction of pNS358, a polylinker containing unique *Bam*HI, *Xba* I, *Not* I, *Sal* I, and *Sna*BI sites was inserted at the unique *Bam*HI site. pNS358 was converted to pNS582 by inserting the P1 lytic replicon (13) as a 2-kbp *Hpa* I fragment into the unique *Sna*BI site of pNS358. The activity of that replicon is regulated by the *lac* operon promoter (13). A final vector used, pNS582*tet*14, is one in which the polylinker in pNS582 is replaced by the tetracycline resistance (*tet*) gene from pBR322. pNS582*tet*14 contains unique *Bam*HI and *Sal* I restriction sites located in *tet*.

Standard DNA Methods. Restriction enzymes, T4 DNA ligase, and calf intestinal alkaline phosphatase (CIP) were purchased from New England Biolabs and were used as specified by the vendor. Plasmid DNA was isolated by the alkaline lysis method of Birnboim and Doly (14). Restriction enzyme-digested DNAs were fractionated by standard agarose gel electrophoresis in 1× TBE buffer as described by Cohen and Sternberg (15) or in field-inversion gels in 0.5× TBE buffer. In the latter case, samples were first electrophoresed into the gel (6 × 6 in; 1 in = 2.54 cm) for 30 min at 100 V and then subjected to a switching regimen of 0.6 sec

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Abbreviations: CIP, calf intestinal alkaline phosphatase; FIGE, field-inversion gel electrophoresis; YAC, yeast artificial chromosome; IPTG, isopropyl β-D-thiogalactopyranoside.

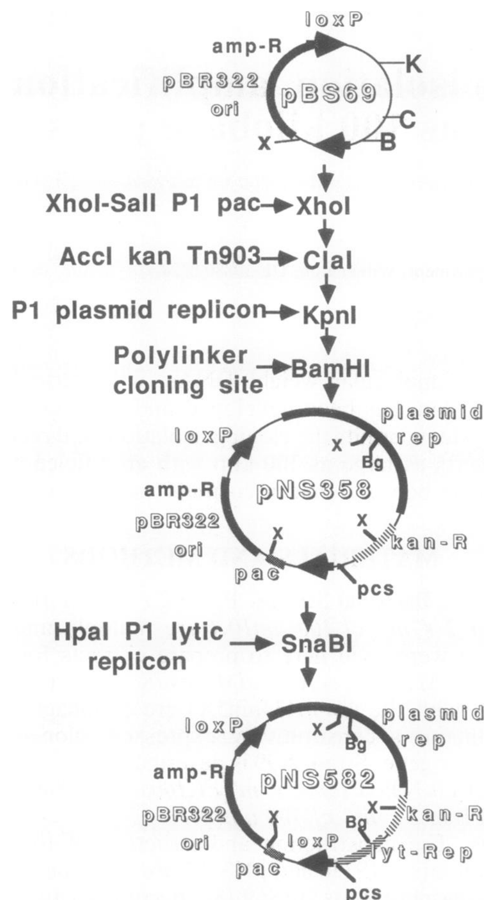

FIG. 1. The construction of P1 cloning plasmids pNS358 and pNS582. The order of steps used to construct pNS358 and pNS582 are shown. The location of restriction sites used to construct pNS358 and pNS582 is shown. The position of *Xho* I and *Bgl* II restriction sites in pNS358 and pNS582 is shown. These two restriction enzymes were used to analyze hybrids containing cloned DNA inserts (see Fig. 4). →, *loxP*; ■, pBR322 and *amp*^{r}; ■, P1 plasmid replicon; ■, *kan*^{r} gene Tn*903*; ■, *pac*; ▨, P1 lytic replicon. K, *Kpn* I; X, *Xho* I; B, *Bam*HI; C, *Cla* I; Bg, *Bgl* II.

forward/0.2 sec backward with a ramp factor of 20 at 220 V using a PC750 (Hoefer) field-inversion gel electrophoresis (FIGE) apparatus. Electrophoresis was carried out at room temperature without cooling for 2 hr.

Preparation of Pacase and Head–Tail Extracts. L broth (500 ml) was inoculated with 5 ml of an overnight culture of either NS2961 or NS2962 and the cultures were grown at 32°C to an OD_{650} of 0.6. The temperature of the cultures was then rapidly raised to 42°C and growth continued at that temperature for an additional 15 min. The temperature was then lowered to 38°C and growth continued at the lower temperature for an additional 35 min (for NS2961) or 165 min (for NS2962). The cultures were then rapidly chilled to 4°C and the cells were pelleted by centrifugation (10 min, 7000 rpm in a Sorvall GSA rotor). Cells derived from the NS2962 culture were resuspended in 1/500th vol of cold buffer containing 20 mM Tris·HCl (pH 8.0), 1 mM EDTA, 50 mM NaCl, and 1 mM phenylmethylsulfonyl fluoride, and then sonicated on ice with the medium tip of a Branson sonifier at setting 5 for four 15-sec intervals. The sonicated extract was centrifuged for 30 min at 17,000 rpm in a Sorvall SS34 rotor and the supernatant (the pacase extract) was aliquoted and stored at −80°C. This extract is stable to several rounds of freezing and thawing. The pelleted cells from the NS2961 culture were resuspended in 1/500th vol of a cold buffer consisting of 50 mM Tris·HCl (pH 8.0) and 10% sucrose. Forty-microliter aliquots were then transferred to 1.5-ml Eppendorf tubes containing 4 μl of a lysozyme solution (10 mg/ml) and the tubes were immediately frozen in liquid nitrogen. The extract in these tubes is the head–tail extract.

***In Vitro* Packaging.** This reaction is divided into two stages. In stage 1 the packaging site (*pac*) in the vector DNA is cleaved by the pacase extract. In stage 2 that DNA is packaged into phage particles.

The stage 1 reaction was performed in 15 μl containing 10 mM Tris·HCl (pH 8.0), 50 mM NaCl, 10 mM $MgCl_2$, 0.1 mM each dTTP, dGTP, dATP, and dCTP, 2 mM dithiothreitol, 1 mM ATP, *pac*-containing vector DNA, or a ligation reaction mixture containing that DNA and 0.1–0.5 μl of the pacase extract. The reaction mixture was incubated for 15 min at 30°C. In stage 2, 3 μl of a buffer containing 6 mM Tris·HCl (pH 8.0), 16 mM $MgCl_2$, 60 mM spermidine, 60 mM putrescine, 30 mM 2-mercaptoethanol, and 1 μl of 50 mM ATP were added to the stage 1 reaction mixture and the entire reaction was transferred to an Eppendorf tube containing a freshly thawed head–tail extract. The contents of the tube were very viscous at this point and were mixed by gently swirling with a pipette tip. The reaction mixture was incubated for 20 min at 30°C and the reaction was stopped by adding 120 μl of a solution of 10 mM Tris·HCl (pH 8.0), 10 mM $MgCl_2$, 0.1% gelatin, and pancreatic DNase I (10 μg/ml). The contents of the tube were Vortex mixed and then incubated for 15 min at 37°C. This constitutes the phage lysate and can be stored at 4°C for weeks without loss of phage titer.

RESULTS

P1 Cloning Vectors pNS358 and pNS582. Each of these vectors (Fig. 1) consists of two domains that are flanked by P1 *loxP* recombination sites that are oriented in the same direction. Recombination between these sites mediated by the P1 Cre recombinase separates the two domains of the vector into discrete circles (13, 16, 17). One of the domains of the vector has the pBR322 plasmid *amp*^{r} gene, the pBR322 multicopy replicon, and the P1 *pac* site. The latter was oriented so as to direct packaging counterclockwise on the vector (Fig. 1). The second vector domain contains a P1 plasmid replicon (12, 18), a *kan*^{r} gene from Tn*903*, and a polylinker cloning site containing unique *Bam*HI, *Sna*BI, *Sal* I, *Not* I, and *Xba* I sites. pNS582 differs from pNS358 in that it contains a *lac* promoter-regulated P1 lytic replicon in the *kan*^{r} domain of the vector.

An *in Vitro* P1 Packaging System. Bacteriophage P1 packages DNA by a headful mechanism. The process is initiated when P1-encoded pacase proteins recognize and cleave *pac* in the phage DNA (9, 10). The DNA on one side of that cleavage is then packaged into an empty phage prohead. Once the head has been filled, a second DNA cleavage event (the headful cut) occurs that separates the DNA inside of the head from that outside of the head. The headful cleavage is not sequence specific. Phage tails are then added to the filled heads to complete particle formation. Note that in this process *pac* is cleaved only during the initiation of packaging and not at its termination. Thus, the two ends of the packaged DNA are different.

The *in vitro* packaging reaction described in this report occurs in two stages. In the first stage, the *pac* site of the vector DNA is cleaved by a sonicated extract that lacks phage heads and tails but contains essential phage *pac* cleavage proteins—the pacase extract. That extract was prepared by induction of a P1 *cm-2 c1.100 r*$^-$*m*$^-$ *am10.1* lysogen (see *Materials and Methods*). The four mutations present in the P1 prophage are important for the production of a functional extract. The *c1.100* mutation (19) is a temperature-sensitive P1 repressor mutation that permits rapid prophage induction by shifting the temperature of an exponentially growing lysogenic culture from 32°C to 42°C. The

cm-2 mutation is a deletion of 10 kbp of phage DNA (20, 21) that causes a delay in cell lysis following prophage induction. This property is useful to produce the highly concentrated pacase extract necessary for efficient *pac* cleavage. The r^-m^- mutation (22) inactivates the P1 restriction and modification enzyme that might otherwise degrade unmodified DNA added to the pacase extract. The P1 *10.1* amber mutation blocks the production of all "late" P1 proteins, including head and tail proteins (23), but permits the production of the *pac* recognition and cleavage proteins (10). When the pacase extract was incubated with a P1 DNA fragment containing *pac*, as much as 20% of the fragment was cleaved (Fig. 2). Details of the cleavage reaction will be reported elsewhere (unpublished data).

In stage 2 of the packaging reaction the *pac*-cleaved DNA generated in stage 1 is packaged into empty phage proheads, and tails are attached to the filled heads to complete the phage assembly process. The stage 2 head–tail extract was prepared by induction of a P1 *cm-2 c1.100* r^-m^- *am9.16* lysogen (see *Materials and Methods*). The 9.16 amber mutation is located in one of three genes necessary for pacase function (9).

***In Vitro* Packaging of Vector DNA.** To assess the efficiency of the *in vitro* packaging reaction, pNS582 vector DNA was cleaved at one of the restriction sites in the polylinker, ligated to produce concatemers consisting of 3–10 vector units (Fig. 3*A*), and that DNA was then packaged *in vitro*. The phage produced generate 2×10^6 *kan*^{r} transformants per μg of vector used when the infected strain (NS2974) contains the Cre recombinase (Table 1, lines 2 and 5). If the infected strain does not contain Cre (JM109) then the yield of *kan*^{r} transformants is reduced ≈50-fold. Presumably, Cre-mediated recombination between *loxP* sites is necessary to cyclize the infecting linear DNA so that it can be faithfully maintained in the transformed cells (refs. 6, 13, and 16; Fig. 3*A*). This contention is supported by the structure of plasmids present in *kan*^{r} *amp*^{s}, *kan*^{s} *amp*^{r}, and *kan*^{r} *amp*^{r} transformants generated by the *in vitro* packaged phage (Fig. 4; see also ref. 10). The *kan*^{r} *amp*^{s} transformants contain a plasmid with only the *kan*^{r} domain of pNS582 (Fig. 4, lane 2), the *kan*^{s} *amp*^{r} transformants contain a plasmid with only the *amp*^{r} domain of pNS582 (lane 4), and the *amp*^{r} *kan*^{r} transformants contain both of these plasmids (lane 3).

For vector DNA to be packaged, its size must be large enough to fill an empty P1 head. Thus, if digested pNS582 DNA (≈16 kb) is not ligated (Table 1, line 1), if it is treated with CIP before ligase is added (lines 3 and 6), or if the ligation products are predominantly monomer or dimer circles (data not shown), then the recovery of *kan*^{r} transformants is reduced ≈200-fold (Table 1).

Cloning of High Molecular Weight DNA Fragments. To assess the ability of the P1 system to clone foreign DNA inserts, *Bam*HI-digested pNS582 DNA treated with CIP was mixed with *E. coli* DNA that was partially digested with *Bam*HI (average fragment size, 40–80 kbp). The mixture was ligated and packaged, and the lysate was then used to infect NS2974. The yield of *kan*^{r} transformants was stimulated ≈6-fold by the presence of the *E. coli* DNA in the ligation reaction mixture (Table 1, lines 6 and 7), and ≈70% of those

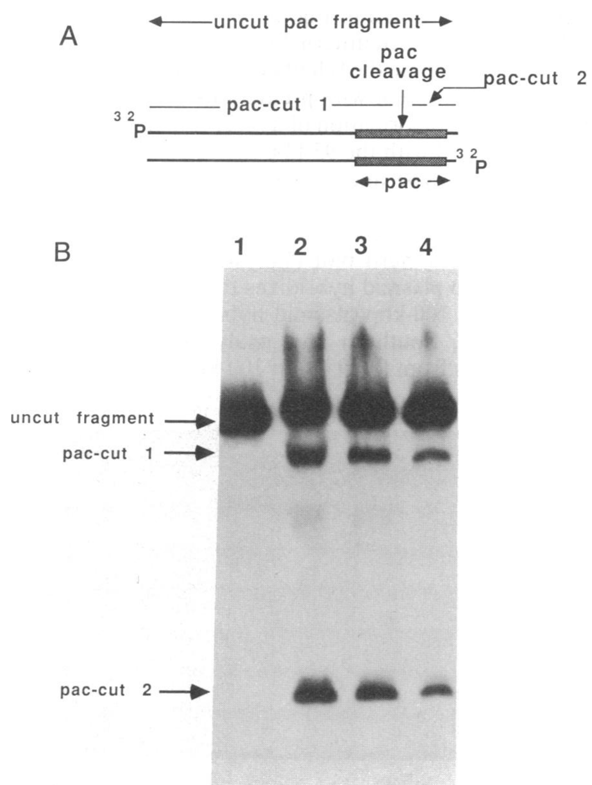

FIG. 2. The *pac* cleavage reaction. (*A*) The *pac*-containing fragment used in this assay is P1 *Eco*RI-20 (9, 10). For these studies, it was end-labeled with [γ-^{32}P]dATP and polynucleotide kinase (Boehringer-Mannheim). Cleavage of this fragment at *pac* generates two smaller end-labeled fragments. (*B*) The fragments generated by *pac* cleavage. The pacase reaction was carried out as described. The reaction was stopped by heating at 70°C for 10 min, and the samples were then applied to a 5% polyacrylamide gel. After electrophoresis for 3 hr at 150 V the gel was dried onto Whatmann 3 MM paper and exposed to Kodak XAR film. Lanes: 1, no pacase extract; 2, 1 μl of the pacase extract; 3, 0.1 μl of the pacase extract; 4, 0.01 μl of the pacase extract.

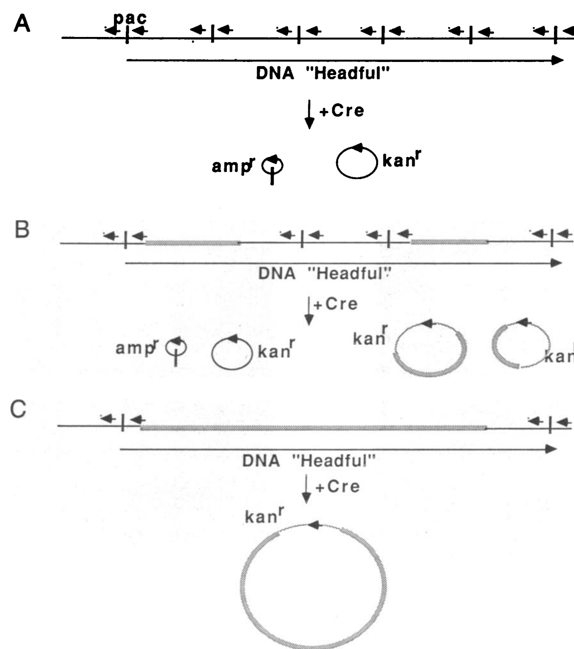

FIG. 3. Products generated by the packaging of various ligated products. |, *pac* site; ◄, *loxP* site; —, vector DNA; ▩, insert DNA; ◄|◄, *amp*^{r} domain plus *pac* site; ◄—◄, *kan*^{r} domain. (*A*) Singly digested vector DNA was ligated to form a long concatemer. Packaging of that DNA into large P1 heads followed by the infection of a Cre$^+$ strain cyclizes the DNA between *loxP* sites and produces compatible *kan*^{r} and *amp*^{r} domain plasmids. (*B*) Vector DNA was ligated to small insert DNA to generate a mixed concatemer. Headful packaging of that DNA from a *pac* site produces an *amp*^{r} domain plasmid and three *kan*^{r} plasmids. Two of those contain different, small inserts and the third does not. The *kan*^{r} plasmids are incompatible and, therefore, segregate away from each other into separate cells. (*C*) A single large insert was ligated between two vector molecules. Headful packaging of that DNA generates only a *kan*^{r} plasmid with the large insert.

Table 1. Cloning of *E. coli Not* I and *Bam*HI fragments into pNS582 DNA

Vector DNA	Insert DNA	Kanamycin-resistant colonies per μg of vector DNA: NS2974	JM109
pNS582 *Not* I	None	$<10^4$	
pNS582 *Not* I	None	2.2×10^6	4×10^4
pNS582 *Not* I CIP	None	2.0×10^4	
pNS582 *Not* I CIP	*E. coli Not* I*	8.0×10^4	2×10^3
pNS582 *Bam*HI	None	1.7×10^6	
pNS582 *Bam*HI CIP	None	1.4×10^4	
pNS582 *Bam*HI CIP	*E. coli Bam*HI†	9.6×10^4	

All of these ligation reactions except the first one were carried out in 20 μl with 400 units of T4 DNA ligase (New England Biolabs). The first reaction mixture contained no ligase. Each reaction mixture contains 1 μg of vector DNA. FIGE of the products of the reactions indicates that the reaction in line 2 produced concatemers ranging in size from 3 to 10 vector units. About 60% of kan^r transformants generated by the reaction in line 4 have inserts. About 70% of the kan^r transformants generated by the reaction in line 7 have inserts.

*E. coli DNA (3 μg) digested with *Not* I.

†*E. coli* DNA (1 μg) partially digested with *Bam*HI (40–80 kbp based on ethidium bromide staining of field-inversion gels).

transformants contained plasmids with inserted *E. coli* DNA fragments. The inserts ranged in size from 25 to 80 kbp.

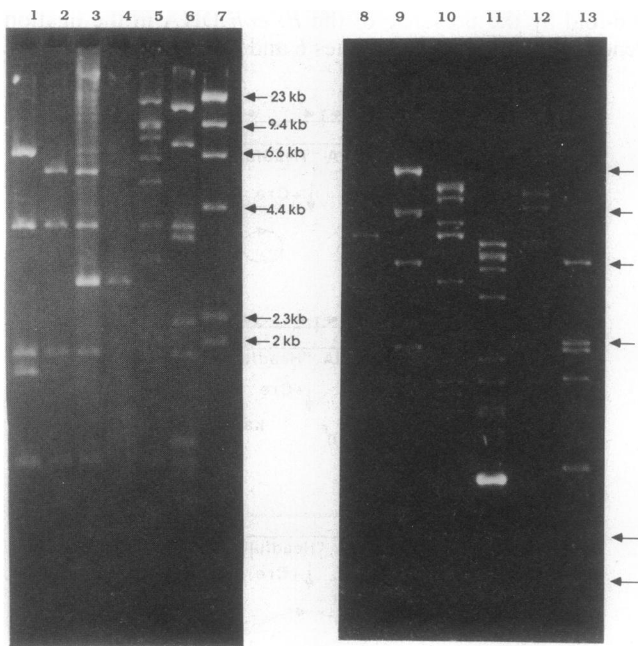

FIG. 4. Agarose gel analysis of plasmids containing cloned inserts. All plasmid DNAs were digested with *Bgl* II and *Xho* I and were analyzed on 1% agarose gels. Lanes: 1, pNS582 DNA; 2, plasmid DNA from a kan^r amp^s pNS582 transformant of NS2974; 3, plasmid DNA from a kan^r amp^r pNS582 transformant of NS2974; 4, plasmid DNA from a kan^s amp^r pNS582 transformant of NS2974; 5 and 6, plasmids containing *Bam*HI inserts of *E. coli* DNA [plasmid in lane 5 is 78–80 kbp (insert size, 65–67 kbp) and plasmid in lane 6 is 42 kbp (insert size, 29 kbp)]; 7, λ *Hin*dIII-digested DNA; 8, *Bgl* II/*Xho* I-digested pNS582*tet14* DNA; 9, λ*Hin*dIII-digested DNA; 10–12, pNS582 with *Bam*HI inserts of *E. coli* DNA [plasmid in lane 10 is 92 kbp (insert size, 77 kbp), plasmid in lane 11 is 55 kbp (insert size, 40 kbp), and plasmid in lane 12 is 84 kbp (insert size, 69 kbp)]. The sizes of the plasmids were determined by summing the sizes of the *Bgl* II/*Xho* I fragments in each lane of the gel. The intense 2.5-kbp band in lane 11 is the *Xho* I-digested amp^r domain of pNS582 *tet14*, which is also present in the clone containing the 55-kbp kan^r plasmid. Lane 13 contains additional DNA size markers. Arrows indicate the positions and sizes of the λ *Hin*dIII marker fragments.

Fragments generated by the digestion of representative insert-containing plasmids with *Bgl* II and *Xho* I are shown in Fig. 4 (lanes 5, 6, and 10–12). These DNAs contain three of the four *Bgl* II/*Xho* I fragments present in the kan^r plasmids without an insert (lanes 2 and 8) but are missing the largest vector *Bgl* II/*Xho* I fragment, which contains the polylinker cloning site. The DNA in lane 11 contains a small 2.5-kbp amp^r domain plasmid in addition to an insert-containing kan^r plasmid (see *Discussion*).

To determine the upper limit of fragment sizes that could be cloned into the P1 vector, *Not* I fragments of *E. coli* DNA were cloned into *Not* I-digested CIP-treated vector DNA and the inserts were analyzed by FIGE (24). As was the case for the cloning of *Bam*HI fragments, the presence of insert DNA in the ligation reaction mixture increased the number of kan^r transformants recovered (Table 1, lines 3 and 4), and ≈60% of those transformants had inserts. The recent *Not* I restriction map of *E. coli* (25) suggests that six or seven *Not* I fragments should be small enough to be cloned into the P1 vector. These fragments are 20, 40, 43, 43, 95, 100, and 106 kbp. The first six have been cloned with the expected efficiency (each is represented in 10–20% of the kan^r transformants containing inserts) but the last one (the 106-kbp fragment) could not be cloned. These results suggest that 113 kbp of DNA (the 100-kbp fragment plus 13 kbp of the kan^r domain of pNS582 DNA) can be packaged into a P1 head, but 119 kbp cannot. FIGE with *Not* I digests of plasmids containing both of the 43-kbp inserts, the 95-kbp insert, and the 100-kbp insert is shown in Fig. 5.

Both genetic and Southern hybridization analyses were carried out to evaluate the fidelity of the cloning process (data not shown). Consistent with predictions from the *Not* I map of *E. coli* (25) transformation of a $recA^-$ *trpE* strain of *E. coli* [NS440*recA* (26)] with the 95-kbp insert plasmid conferred a trp^+ kan^r phenotype and transformation of a $recA^-$ *nadA* strain of *E. coli* (NS523*recA* (26)] with the 100-kbp insert plasmid conferred a nad^+ kan^r phenotype. This result was confirmed by Southern blot analysis. Thus, DNA derived from the 95-kbp plasmid hybridizes to a *trpE* gene probe and DNA from the 100-kbp plasmid hybridizes to a *nadA* gene probe. Finally, Southern blot analyses of *Bgl* II/*Xho* I-digested DNAs from the 95- and 100-kbp plasmids and from

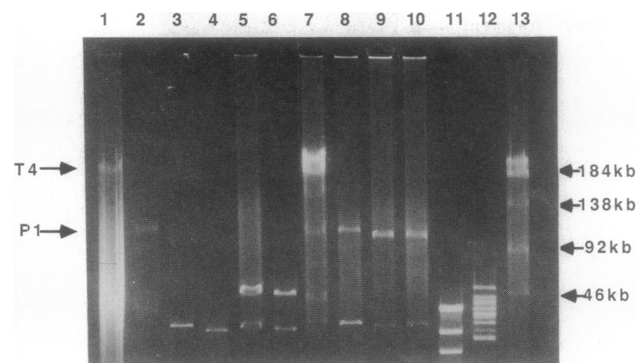

FIG. 5. FIGE of cloned *E. coli Not* I fragments. A pulse-field gel analysis of cloned *E. coli Not* I fragments. Markers used are shown in lanes 1, 2, 7, and 11–13. Lanes: 1, T4 DNA (≈160 kbp); 2, P1 DNA (≈115 kbp); 7, a *Not* I digest of *E. coli* DNA; 11, λ DNA digested with *Hin*dIII; 12, BRL high molecular weight marker (largest fragment is 49 kbp); 13, a λgt11 DNA ladder (46-, 92-, and 138-kbp markers). All other lanes contain DNA digested with *Not* I. Lanes: 3, pNS582 DNA; 4, pNS358 DNA; 5, the 43-kbp fragment cloned in pNS582 DNA; 6, the other 43-kbp fragment cloned in pNS358 DNA; 8, the 100-kbp fragment cloned in pNS582; 9, the 95-kbp fragment cloned in pNS358 prepared 100 generations after transformation; 10, the same plasmid DNA as in lane 9 prepared 200 generations after transformation.

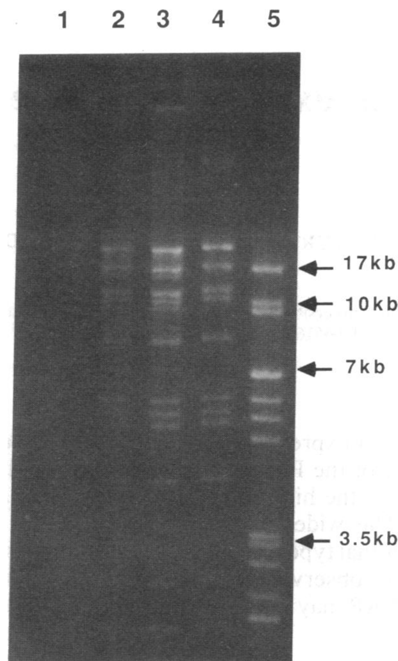

FIG. 6. Amplification of pNS582 DNA containing the 100-kbp *E. coli nadA Not* I insert. Ten milliliters of NS2974 (F′ *lacI*^{q}, *cre*$^+$) harboring this plasmid was grown to a density of 2×10^7 cells and then divided in half. One half of the culture received 1 mM IPTG and the other half did not. After an additional 6 generations of growth, plasmid DNA was isolated by the method of Birnboim and Doly (14). The growth rate of culture was the same with or without IPTG. Lanes 1, 2, and 3 contain 3%, 15%, and 65%, respectively, of the plasmid DNA from the cells grown without IPTG. Lane 4 contains 3% of the DNA isolated from the cells grown with IPTG. All DNAs were digested with *Bgl* II and *Xho* I. The minor bands in lanes 2 and 3 come from the unit copy F′ *lacI*^{q} plasmid present in NS2972. Lane 5 contains an *Eco*RI digest of P1 DNA.

E. coli DNA showed that these plasmids contain the same *E. coli* DNA fragments as does the *E. coli* genome.

Amplification of Chimeric Plasmid DNA by the P1 Lytic Replicon. The rationale for placing two replicons in the *kan*^{r} domain of pNS582 is to provide alternative replication modes for insert maintenance and insert recovery. To achieve this end, the cell line used as recipient for packaged DNA (NS2974) contains a *lacI*^{q} repressor, which renders the high copy number *lac* promoter-regulated lytic replicon in the plasmid nonfunctional. Under these conditions, the plasmid is stably maintained at one copy per cell by the P1 plasmid replicon. To recover large amounts of plasmid DNA for restriction analysis and possible subcloning, isopropyl β-D-thiogalactopyranoside (IPTG) (1 mM) was added to the culture to inactivate the *lacI*^{q} repressor before the DNA was isolated. A comparison of the relative amounts of insert-containing plasmid DNA recovered with and without IPTG (Fig. 6) indicates that cells grown for 6 generations with IPTG contain 25 times as much plasmid DNA as do cells grown without IPTG. Thus, lanes 3 (−IPTG) and 4 (+IPTG) contain nearly equal amounts of plasmid DNA despite the fact that the DNA in lane 3 is from 25 times as many cells.

DISCUSSION

We describe here a two-domain P1 cloning vector and an *in vitro* P1 packaging system that permits the cloning of fragments up to 100 kbp. The essence of the vector is a P1 packaging site (*pac*), which is needed to initiate the packaging process and two P1 *loxP* recombination sites flanking the cloned insert, which are needed to cyclize the packaged linear DNA following phage infection of cells containing the P1 Cre recombinase. Cyclization is necessary for the propagation and maintenance of the vector plasmid. In strains containing a *lacI*^{q} repressor, the P1 plasmid replicon maintains the DNA at about one copy per host chromosome. If IPTG is added to the medium the P1 lytic replicon in the vector is induced and the copy number of the chimeric plasmid increases ≈25-fold in 6 generations. Under these conditions, ≈4 μg of cloned fragment is normally recovered from 3×10^9 exponentially growing cells.

The size of the DNA that is cloned in the P1 vector is determined by the headful size of P1 (110–115 kbp of DNA) and by the amount of DNA between any two directly repeated *loxP* sites in the ligated concatemer (Fig. 3). If the inserted DNA fragment is >100 kbp, then the distance between *loxP* sites in the *kan*^{r} domain of the plasmid will be greater than a P1 headful, and the packaged DNA will not contain two *loxP* sites. When that DNA is injected into a Cre-containing host, it will not be cyclized and, therefore, will not be recoverable as a *kan*^{r} transformant. If the inserted DNA fragment is smaller than 100 kb (Fig. 3 *B* and *C*) it can be packaged between *loxP* sites and will be cyclized and recovered after injection. Moreover, as P1 packages by a headful mechanism, it is possible to package multiple vector units from a ligated concatemer, each of which may contain only a small or, in fact, no insert (Fig. 3*B*). When that DNA is injected into a Cre-containing host, each of the DNAs flanked by directly repeated *loxP* sites will be cyclized. It is clear from this discussion that to ensure the cloning of mostly high molecular weight fragments, those fragments should be size selected prior to the ligation step.

The maximum size fragment that can be cloned by the P1 vector is ≈100 kbp. Since this is more than twice the size of the fragments that can be cloned in cosmid vectors, it should be possible to use the P1 clones to walk or jump along genomic DNA at least twice as fast, and with greater fidelity, than is now possible with cosmid clones. Moreover, it should be possible to directly clone genomic copies of genes that are too big to be cloned in cosmids—genes in the 40- to 80-kbp range.

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