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Biochemical construction of specific chimeric plasmids from ColE1 DNA and unfractionated *Escherichia coli* DNA

(*Eco*RI restriction endonuclease/recombinant DNA methodology/cloning and amplification/ligase/calcium transformation)

CAROLYN J. COLLINS, DAVID A. JACKSON*, AND F. ALBERT J. DEVRIES†

Department of Microbiology, University of Michigan Medical School, Ann Arbor, Mich. 48109

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ABSTRACT **A series of chimeric plasmids was constructed using colicinigenic factor E1 (ColE1) DNA as the replicon and DNA fragments carrying the galactose or tryptophan operons from *E. coli*. Restriction endonuclease *Eco*RI digests of ColE1 DNA and various DNAs containing the *trp* or *gal* operons were joined by T4 polynucleotide ligase [polynucleotide synthetase (ATP), poly(deoxyribonucleotide):poly(deoxyribonucleotide) ligase (AMP-forming), EC 6.5.1.1]. Chimeric plasmids carrying the desired genes were selected after transformation of Trp^- or Gal^- cells with ligated DNA. By using this method, we constructed ColE1-*gal* and ColE1-*trp* chimeric plasmids in which the source of the bacterial *gal* and *trp* operons was an *unfractionated Eco*RI digest of total *E. coli* DNA. The frequency of recovery of such chimeric plasmids is 10 to 20 colonies per μg of ligated DNA used in the transformation step.**

The method utilized in this report for constructing specific chimeric plasmids from total *E. coli* DNA is very simple. It requires only endonuclease R·*Eco*RI and T4 polynucleotide ligase, both of which are commercially available. The yield of transformants suggests that this method will be useful for cloning and amplifying a wide variety of functionally defined genes from *E. coli* and other prokaryotic organisms.

The development of an experimental system for the isolation and amplification of any desired genetic region from unfractionated prokaryotic or eukaryotic DNA would be extremely useful in the study of gene organization and regulation. The restriction enzyme *Eco*RI cleaves DNA at unique sites to produce linear fragments with short complementary single-stranded ends that can be covalently joined by DNA ligase (1, 2). Chimeric plasmid molecules, in which a DNA fragment containing specific genetic sequences is joined to a self-replicating vector molecule, can be introduced into *Escherichia coli* by transformation, and the inserted DNA fragment can then be propagated as part of the functional plasmid replicon (3).

Many laboratories have reported the cloning and amplification of specific genes by the construction and propagation of such chimeric plasmid molecules (4–12). We wished to clone specific genes from complex sources of DNA, such as bacterial chromosomes, without a requirement for prior physical or genetic fractionation of the DNA. To simplify the technical aspects of the cloning as much as possible, we utilized the previously described ligation of DNA fragments at *Eco*RI termini (1, 9) to accomplish this objective. We report here the construction and propagation of chimeric plasmid DNAs containing the galactose (*gal*) or tryptophan (*trp*) operons of *E. coli* and the DNA of colicinigenic factor E1 (ColE1). The *gal* and *trp* DNAs were derived from an unfractionated *Eco*RI digest of total *E. coli* DNA. Neither the *gal* nor the *trp* operon contains an *Eco*RI site (4, 13). ColE1 or its derivative ColE1amp (14) were chosen as the plasmid molecular vehicles because of their single *Eco*RI sites, their relaxed modes of replication, in which 20 to 30 copies of the plasmid are found in each cell, and because ColE1 and its derivatives can be selectively amplified by growing cells that contain it, in the presence of chloramphenicol (15).

We also report the construction of three other species of chimeric DNA with ColE1 DNA as the vector, which include one composed of genetic elements from three different parental DNA species.

MATERIALS AND METHODS

Bacterial Strains. *E. coli* K12 strains C600*trpR*Δ*trpE5 gal1,2* (MV1) and C600Δ*trpE5gal1,2 recA* (MV12) were obtained from C. Yanofsky (4). *E. coli* JC411 thy^- (ColE1) was obtained from D. B. Clewell (15) and was used as the source of both ColE1 DNA and colicin E1. JC411(RSF2124) (14) was obtained from R. B. Helling and was used as the source of ColE1amp DNA. DB 866 (λ*dvgal* 120 clone B) has been described previously (16). *E. coli* W3110 is prototrophic.

DNA Purification. Bacterial chromosomal DNA was purified by the procedure of Marmur (17). *ϕ80ptA-E* (18) DNA was kindly supplied by E. Jackson. ColE1 [^{14}C]DNA was provided by H. Whitfield. Plasmid DNAs were prepared as follows. Purified transformed clones were grown in minimal selective media (Vogel-Bonner media plus required but unselected amino acids, vitamin B_1, and 0.4% glucose or galactose) to mid-logarithmic phase (OD_{590} = 0.6) at which time chloramphenicol (150 μg/ml) and [^3H]thymidine (10–20 μCi/ml) were added, and the cells incubated further at 37° for 4 hr. The cells were then harvested by centrifugation and a "cleared lysate" prepared as described by Clewell and Helinski (19). The lysates were centrifuged for 48–60 hr at 37,000 rpm in CsCl-ethidium bromide gradients (20) in a Beckman type 50 rotor at 15°. Covalently closed DNA was isolated, extracted with NaCl-saturated isopropanol to remove ethidium bromide, and then dialyzed extensively against 10 mM Tris·HCl at pH 8.1, 1 mM EDTA, and 10 mM NaCl (TEN).

Enzyme Reactions. Digestion of DNA using *Eco*RI (Miles) has been described (13). A portion of the enzyme used was the kind gift of K. Berkner. The reaction conditions for T4 polynucleotide ligase [polynucleotide synthetase (ATP), poly(deoxyribonucleotide):poly(deoxyribonucleotide) ligase (AMP-forming), EC 6.5.1.1] (Miles) have been described elsewhere (21, 22). Ligase incubations were for 4 hr at 16°. DNA concentrations are given in the *text* and table legends.

Transformation. MV1 or MV12 cells were transformed as described by Chang, Cohen, and Hsu (23). After exposure to DNA (1–6 μg of ligated DNA per 3×10^{10} cells in 0.4 ml of 0.05 M $CaCl_2$), the recipient cells were incubated for 90 min in L-

Abbreviations: (L_{RI}), linear DNA molecules prepared by *Eco*RI limit digestion; ColE1, colicinigenic factor E1; M_r, molecular weight.

* To whom reprint requests should be addressed.

† Present address: Laboratory for Physiological Chemistry, Sylvius Laboratories, State University of Leiden, Leiden, The Netherlands.

Table 1. Properties of the chimeric plasmids pCC1–pCC4

Chimeric plasmid DNA	Parental DNAs	Molecular weight × 10^{-6}: Sedimentation coefficient in sucrose gradient*	Molecular weight × 10^{-6}: *Eco*RI digestion and electrophoresis	Molecular weight × 10^{-6}: Electron microscopy of intact plasmid	*Eco*RI fragments	Molar ratios of *Eco*RI fragments†	Approximate colony-forming units/μg of DNA in calcium transformation
pCC1	ColE1 λ*dvgal-120B*	11.6 (36S)	11.0	11.2	4.2 6.8	1.00‡ 1.00	10^4
pCC2	ColE1 *ϕ80pt(A-E)*	20.7 (46S)	20.1	20.8	1.6 4.2 8.5	2.00 2.07 1.00‡	10^3
pCC3	ColE1 λ*dvgal-120B* *ϕ80pt(A-E)*	20.7 (46S)	21.1	21.9	1.6 4.2 6.8 8.5	1.05 1.00‡ 1.10 1.03	10^3
pCC4	ColE1 *E. coli* 3110	32.8 (56S)	31.2	31.2	4.2 6.9 20.1	1.00‡ 0.91 0.98	10^2

Plasmids pCC1–pCC4 were constructed from the indicated *Eco*RI linear fragments of parental DNAs as described in the *text*. Sedimentation coefficients of the form I plasmid DNAs were calculated from sedimentation in neutral sucrose gradients. Plasmid molecular weights were derived from the sedimentation coefficients by using the formula described by Bazaral and Helinski (26). The *Eco*RI digestion products of the plasmid DNAs are shown in Fig. 2. The molecular weight of the intact plasmid is estimated from the summation of the fragment molecular weights obtained from agarose gel electrophoresis and their molar yields. In the case of pCC4, the molecular weight estimate from *Eco*RI fragments is based on contour length measurements of the three *Eco*RI linear fragments in the electron microscope, using ColE1(L_{RI}) as an internal standard of 4.2×10^6. The molecular weight estimates calculated from electron microscopic analysis of the intact plasmids were obtained as described in *Materials and Methods*. The transforming activity of each plasmid DNA was assayed by using the calcium technique.

* Sedimentation coefficients are given in parentheses.

† Molar yield defined as 1.00.

‡ Molar ratios determined by slicing out bands from agarose gels containing *Eco*RI fragments of ^{3}H-labeled plasmid DNA, determining the radioactivity, and dividing the radioactivity by the molecular weight of the fragment.

broth to allow for expression of immunity to colicin E1 or resistance to ampicillin. Cells simultaneously transformed to colicin E1 immunity (ColE1$^+$) and Gal$^+$ or Trp$^+$ were selected by exposing the culture for 30 min to five times the amount of colicin E1 required to kill all sensitive cells and then plating on MacConkey-galactose agar (Difco) or on media lacking tryptophan. Cells transformed to ampicillin resistance (Amp^R) and Gal$^+$ or Trp$^+$ were selected by plating directly on the above media containing 50 μg/ml of ampicillin. A maximum of 10^{10} cells was added to any one plate and approximately 10% of these cells were viable.

Agarose Gel Electrophoresis. DNA was analyzed by electrophoresis in 0.6 × 15 cm cylindrical gels containing 0.7% agarose (Seakem) as described by Helling *et al.* (24).

Electron Microscopy. Plasmid DNA was mounted for electron microscopy by the aqueous method of Davis *et al.* (25). The relaxed circular form of SV40 DNA was used as an internal length standard.

Biohazard Containment. These experiments were performed in a P2 containment laboratory.

RESULTS

Construction of Chimeric Plasmids. To ascertain whether specific chimeric plasmids could be recovered from ligations of a vector DNA with *Eco*RI digests of complex DNAs, we constructed a series of four plasmids, pCC1 through pCC4, from increasingly complex sources of DNA (see Table 1). All utilized ColE1 DNA as the vector.

ColE1 DNA and the DNAs of λ*dvgal*-120B (Gal$^+$), *ϕ80ptA-E* (Trp$^+$), and *E. coli* W3110 (Gal$^+$Trp$^+$) were separately digested to completion with *Eco*RI. In the construction of plasmid pCC1 (Gal$^+$ColE1$^+$), ColE1(L_{RI}) DNA was mixed with λ*dvgal*(L_{RI}) in a 1:1 molecular ratio (13 μg/ml of total DNA), ligated, and used to transform *E. coli* MV1 [(L_{RI}) refers to linear DNA molecules prepared by *Eco*RI limit digestion.] Seventy-five Gal$^+$ColE1$^+$ transformants per μg of ligated DNA were recovered. A single colony was selected and used as the source of pCC1 DNA. Plasmid DNA prepared from three independent Gal$^+$ColE1$^+$ isolates displayed the same properties as pCC1 DNA.

Plasmid pCC2 (Trp$^+$ColE1$^+$) was constructed by using a 1:1 molecular mixture of ColE1 (L_{RI}) DNA and *ϕ80ptA-E*(L_{RI}) DNA (6 μg/ml of total DNA) to transform *E. coli* MV12. In this case, the parental *Eco*RI linear fragments were *not* ligated prior to use in transformation. Three Trp$^+$ColE1$^+$ transformants were found, and one was used to prepare pCC2 plasmid DNA.

Three parental DNA species were used in the construction of plasmid pCC3 (Gal$^+$Trp$^+$ColE1$^+$). A mixture of ColE1 (L_{RI}) DNA, λ*dvgal* (L_{RI}) DNA, and *ϕ80ptA-E*(L_{RI}) DNA (1:1:1 molar ratio, 6 μg of total DNA per ml) was ligated and used to transform *E. coli* MV12. After initial selection in broth for ColE1$^+$ cells, Trp$^+$Gal$^+$ colonies were selected on media lacking tryptophan with galactose as the carbon source. Plasmid DNAs (pCC3) prepared from two independent isolates had the same properties.

The *gal* or *trp* operons used to construct pCC1, pCC2, and pCC3 were supplied to the ligation mixture at relatively high purity as part of the DNA of specialized transducing phages or plasmids. It would be desirable if an unfractionated *Eco*RI digest of total *E. coli* DNA could serve as the source of any particular gene. It would then, in principle, be possible to clone and amplify any bacterial gene for which a sufficiently strong selection was available. If *Eco*RI sites are randomly distributed in *E. coli* DNA, then the DNA will be cut by *Eco*RI about once

Table 2. Frequency of isolation of cells containing ColE1-*trp* or ColE1amp-*trp* with W3110(L_{RI}) DNA as a source of *trp* genes

Experiment	Donor DNAs*	μg of DNA†	Phenotype selected	Colonies obtained	Transformants/μg donor DNA
1	ColE1	1	ColE1$^+$,	70	18
	W3110	3	Trp$^+$		
2	ColE1	2	ColE1$^+$,	131	22
	W3110	4	Trp$^+$		
3	ColE1amp	1	Amp^R,	74	19
	W3110	3	Trp$^+$		
4	ColE1amp	2	Amp^R,	59	10
	W3110	4	Trp$^+$		

In each experiment, the total ligated DNA was added to 3×10^{10} MV12 cells in 0.4 ml of 0.05 M $CaCl_2$, and the transformation was performed as described in *Materials and Methods*. All colonies which appeared on the selective plates were retested for phenotype. About 95–97% of the colonies maintained their phenotype on retesting. All Amp^{R}Trp$^+$ colonies were also ColE1$^+$. No Trp$^+$ transformants were recovered if W3110(L_{RI}) DNA alone was used as the donor DNA.

* All donor DNAs were *Eco*RI limit digests.

† Volume of each ligation mixture was 100 μl.

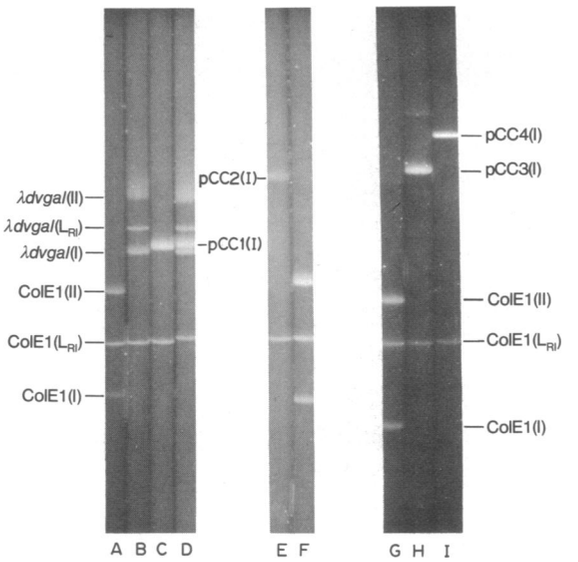

FIG. 1. Agarose gel electrophoresis of intact chimeric plasmid DNAs. All gels contained ColE1(L_{RI}) as an internal marker. Migration was from top to bottom. Electrophoresis was performed at 100 V for 4 hr. The three groups of gels shown here (A–D, E–F, and G–I) were run separately. (A) ColE1; (B) λ*dvgal*-120B; (C) pCC1; (D) λ*dvgal* + pCC1; (E) pCC2; (F) ColE1; (G) ColE1; (H) pCC3; (I) pCC4.

every 4100 base pairs, which will generate roughly 1000 fragments. The *gal* operon will therefore be found only on one out of about every 1000 fragments in an *Eco*RI limit digest of total *E. coli* DNA. Thus, in order to use an *Eco*RI limit digest of *E. coli* DNA as the source of the *gal* operon, one must be able to link a plasmid vector molecule to a fragment that represents approximately 0.1% of the DNA fragments in the limit digest at a high enough frequency so that at least one cell containing the desired chimeric plasmid can be isolated after transformation.

To test the feasibility of utilizing total *E. coli* DNA as the source of specific genes, we attempted to construct a ColE1-*gal* plasmid where the source of the *gal* operon was an *Eco*RI limit digest of W3110 (Gal$^+$) DNA. One μg of ColE1(L_{RI}) was mixed and ligated with 3 μg of *E. coli* W3110(L_{RI}) (Gal$^+$) DNA in a volume of 100 μl and used to transform 10^{10} MV12 (Gal$^-$) cells. A single Gal$^+$ColE1$^+$ colony was isolated and used as the source of pCC4 DNA.

Frequency of Isolation of Specific Chimeric Plasmids. A series of four experiments was performed to determine (*a*) the frequency of recovery of a specific marker from an *Eco*RI limit digest of *E. coli* DNA, (*b*) whether a marker other than *gal* could be recovered, and (*c*) whether a vector other than ColE1 could be employed (see Table 2). In these experiments, the yield of ColE1$^+$Trp$^+$ or ColE1$^+$Trp$^+$Amp^R colonies after transformation of MV12 with ligated mixtures of W3110(L_{RI}) DNA and ColE1(L_{RI}) or ColE1amp(L_{RI}) DNA was measured. As can be seen, in four separate experiments between 10 and 20 colonies with the desired phenotype were isolated for each μg of ligated DNA used in the transformation. These frequencies are probably over-estimates of the number of colonies of independent origin since the cells must be grown under nonselective conditions for 90 min post-transformation. Nonetheless, it is clear that it is routinely possible to isolate significant numbers of colonies containing a chimeric plasmid carrying a specific gene when the source of that gene is a pool of DNA fragments representing an entire bacterial genome.

Characterization of Chimeric Plasmids. Covalently-closed circular plasmid DNA$^+$ (form I) was isolated from pure clones containing plasmids pCC1, pCC2, pCC3, or pCC4 as described in *Materials and Methods*. No covalently closed DNA was seen in cleared lysate preparations made from control recipient cells or from Gal$^-$ or Trp$^-$ segregants. Molecular weights for these DNAs were determined by sedimentation in neutral sucrose gradients employing ColE1 [^{14}C]DNA as an internal marker, by electrophoresis in 0.7% agarose gels, and by electron microscopy. The results of these analyses are summarized in Table 1. As expected, each of the chimeric plasmid DNAs is larger than the ColE1 replicon, corresponding to the addition of from 6.8×10^6 (pCC1) to 27×10^6 (pCC4) daltons of DNA to the ColE1 vector molecule.

Electrophoretic Analysis of *Eco*RI Fragments of Chimeric Plasmids. Each chimeric plasmid DNA and its *Eco*RI cleavage products were analyzed by electrophoresis in 0.7% agarose gels. Fig 1 (gel C) shows the migration pattern obtained from pCC1 DNA. When pCC1 DNA is co-electrophoresed with λ*dvgal*-120B DNA [molecular weight (M_r) = 10.8×10^6] it can be seen (Fig. 1, gel D) that pCC1(I) DNA migrates slightly more slowly than the λ*dvgal*-120B DNA, confirming the 11×10^6 molecular weight estimate for pCC1 DNA determined from sucrose gradient and electron microscopic measurements. *Eco*RI digestion of pCC1 DNA yields two linear fragments (Fig. 2, gel C). One corresponds to ColE1(L_{RI}) ($M_r = 4.2 \times 10^6$) and the other to λ*dvgal*(L_{RI}) ($M_r = 6.8 \times 10^6$).

Plasmid pCC2 (I) DNA migrates on agarose gels as shown in Fig. 1 (gel E). *Eco*RI cleavage of pCC2 DNA yields three size classes of linear fragents (Fig. 2, gel E). One class corresponds to ColE1 (L_{RI}), and the other two correspond to the *ϕ80ptA-E* linear fragments of 1.6×10^6 and 8.5×10^6 daltons (4). The 8.5×10^6 dalton *ϕ80ptA-E* fragment carries the tryptophan operon (24). The 1.6×10^6 dalton *ϕ80ptA-E* fragment that co-clones with the *trp* operon fragment was previously found by Hershfield *et al.* (4) in pVH5, a chimeric plasmid which also carries the *trp* operon and ColE1. Hershfield *et al.* have suggested that the 1.6×10^6 fragment is required for the viability of a plasmid containing the 8.5×10^6 fragment. In contrast to pCC2, pVH5 has a M_r of 14.3×10^6. It is clear from the sucrose gradient and electron microscopic analysis that pCC2 is much

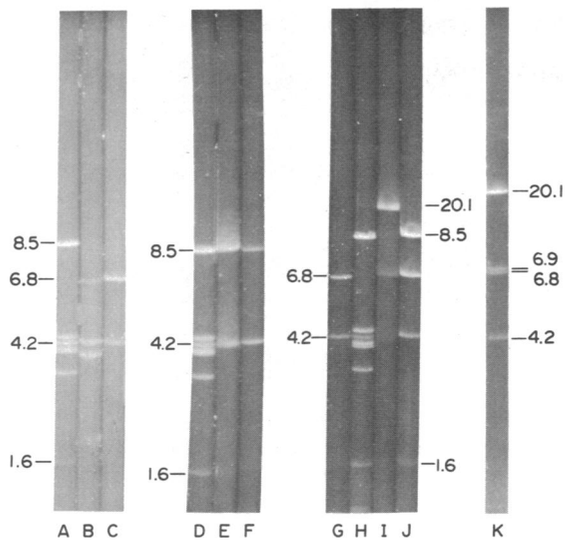

FIG. 2. Agarose gel electrophoresis of the *Eco*RI fragments of pCC1 – pCC4. Electrophoresis conditions were as in Fig. 1. The four groups of gels shown (A–C, D–F, G–J, and K) were run separately. *Eco*RI limit digests of the following DNAs were run. (A) *ϕ80ptA-E* (six fragments are shown here, six smaller fragments have migrated off the gel) + ColE1; (B) λ*dvgal*-120B (two fragments, 6.8×10^6 and 4.0×10^6 M_r) + ColE1; (C) pCC1; (D) *ϕ80ptA-E* + ColE1; (E) pCC2; (F) pCC2 + ColE1; (G) pCC1; (H) *ϕ80ptA-E* + ColE1; (I) pCC4; (J) pCC3; (K) pCC4 + λ*dvgal* (6.8×10^6 fragment only) + ColE1.

larger than pVH5. Accordingly, the molar yield of each *Eco*RI fragment derived from pCC2 was determined by slicing out and measuring the radioactivity of individual *Eco*RI fragments from agarose gels. It was found that pCC2 DNA has two copies each of the 1.6×10^6 *ϕ80* fragment and the 4.2×10^6 ColE1 fragment, and one copy of the 8.5×10^6 *ϕ80* fragment per 21×10^6 pCC2 molecule (see Table 1). The sum of the fragment molecular weights when they are present in 2:2:1 ratio is 20.1×10^6 which agrees well with the molecular weight determined for intact pCC2.

Agarose gel analysis of plasmid pCC3 DNA revealed that the form I species of this DNA is slightly larger than pCC2(I) (Fig. 1, gel H). Digestion of pCC3 DNA with *Eco*RI yields four linear DNA fragments (Fig. 2, gel J). One fragment corresponds, as expected, to ColE1(L_{RI}), two fragments correspond to the 8.5×10^6 and the 1.6×10^6 *ϕ80ptA-E* fragments seen in the pCC2 DNA, and the fourth fragment corresponds to the 6.8×10^6 λ*dvgal*(L_{RI}) fragment. The sum of the molecular weights of the four *Eco*RI fragments gives a plasmid molecular weight for pCC3 DNA of about 21×10^6. Molar ratio analysis confirmed that the four *Eco*RI fragments of pCC3 DNA are present in equimolar amounts.

Plasmid pCC4(I) DNA, constructed from ColE1(L_{RI}) and unfractionated *E. coli* W3110(L_{RI}) DNA, migrates on agarose gels as a single high-molecular-weight species (Fig. 1, gel I). Complete digestion of pCC4 DNA with *Eco*RI produced three fragments (Fig. 2, gel I). The fragment corresponding to ColE1(L_{RI}) was obtained as well as fragments of 6.9×10^6 and 20×10^6. The molecular weight of the 6.9×10^6 pCC4 fragment, as well as its non-identity to the 6.8×10^6 λ*dvgal* fragment used in other experiments in this paper, was established by co-electrophoresing an *Eco*RI digest of pCC4 with the 6.8×10^6 λ*dvgal* fragment (Fig. 2, gel K). It can be seen that the λ*dvgal* (L_{RI}) fragment is clearly distinguishable from the 6.9×10^6 fragment of the pCC4 plasmid. The molecular weight of the 20×10^6 fragment was established by electron microscopy (see legend to Table 1). Molar ratio analysis of the *Eco*RI fragments of pCC4 revealed that the plasmid contains the three fragments in equimolar proportions, yielding a plasmid molecular weight of 32.1×10^6. Four different plasmid DNA preparations have been made from the original MV12(pCC4) colony after about 15, 40, 65, and 100 generations of subculturing in galactose minimal media. All four DNA preparations had the properties described for pCC4 plasmid DNA.

Table 3. Hybridization of pCC4 and pCC4 *Eco*RI fragments to λ and λ*dvgal* DNA

Challenging DNA	cpm challenging DNA	cpm and % of input cpm bound to filters		
		No DNA	λ DNA	λ*dvgal* DNA
pCC4	12,850	39 (0.3%)	68 (0.5%)	979 (7.6%)
pCC4-6.9	4,000	3 (0.1%)	7 (0.2%)	27 (0.7%)
pCC4-20.1	3,200	2 (0.1%)	10 (0.3%)	228 (7.1%)
λ*dvgal*	8,020	21 (0.3%)	6268 (78%)	7780 (97%)

Hybridization was performed at 65° for 36 hr as described by Denhardt (27). Filters contained 3 μg of λ DNA or λ*dvgal* DNA. The challenging ^{3}H-labeled DNA was sheared by sonication prior to hybridization. The pCC4-6.9 DNA preparation contained approximately 10%, by weight, of the pCC4-20.1 fragment.

Location of the *gal* Operon in pCC4. The identity of the pCC4 *Eco*RI fragment carrying the *gal* operon was established by purifying the fragments by zone sedimentation in neutral sucrose gradients and hybridizing the purified fragments to filters to which λ or λ*dvgal* DNA had been fixed (27). The results of these experiments (Table 3) confirm that pCC4 does carry the *gal* operon and demonstrate that it is located on the 20×10^6 dalton *Eco*RI fragment. There is, thus, a stretch of roughly 31,000 base pairs surrounding the *gal* operon in the *E. coli* chromosome which does not contain an *Eco*RI site.

Genetic Analysis of Hybrid DNAs. To test the biological activity of the recombinant plasmid DNAs, we transformed *E. coli* MV12 with each of the purified DNA species. All four plasmid DNAs were found to transfer the expected markers to MV12 (see Table 1). It should be noted that the efficiency of transformation appears to decrease substantially with increasing size of the chimeric plasmid DNA. The reasons for this phenomenon are currently unclear.

DISCUSSION

A number of laboratories have reported the construction of chimeric plasmid (4–6, 9–12) and bacteriophage (7, 8, 28) DNAs. Most of the chimeric DNAs containing specific, functionally defined genes have been constructed from DNAs in which those genes were present in a relatively high state of purity. Recently, however, Clarke and Carbon (12) have described a general method for isolating chimeric plasmids containing specific genes from total *E. coli* DNA. Their procedure utilizes the method first developed by Jackson *et al.* (13) and Lobban and Kaiser (29) of joining DNA molecules using single-stranded homopolymeric oligo(dA) and oligo(dT) connector sequences on the two populations of DNA molecules to be joined (12, 13).

The procedure we have described in this paper complements that described by Clarke and Carbon (12). To simplify the technical aspects of the isolation and amplification of specific, functionally defined genes from complex sources of DNA as much as possible, we decided to rely on enzymes which are both easy to use and readily available from commercial sources, as are both *Eco*RI and T4 ligase.

The procedure of Clarke and Carbon insures that vector DNA molecules can join only with nonvector DNA fragments. In principle, a disadvantage of relying on *Eco*RI-generated cohesive ends for linking DNA molecules is that any DNA molecule with an *Eco*RI terminus can interact with any other molecule with a similar terminus. Thus, vector molecules can join to each other as well as to nonvector DNA fragments. In practice, however, the yield of transformants carrying a ColE1-*trp* chimeric plasmid which we have observed after relying on joining at *Eco*RI termini compares favorably with that reported by Clarke and Carbon for the same chimeric plasmid joined by oligo(dA-dT) connectors (12).

Besides technical simplicity, another major advantage of using *Eco*RI termini in the joining reaction is that *Eco*RI sites are reformed in the ligation process which generates the chimeric plasmid. Thus, the inserted fragment can be recovered free from the vector DNA molecule after cloning and amplification by digesting the chimeric plasmid with *Eco*RI.

A potential disadvantage of the use of the *Eco*RI termini to link DNA molecules is that it will not be possible to isolate any gene containing an *Eco*RI site from a limit digest. The use of partial digests rather than limit digests or the use of any one of several other restriction enzymes with different specificities which also leaves cohesive termini may provide solutions for this problem.

Another useful property of utilizing *Eco*RI termini for linking different DNAs is that it allows construction of complex plasmids from more than two sources of DNA in a single step. Plasmid pCC3, which carries both the *gal* and the *trp* operons, was constructed from three different parental DNAs in a single reaction. The frequency with which this type of plasmid could be isolated suggests that considerably more complex plasmids could be constructed in this same manner, particularly if the genes to be selected for are relatively abundant in the total pool of DNA fragments to be ligated.

pCC2, which was constructed without ligation *in vitro*, is also a complex plasmid. It is composed of three different *Eco*RI fragments, two of which are present in two copies each. The relatively complex composition of pCC2 could either be the result of chance interactions among five separate *Eco*RI fragments, or it could be the result of a partial duplication of an initially monomeric plasmid containing one copy of each fragment. The isolation of pCC2 demonstrates that ligation *in vitro* is not required for formation of chimeric plasmids at *Eco*RI termini, although in the absence of *in vitro* ligation, the frequency with which recombinant plasmids are recovered is several orders of magnitude lower than when ligation is performed. Cohen *et al.* (3) have previously reported formation of recombinant plasmids without *in vitro* ligation.

The ability to isolate and amplify specific genes from DNA molecules as complex as a bacterial chromosome should have many useful experimental applications. The chimeric plasmids generated can serve as convenient sources of large amounts of DNA from specific bacterial genes for use in a wide variety of experiments on gene transfer, DNA sequencing, and study of gene expression and regulation. Although the experiments reported in this paper were performed with only *Eco*RI, any other restriction enzyme which leaves sufficiently long cohesive ends should work equally well.

It should be noted that this method, which utilizes ColE1 and *E. coli*, should be useful in isolating functionally defined genes from species of bacteria other than *E. coli*. Chang and Cohen (6) have shown that fragments of DNA from Gram positive bacteria can be expressed normally in *E. coli*. It is likely that appropriate mutants of *E. coli* can be used in selecting for the expression of many genes from a wide range of prokaryotic organisms.

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