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Shuttle Vectors for Cloning Recombinant DNA in *Escherichia coli* and *Streptomyces griseofuscus* C581

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The replicon of the *Streptomyces* plasmid SCP2* was located on a 5.9-kilobase *Eco*RI-*Sal*I restriction fragment. The SCP2* replicon was combined with *Escherichia coli* plasmid pBR322 and genes specifying neomycin resistance and thiostrepton resistance in streptomycetes to construct shuttle vectors that are useful for cloning in *E. coli* and streptomycetes.

The streptomycete plasmid SCP2 and its mutant SCP2* (3, 4, 15) are 31-kilobase (kb) (10) sex factors (3) with one to five copies per cell (16). The SCP2 sequence can be stably cloned in *Escherichia coli* (12). SCP2* is suitable as a cloning vector in streptomycetes (2). Further developments of SCP2* into useful cloning vectors are described in this report.

(Portions of this work were presented at the annual meeting of the Indiana branch of the American Society of Microbiologists, 23 April 1983, Crawfordsville, Ind., and the Engineering Foundation Conference, 19 to 24 September 1982, Santa Barbara, Calif. [Ann. N.Y. Acad. Sci., in press].)

E. coli K-12 C600 *hsdR hsdM* (11) (designated *E. coli*) was used throughout. *Streptomyces coelicolor* M110 (5) was used to isolate plasmid SCP2* (3, 4). Methods for growth, isolation of DNA, analysis of DNA, construction of recombinant plasmids, and transformation have been described previously (8; P. R. Rosteck, Jr., and C. L. Hershberger, Gene [Amsterdam], in press). Plasmids were initially designated pJL; however, pHJL was approved as the official designation by the Plasmid Reference Center.

Streptomyces griseofuscus C581 is the preferred host for transformation and propagation of the streptomycete plasmids. It was selected because it grows rapidly, sporulates well, and is easily protoplasted and regenerated (1). Additionally, it appears to be nonrestricting (K. Cox and R. Baltz, manuscript in preparation).

A collection of chimeric plasmids was constructed with deletions in the SCP2* sequence so that the segment responsible for self-propagation in strain C581 could be identified. Two chimeric plasmids, pHJL120 and pHJL121, contained the entire sequence of SCP2*. They were either completely or partially digested with restriction enzyme *Bgl*II, *Pst*I, *Sal*I, *Sst*I, or *Xor*II, ligated under conditions that favored circularization rather than formation of recombinants (7), and transformed into *E. coli*. Eighteen isolates were retained for further investigations. Additional deleted derivatives of SCP2* were generated by shotgun cloning of *Pst*I fragments and *Bam*HI fragments into the *Pst*I site or the *Bam*HI site of pBR322 or pBR325. Restriction maps for the plasmid derivatives are summarized in Fig. 1.

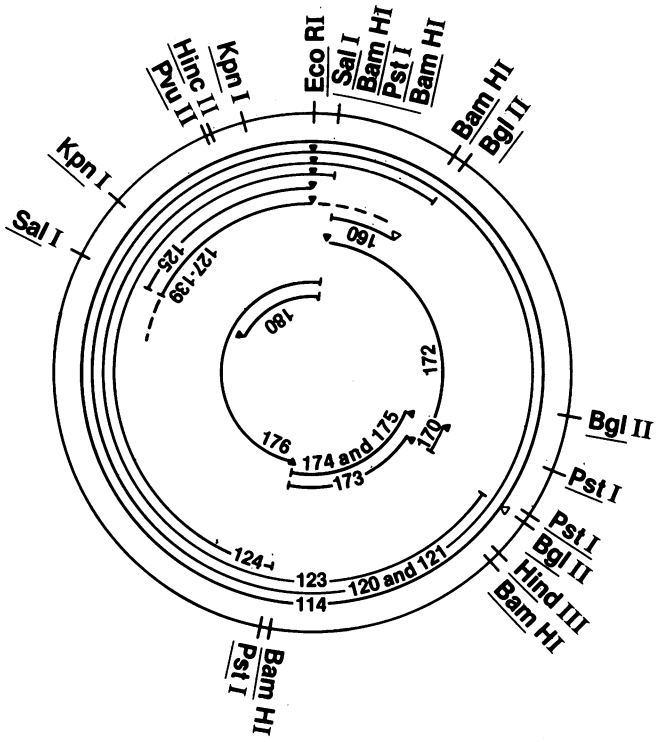

FIG. 1. Restriction maps of chimeric plasmids with deletions in SCP2*. The outer circle is the restriction map of SCP2*. △ identifies the position where pBR322 is inserted, and ▲ identifies the position where pBR325 is inserted. The plasmids containing pBR322 were generated by shotgun cloning. The plasmids containing pBR325 were generated by the deletion of pHJL120 or pHJL121 or by shotgun cloning. The arcs, extrapolated to the outer circle, define the portions of SCP2* that are present in the deleted derivatives. The dashed arcs identify a family of plasmids deleted to different *Sal*I sites. The locations of most of the *Sal*I sites have not been accurately determined on the restriction map. The *Kpn*I site at ca. 11 o'clock on the map actually represents two *Kpn*I sites. The scale of the restriction map does not allow accurate resolution of the two sites. The offset line between 12 and 1 o'clock on the restriction map shows the order of four restriction sites in SCP2* which are too closely spaced for accurate resolution on the scale of the restriction map. Their approximate position is denoted by a single line crossing the circle.

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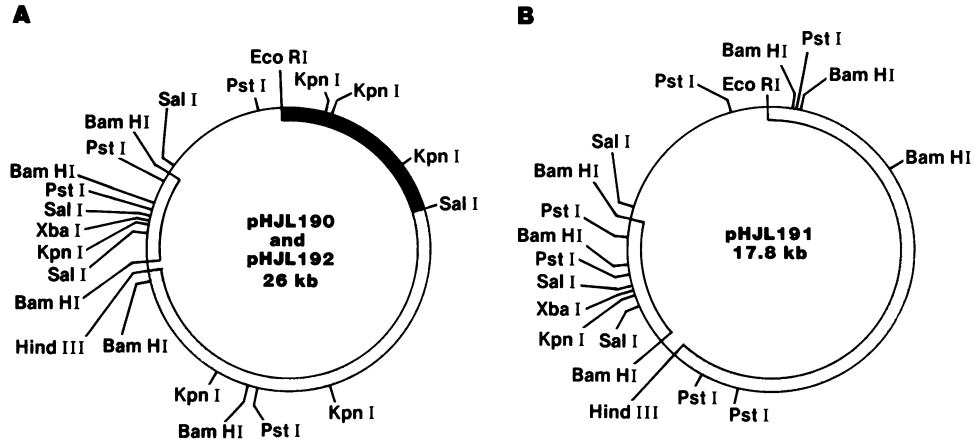

FIG. 2. Restriction maps of pHJL190, pHJL191, and pHJL192. The restriction maps of pHJL190 and pHJL192 are identical. The broad solid line denotes the 5.9-kb *Eco*RI-*Sal*I fragment of SCP2*. The broad open line denotes the fragment containing the *aph* gene that specifies neomycin resistance in streptomycetes. The broad stipled line denotes the segment of SCP2* which is not essential for replication in streptomycetes. The thin line denotes the sequence from pBR322.

All of the plasmids that contained the 5.9-kb *Eco*RI-*Sal*I fragment from SCP2* caused the formation of pocks in strain C581 and could be recovered after back transformation to *E. coli*. pHJL125 was generated by complete *Sal*I digestion of pHJL121 and was the smallest plasmid that propagated in C581 (Fig. 1). It contains only the 5.9-kb *Eco*RI-*Sal*I fragment from SCP2*. This 5.9-kb fragment is contained within the larger replicon fragments identified previously (2).

Plasmid pFJ165 (13) was used to insert neomycin resistance into derivatives of SCP2*. *Eco*RI-*Hin*dIII fragments of pFJ165 and pHJL120 were joined to construct pHJL190 and pHJL191. Both pHJL190 and pHJL191 contained the 7.7-kb *Eco*RI-*Hin*dIII fragment from pFJ165. pHJL190 contained the 19-kb *Eco*RI-*Hin*dIII fragment from pHJL120, whereas pHJL191 contained the 12-kb *Eco*RI-*Hin*dIII fragment from pHJL120 (Fig. 2). Transformation of strain C581 by pHJL190 resulted in the appearance of pocks which correlated to neomycin-resistant growth; however, pHJL191 did not yield transformants of C581 that were detectable by pocks or neomycin resistance.

Strain C581(pHJL190) grew on modified R2 medium supplemented with 1.0 μg of neomycin per ml and did not grow in Trypticase soy broth (BBL Microbiology Systems) with 1.0 μg of neomycin per ml. A spontaneous mutant with resistance to an elevated concentration of neomycin was selected by spreading C581(pHJL190) on modified R2 medium containing 10.0 μg of neomycin per ml. Also, the mutant grew in Trypticase soy broth containing 1.0 μg of neomycin per ml. All phenotypic traits of this mutant except the level of resistance to neomycin were identical to the phenotypes imparted by pHJL190. The plasmid isolated from this mutant was transformed into virgin C581 and yielded transformants that were resistant to neomycin at the elevated level of 10 μg/ml. This new plasmid, pHJL192, has the same restriction map as pHJL190 (Fig. 2).

Two plasmids were constructed to determine whether the mutation occurred in the fragment containing the *aph* gene which specifies neomycin resistance in streptomycetes (17, 18). The 5.9-kb *Eco*RI-*Sal*I fragment from pHJL125 was joined to corresponding 6.7-kb *Eco*RI-partial *Sal*I fragments containing the *aph* gene and most of the sequence of pBR322 from pHJL192 and pFJ165. Restriction maps of pHJL195 containing the same *aph* allele as pHJL190 and restriction maps of pHJL196 containing the *aph* allele from pHJL192 were identical (Fig. 3). Both pHJL195 and pHJL196 caused the formation of pocks in strain C581 which corresponded to neomycin-resistant growth on 1.0 μg of neomycin per ml. C581(pHJL196) grew on modified R2 medium containing 10 μg of neomycin per ml and in Trypticase soy broth containing 1.0 μg of neomycin per ml; however, C581(pHJL195) did not grow in Trypticase soy broth containing neomycin and did not grow on modified R2 medium with 10 μg of neomycin per ml.

The 1.0-kb *Bcl*I fragment of pFJ105 (13) containing the *tsr* gene which specifies resistance to thiostrepton in streptomycetes (17, 18) was cloned into pHJL196 which had been partially digested with *Bam*HI and separated by agarose gel electrophoresis to isolate full-length linear molecules. Two

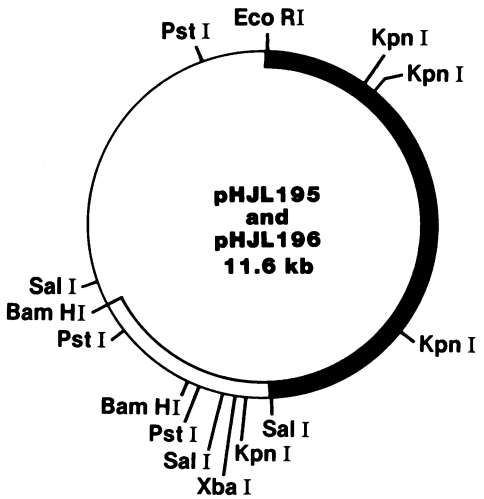

FIG. 3. Restriction maps of pHJL195 and pHJL196. The restriction maps of pHJL195 and pHJL196 are identical. The broad solid line denotes the 5.9-kb *Eco*RI-*Sal*I fragment from SCP2*. The broad open line represents the fragment containing the *aph* gene which specifies neomycin resistance in streptomycetes. The thin line represents the sequence from pBR322.

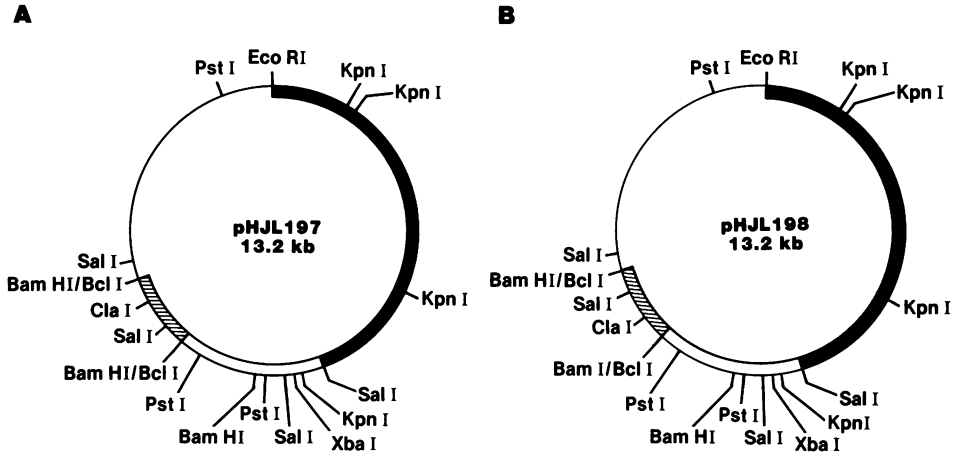

FIG. 4. Restriction maps of pHJL197 and pHJL198. The *Bcl*I fragment containing the *tsr* gene has opposite orientations in pHJL197 and pHJL198; otherwise, the restriction maps are identical. The broad solid line denotes the 5.9-kb *Eco*RI-*Sal*I fragment from SCP2*. The broad open line denotes the fragment containing the *aph* gene which specifies neomycin resistance in streptomycetes. The broad striped line denotes the fragment containing the *tsr* gene which specifies resistance to thiostrepton in streptomycetes. The thin line denotes the sequence from pBR322.

plasmids, pHJL197 and pHJL198, were isolated. Restriction maps of pHJL197 and pHJL198 were identical except that they contained reciprocal orientations of the fragment with the *tsr* gene (Fig. 4).

Comparable transformation frequencies (1.2×10^6 per μg) and cloning efficiencies (3×10^3 to 10×10^3 recombinants per μg of vector) were obtained in both *E. coli* and strain C581 when pHJL197 was isolated from either species. These shuttle vectors allow transfer of recombinant plasmids into *E. coli* from which large quantities of the DNA can be isolated easily and cloning of *Streptomyces* genes in C581 whose expression can be analyzed. These vectors can be shuttled between the hosts without any detectable deletions or rearrangements.

The parental plasmid contains a low-copy-number replicon (16). The copy numbers of pHJL197 and pHJL198 were not measured directly; however, the yields of plasmids seen by electrophoresis of whole-cell lysates and by isolation of covalently closed circular DNA indicated that the low copy number was preserved. The low copy number may be an important feature if the vectors are used to clone genes that specify synthesis of products, such as antibiotics, which may be toxic in high concentrations to the producing organisms.

pHJL197 and pHJL198 contain four single restriction sites (*Eco*RI, *Xba*I, *Bam*HI, and *Cla*I) that should be useful for inserting DNA restriction fragments. Cloning into the *Cla*I site should insertionally inactivate thiostrepton resistance (6, 9), and cloning into the *Bam*HI site insertionally inactivated neomycin resistance as was expected (6, 9). The *Bam*HI site should be particularly useful for constructing recombinant plasmids because it can accept fragments that are produced with *Bam*HI, *Bcl*I, *Bgl*II, *Mbo*I, *Sau*3A, and *Xho*II (14).

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