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Amplification in *Escherichia coli* of enzymes involved in genetic recombination: Construction of hybrid ColE1 plasmids carrying the structural gene for exonuclease I

(*sbcB*/plasmid maintenance/restriction endonucleases/*recB recC*)

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ABSTRACT **Endo·R·*Hin*dIII restriction endonuclease fragments obtained from F30 and pMB9 plasmid DNAs were ligated *in vitro* and used to transform a *recB21 recC22 sbcB15* strain of *E. coli* K-12. The inability of this strain to stably maintain pMB9 alone permitted the isolation of transformants that carried hybrid plasmids containing the *sbcB*$^+$ allele. These transformants became sensitive to ultraviolet light and recombination deficient, and showed a 25-fold increase in the level of exonuclease I activity.**

The stability of the *sbcB* hybrid plasmids and their effects on exonuclease I activity have also been determined in wild-type and *recA1* genetic backgrounds. The presence of the plasmids results in a 7-fold increase in the level of exonuclease I in a wild-type strain and a 15-fold increase in a *recA1* strain. The increased activity in the *recA1* mutant appears to be a result of increased plasmid stability in this genetic background.

Although the genetic analysis of chromosomal recombination in *Escherichia coli* has made tremendous strides in recent years (1, 2), with the exception of *recB recC* (exonuclease V; refs. 3 and 4) and *sbcB* (exonuclease I; refs. 5 and 6) the majority of gene products have eluded identification and purification. Even in the case of exonuclease I (7) and exonuclease V (5), poor recoveries upon purification have prevented obtaining large quantities of homogeneous enzyme. The inability to detect other enzymes may result from their presence within the cell in extremely small amounts. Amplification of the gene products involved in genetic recombination would thus provide a means of circumventing many of these problems.

Recently, Cameron *et al.* reported the isolation of a bacteriophage λ carrying the gene for *E. coli* DNA ligase (8). In addition, by using the autonomously replicating plasmid ColE1 as a vehicle (9) and sheared *E. coli* chromosomal DNA, Clarke and Carbon have successfully cloned the *trp*, *ara*, and *leu* operons (10). In this communication we describe the amplification of a gene involved in genetic recombination (*sbcB*, exonuclease I) by employing restriction endonuclease *Hin*dIII, ColE1, and F plasmid DNA carrying the desired sequence.

MATERIALS AND METHODS

Materials. Reagents were obtained from the following sources: CsCl (high purity), Penn Rare Metals; Sarkosyl NL-97, Geigy; lysozyme, Worthington Biochemical Corp.; "Ultra Pure" $(NH_4)_2SO_4$, Schwarz/Mann; carrier-free $H_3{}^{32}PO_4$, New England Nuclear; agarose, Seakem; bovine serum albumin, Miles Research Laboratories; ethidium bromide, Calbiochem; calf thymus DNA, chloramphenicol, tetracycline, and bromphenol blue, Sigma Biochemical Co. Phage T_4 DNA ligase was purified by the method of Weiss *et al.* (11). Endo·R·*Hin*dIII was obtained from either Miles Research Laboratories or Bethesda Research Laboratories. Endo·R·*Eco*RI was purified by the method of Green *et al.* (12). One unit of either enzyme is defined as that amount required to completely digest 1 μg of λ DNA in 1 hr at 37°. All other chemicals were of reagent grade. Bacteriophage λ DNA was graciously supplied by R. Mural.

Bacterial Strains and Plasmids. The relevent genotypes and origins of the plasmids and bacterial strains are listed in Tables 1 and 2. Bacterial nomenclature conforms to the suggestions of Demerec *et al.* (13) and Bachmann *et al.* (14). A minus sign is used to designate a general mutant allele.

Methods. The procedures and media for conjugational crosses and for the discrimination of recombination-defective (Rec$^-$) from recombination-proficient (Rec$^+$) strains have been described previously (16, 17). Cells were grown in Luria (L) broth (18), K medium (19), or LT broth (Luria broth with 20 μg/ml of tetracycline). For solid media 2% agar was added. For UV sensitivity tests plates received approximately 1000 ergs/mm^2 from a GE germicidal lamp. Quantitative UV survival analysis was carried out as described by Kushner (18). Doses were determined with a GE germicidal ultraviolet intensity meter no. 16569.

^{32}P-Labeled *E. coli* B DNA was prepared as previously described (20). Exonuclease I activity was measured at pH 9.2 as outlined by Kushner *et al.* (5). One unit of exonuclease I is defined as that amount of protein that catalyzes the release of 1.0 nmol of nucleotide in 30 min at 37°. All enzyme assays were carried out in crude lysates which were prepared by the method of Wickner *et al.* (21). Colicin E1 and colicin E2 were purified by the method of Herschman and Helinski (22). The method of Lowry *et al.* was used to determine protein concentrations (23).

Bacterial Transformation. Recipient strains were transformed by the technique of Cohen *et al.* (24) with the exception that strains to be transformed were grown in L broth to a density of 1×10^8 cells per ml and DNA uptake was allowed to take place during a 20 min incubation at 37°. After expression in L broth for 1.5–4 hr at 37°, the culture was spread on LT agar plates. Transformants were allowed to grow 24–48 hr prior to analysis.

Purification of Plasmid DNA. Unlabeled F$^+$ and F30 DNA were prepared from 2 liter cultures (K medium) of AB4014 and JC7653, respectively, by the method of Davis and Vapnek (25). Unlabeled pMB9 DNA was obtained from SK1163 grown in LT broth to a density of 5×10^8 cells per ml. At this point chloramphenicol was added to a final concentration of 200 μg/ml and the culture was shaken overnight at 37° to permit amplification of the plasmid DNA. The DNA was isolated by the method described above (25).

Purification of Recombinant DNA Plasmids. Cultures (20

Abbreviations: Rec, recombination phenotype; ^{R}, ^{S}, resistant and sensitive, respectively.

Table 1. Bacterial strains

Strain no.	Sex	*recB*	*recC*	*sbcB*	*recA*	*str*	*leu*	*thr*	Other	Source
AB1157	F⁻	+	+	+	+	*31*	*6*	*1*		A. J. Clark
AB4014	F′	+	+	+	+	S	+	+	F⁺, *thyA*, *drm*	D. Vapnek
C600	F⁻	+	+	+	+	S	—	—		H. Boyer
E3	F′	+	+	+	+	S	+	+	F′ *lac*	A. J. Clark
HB129*	F⁻	+	+	+	+	R	+	+	pMB9	H. Boyer
Hfr H	Hfr	+	+	+	+	S	+	+		B. Bachmann
JC411	F⁻	+	+	+	+	R	−	+	ColE1, *thyA*	W. D. Rupp
JC2921	F⁻	+	+	+	1	*31*	*6*	*1*		A. J. Clark
JC5519	F⁻	*21*	*22*	+	+	*31*	*6*	*1*		A. J. Clark
JC7623	F⁻	*21*	*22*	*15*	+	*31*	*6*	*1*		A. J. Clark
JC7653	F′	+	+	+	+	*31*	*6*	*1*	F30 *his-323*, *sbcB*⁺	A. J. Clark
JC7689	F⁻	+	+	*15*	+	*31*	*6*	*1*		A. J. Clark
SK1163	F⁻	+	+	+	+	S	—	—	pMB9	pMB9 transformant of C600
SK1188	F⁻	+	+	+	+	*31*	*6*	*1*	pMB9	This paper
SK1199	F⁻	*21*	*22*	*15*	+	*31*	*6*	*1*	pVK10	This paper
SK1404	F⁻	*21*	*22*	*15*	+	*31*	*6*	*1*	pVK14	This paper
SK1405	F⁻	*21*	*22*	*15*	+	*31*	*6*	*1*	pVK15	This paper
SK1414	F⁻	*21*	*22*	*15*	+	*31*	*6*	*1*	pVK24	This paper
SK1415	F⁻	*21*	*22*	+	+	*31*	*6*	*1*	pMB9	This paper
SK1430	F⁻	+	+	+	+	*31*	*6*	*1*	pVK10	This paper
SK1435	F⁻	*21*	*22*	*15*	+	*31*	*6*	*1*	pVK10	This paper
SK1440	F⁻	*21*	*22*	*15*	+	*31*	*6*	*1*	pVK24	This paper
SK1447	F⁻	+	+	+	1	*31*	*6*	*1*	pVK10	This paper
SK1450	F⁻	+	+	+	1	*31*	*6*	*1*	pMB9	This paper
W3110 (ColE2)	F⁻	+	+	+	+	R	+	+	ColE2, *thyA*	J. Inselburg

* *E. coli* B derivative. All other strains are *E. coli* K-12 derivatives. S and R indicate sensitive and resistant.

ml) of strains thought to contain recombinant DNA plasmids were grown to a cell density of 5×10^8 cells per ml in LT broth. Chloramphenicol was then added to a final concentration of 200 μg/ml and the cells were allowed to grow overnight with shaking at 37°. Chromosomal DNA was removed from the lysed cultures by the method of Davis and Vapnek (25). The lysates were treated with an equal volume of phenol equilibrated with 10 mM Tris·HCl, pH 8.0, to remove proteins. The aqueous phase containing the plasmid DNA was precipitated with 2 volumes of 95% ethanol and left overnight at −20°. The precipitated DNA and RNA was pelleted in a Sorvall SS-34 rotor for 10 min at 12,000 × *g*, resuspended in 0.2 ml of TE buffer (10 mM Tris·HCl, pH 8.0, 1 mM EDTA), and dialyzed overnight against 1 liter of the same buffer. Samples (20 μl) of the DNA were routinely used for Endo-R·*Hin*dIII digestion and electrophoretic analysis.

Construction of Recombinant ColE1 Plasmids. F30 DNA and pMB9 were simultaneously digested to completion with Endo-R·*Hin*dIII. The reaction mixtures (150 μl) containing 2 μg of either F30 DNA or pMB9 DNA, 6 mM Tris·HCl, pH 7.5, 50 mM NaCl, 6 mM $MgCl_2$, 20 μg of bovine serum albumin, and 8 units of Endo-R·*Hin*dIII were incubated for 1 hr at 37°. Following heating at 65° for 10 min to inactivate the enzyme, the mixture was further incubated for 5 min at 37° prior to addition of phage T_4 ligase. Ligation was performed in a final volume of 200 μl by the method of Tanaka and Weisblum (26). Transformants of JC7623 were purified and tested by replica plating for auxotrophic requirements, colicin E1 immunity, tetracycline resistance, UV sensitivity, and conjugal proficiency.

Table 2. Plasmids used

Plasmid	Genotype*	Derivation or source
F30	*sbcB*⁺ *his-323 tra*⁺	A. J. Clark (41)
pMB9	*tet cea*⁻	H. B. Boyer
F⁺	*tra*⁺	D. Vapnek (42)
pVK10	*tet cea*⁻ F30 [*sbcB*⁺]	pMB9 × F30
pVK14	*tet cea*⁻	pMB9 × pMB9
pVK15	*tet cea*⁻	pMB9 × pMB9
pVK24	*tet cea*⁻ F30 [*sbcB*⁺]	pMB9 × F30

* Nomenclature conforms to that proposed by Novick *et al.* (15) and Smith and Nathans (43).

Analysis of Recombinant Plasmid DNA. Plasmid DNAs were digested in a solution (30 μl) containing 6 mM Tris·HCl, pH 7.5, 50 mM NaCl, 6 mM $MgCl_2$, 3 μg of bovine serum albumin, and 2 units of Endo-R·*Hin*dIII. Following incubation for 1 hr at 37°, the reaction was terminated by addition of 10 μl of a solution containing 5% sodium dodecyl sulfate, 25% (wt/vol) glycerol, and 0.025% bromphenol blue. The samples were applied to the wells of a 0.8% agarose slab gel (170 mm × 150 mm × 3 mm). The gels were run overnight at 25 V in TEA buffer (40 mM Tris·HOAc, pH 8.05, 20 mM NaOAc, 2 mM EDTA, and 18 mM NaCl) (27). The gels were stained for 30 min in TEA buffer containing 1 μg/ml of ethidium bromide. DNA bands were visualized on a UV Products Blak-Ray View Box and photographed using Polaroid type 55 P/N film with a Polaroid MP-4 camera equipped with a Wratten no. 9 filter.

RESULTS

The loss of exonuclease I activity (*sbcB*⁻) in *recB*⁻ and/or *recC*⁻ (exonuclease V⁻) mutants of *E. coli* results in return of normal recombination proficiency and resistance to ultraviolet light (5). Such *recB*⁻ *recC*⁻ *sbcB*⁻ strains are phenotypically exonuclease I⁻, exonuclease V⁻, UV^R, and Rec⁺. However, because *sbcB*⁺ (exonuclease I⁺) is dominant to *sbcB*⁻ (5), the presence of a recombinant ColE1 plasmid carrying the struc-

Table 3. Transformation proficiency of strains

Strain	Genotype			Transformants/μg DNA		% Transformants maintaining plasmid*
	recB	*recC*	*sbcB*	Exp. 1	Exp. 2	
AB1157	+	+	+	54,000	1,100	100
JC7689	+	+	*15*	2,900	1,900	100
JC5519	*21*	*22*	+	18,000	870	100
JC7623	*21*	*22*	*15*	1.6	0	0

Transformation with pMB9 DNA was carried out on the strains simultaneously as described in *Materials and Methods.*

* One hundred transformants from each strain (if obtained) were transferred to Luria agar plates and allowed to grow at 37° for 24 hr. They were subsequently tested by replica plating for tetracycline resistance, colicin E1 immunity, UV sensitivity, and auxotrophic requirements.

tural gene for exonuclease I (*sbcB*$^+$) in a *recB*$^-$ *recC*$^-$ *sbcB*$^-$ genetic background should produce a phenotypically Rec$^-$, UV^S, and exonuclease I$^+$ strain.

As a control a series of isogenic strains carrying *recB21 recC22* and/or *sbcB15* were tested for transformation proficiency with a tetracycline-resistant derivative of *colE1* (pMB9). Both *recB21 recC22* and *sbcB15* mutant strains were readily transformed and stably maintained the plasmid as ascertained by their ability to retain pMB9 in the absence of tetracycline (Table 3). On the other hand, the *recB21 recC22 sbcB15* triple mutant yielded markedly fewer transformants. Those which did appear after several days of growth at 37° completely lost the plasmid after one purification in the absence of antibiotics. Similar results to those described in Table 3 have been obtained with ColE1*amp* (2124), ColE1*kan* (pML21), and ColE1. These findings are in contrast to those of Cosloy and Oishi, who found that transformation of *E. coli* with linear bacterial DNA occurred most efficiently in *recB*$^-$ *recC*$^-$ *sbcB*$^-$ strains (28).

The inability of the *recB21 recC22 sbcB15* strain to maintain ColE1 appeared to offer a unique positive selection for recombinant ColE1 plasmids that had incorporated the structural gene for exonuclease I (*sbcB*$^+$), since the triply mutant strain carrying such plasmids would be genotypically *recB21 recC22*, thereby theoretically permitting stable maintenance.

Recombinant Plasmid Formation. When a mixture of F30 (*sbcB*$^+$) and pMB9 DNA totally digested with Endo-R·*Hin*dIII was treated with phage T_4 DNA ligase and used to transform JC7623, a small number of tetracycline-resistant transformants appeared. These transformants were purified and tested by replica plating for UV sensitivity, conjugation proficiency, resistance to tetracycline, and immunity to colicin E1.

Electrophoretic Analysis of Recombinant DNA Plasmids. Plasmid DNA was isolated from several strains that appeared UV^S, Rec$^-$, colicin E1 immune, and tetracycline resistant. As shown in Fig. 1, two of the recombinant plasmids (pVK10 and pVK24) contained a sequence unique to F30, while pVK14 and pVK15 consisted entirely of pMB9 DNA. The molecular weight of the bacterial DNA was estimated at 11 to 12 × 10^6 based on an Endo-R·*Eco*RI digest of bacteriophage λ DNA (Fig. 1) (27).

Genetic Properties of Strains Carrying Recombinant Plasmids. The presence of either pVK14 (*tet cea*$^-$) or pVK10 (*tet cea*$^-$ *sbcB*$^+$) in JC7623 resulted in UV sensitivity similar to that of the *recB21 recC22* control strain as long as tetracycline was present in the Luria agar. However, strains (SK1199) containing the bacterial recombinant plasmids appeared considerably more resistant to UV light when survivors were scored on Luria agar not containing added tetracycline. pVK10 did not alter the UV sensitivity of a *recA1* strain.

recB21 recC22 sbcB15 strains carrying pVK10 or pVK24 showed a high degree of spontaneous curing (>90%) when grown for 24 hr in the absence of tetracycline. On the other hand, strains containing pVK14 or pVK15 appeared to have stabilities approaching those shown by wild-type strains of *E. coli* carrying pMB9 (<50% cured). The apparent stability of strains carrying pVK14 and pVK15 coupled with their UV sensitivity suggested that some additional chromosomal alteration had occurred.

The effect of pVK10 and pVK24 on conjugation proficiency was also tested. The presence of either plasmid resulted in over 100-fold decrease in the level of recombinant formation without affecting the ability of the strains to act as recipients for F'lac (Table 4). The presence of pVK10 had no effect on the level of recombination in a *recA1* strain (Table 4).

Enzymatic Activity Associated with Recombinant Plasmids. Since exonuclease I is highly specific for single-stranded

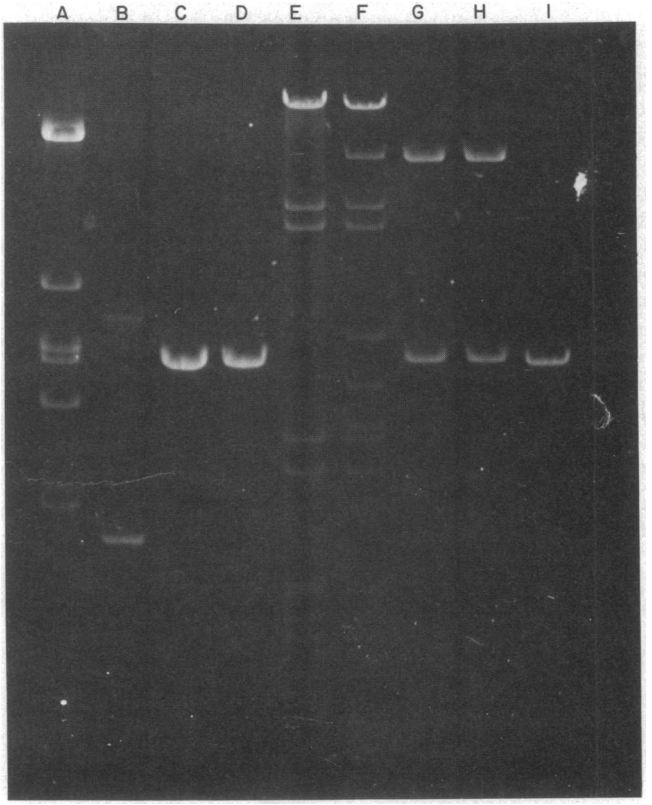

FIG. 1. Electrophoretic analysis of recombinant plasmids. Plasmid DNAs were isolated and digested with either Endo-R·*Hin*dIII (C-I) or Endo-R·*Eco*RI (A) as described in the *Materials and Methods.* (A) λ DNA digested; (B) pMB9 undigested; (C) pVK14 digested; (D) pVK15 digested; (E) F$^+$ digested; (F) F30 digested; (G) pVK10 digested; (H) pVK24 digested; (I) pMB9 digested.

Table 4. Recombination proficiency of various strains

Strain	Genotype *recB*	*recC*	*sbcB*	*recA*	Plasmid	Deficiency indices* HfrH	E3 (F'*lac*)
AB1157	+	+	+	+	—	1	1
SK1188	+	+	+	+	pMB9	1	1
JC7623	*21*	*22*	*15*	+	—	1.2	1
SK1415	*21*	*22*	+	+	pMB9	120	5
SK1199	*21*	*22*	*15*	+	pVK10	450	2
SK1414	*21*	*22*	*15*	+	pVK24	100	1
SK1447	+	+	+	*1*	pVK10	6100	—
SK1450	+	+	+	*1*	pMB9	4200	—

Matings were performed as described in *Materials and Methods*. With HfrH [threonine+ leucine+] [streptomycin-resistant] recombinants were selected, while with E3 [lactose+] [streptomycin-resistant] merozygotes were scored. Representative frequencies with AB1157 were 28% and 65%, respectively.

* Deficiency index is defined as the frequency of formation of either recombinants (HfrH) or merozygotes (E3) with a wild-type control (AB1157) divided by the frequency obtained with a particular mutant strain. Matings with HfrH represent a measure of both recombination and conjugation proficiency, while crosses with E3 (F'*lac*) serve as a control for measuring conjugation proficiency alone.

DNA (29), its presence or absence can easily be detected in crude lysates (5). The results of the exonuclease I assays are presented in Table 5. The presence of pVK14 or pVK15 (SK1404 or SK1405) did not increase nuclease activity. On the other hand, strains carrying pVK10 or pVK24 exhibited a 7-fold increase in specific activity compared to wild type against single-stranded DNA. Transformants of JC7623 obtained with purified pVK10 (SK1435) or pVK24 (SK1440) DNA showed identical increases in nuclease activity.

When pVK10 was used to transform AB1157 (wild type), 2/8 transformants tested had increased levels of activity, as represented by SK1430 (Table 5). However, 2 weeks later both transformants, although kept in frozen culture, no longer showed increased exonuclease I activity. Accordingly, pVK10 DNA was used to transform a *recA1* mutant. Not only did 6/8 transformants have increased nuclease levels, but activity was consistently almost twice as great as in any previously tested strain (Table 5). In addition, in the *recA1* genetic background the amount of enzyme did not appear to decrease over a period of several months.

Although the increased enzymatic activity was most probably directly related to the presence of the *sbcB*+ gene in pVK10 and pVK24, reaction specificity was measured on both native and denatured DNA under identical conditions. While the specific activity on denatured DNA varied from 38 to 792 for the strains tested, activity on native DNA differed by less than a factor of two. In addition, nucleolytic digestion of single-stranded DNA showed an absolute requirement for Mg^{++}.

DISCUSSION

The results presented suggest that the structural gene for exonuclease I has been isolated as part of a recombinant ColE1 plasmid. This conclusion is based on the published properties of F30 (5) and the properties of other *E. coli* exonucleases. The 3'-5' and 5'-3' exonucleases of DNA polymerase I (formerly exonuclease II and exonuclease VI, respectively) map approximately 35 min away on the chromosome (30) and degrade

Table 5. Levels of exonuclease I in transformed strains

Strain	Genotype *recB*	*recC*	*sbcB*	*recA*	Plasmid	Specific activity*
AB1157	+	+	+	+	—	105
SK1188	+	+	+	+	pMB9	108
JC7623	*21*	*22*	*15*	+	—	32
SK1404	*21*	*22*	*15*	+	pVK14	26
SK1405	*21*	*22*	*15*	+	pVK15	29
SK1199	*21*	*22*	*15*	+	pVK10	795
SK1414	*21*	*22*	*15*	+	pVK24	787
SK1435	*21*	*22*	*15*	+	pVK10	749
SK1440	*21*	*22*	*15*	+	pVK24	734
SK1430	+	+	+	+	pVK10	873
SK1450	+	+	+	*1*	pMB9	130
SK1447	+	+	+	*1*	pVK10	1535

Exonuclease I activity was measured in crude lysates as described in *Materials and Methods*.

* Units exonuclease I/mg protein.

either double-stranded DNA (5'-3') or both single- and double-stranded DNA (3'-5') (31–33). Exonucleases III and VIII show strong specificity for duplex DNA (34–36), while exonuclease V, again mapping a considerable distance away from *sbcB*, (37), has an absolute requirement for ATP (38). Exonuclease VII, which preferentially digests single-stranded DNA, does not require magnesium for nucleolytic activity (39).

The instability of *sbcB*+ recombinant plasmids in the *recB21 recC22 sbcB15* (JC7623) tester strain may result from the increased levels of exonuclease I these cells contain. JC7623 transformed with either pVK10 or pVK24 is genotypically *recB21 recC22*, placing these cells at a relative growth disadvantage compared to the parental strain, since *recB*− *recC*− strains have considerably longer generation times than Rec+ analogs (40). Thus, the increased UV resistance observed in the absence of tetracycline represented, almost exclusively, survival of cured cells. On the other hand, the disappearance of increased exonuclease I activity from AB1157 (wild type), appeared to occur through selective elimination of the bacterial sequence, since plasmid DNA isolated from SK1430 at various time intervals showed a progressive loss of the bacterial fragment as measured by agarose gel electrophoresis. The stability of pVK10 in a *recA1* host suggests that loss of the bacterial fragment in the wild-type genetic background occurred by a recombination event that eliminated not only *sbcB*+ but also the entire bacterial DNA sequence. Of interest, however, is the fact that pVK10 derivatives of *recA1* strains show an almost 15-fold increase in activity without significantly altered cell viability or growth rate.

An unexplained result concerns the difficulty of *recB*− *recC*− *sbcB*− strains to be stably transformed by ColE1 plasmids. Those transformants that were isolated (SK1404 and SK1405) appeared to contain an additional chromosomal mutation, based on the strain's genetic properties when cured of plasmid DNA (data not shown). These results suggested that perhaps enzymes involved in the "*recF*" pathway of genetic recombination played a role in the elimination of *colE1* derivatives from the cell. In fact, strains genotypically *recB21 recC22 sbcB15 recJ148* or *recB21 recC22 sbcB15 recF143* are stably transformed by ColE1 and its drug-resistant derivatives (C. L. Bassett and S. R. Kushner, unpublished results).

The use of F' DNA to clone specific *E. coli* chromosomal sequences offers several unique advantages. It provides an initial enrichment for the desired genes as well as making

electrophoretic detection of their presence in recombinant plasmids straightforward. We expect that other nucleic acid enzymes of *E. coli* can be cloned using these techniques.

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1. Clark, A. J. (1973) *Annu. Rev. Genet.* **7**, 67–86.
2. Hori, Z. I. & Clark, A. J. (1973) *J. Mol. Biol.* **80**, 327–344.
3. Goldmark, P. J. & Linn, S. (1972) *J. Biol. Chem.* **247**, 1849–1860.
4. Tomizawa, J. & Ogawa, H. (1972) *Nature New Biol.* **239**, 14–16.
5. Kushner, S. R., Nagaishi, H., Templin, A. & Clark, A. J. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 824–827.
6. Templin, A., Kushner, S. R. & Clark, A. J. (1972) *Genetics* **72**, 205–215.
7. Ray, R. K., Reuben, R., Molineux, I. & Gefter, M. (1974) *J. Biol. Chem.* **249**, 5379–5381.
8. Cameron, J. R., Panasenko, S. M., Lehman, I. R. & Davis, R. W. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3416–3420.
9. Hershfield, V., Boyer, H. W., Yanofsky, C., Lovett, M. A. & Helinski, D. R. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3455–3459.
10. Clarke, L. & Carbon, J. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4361–4365.
11. Weiss, B., Jacquemin-Sahlor, A., Livo, T. R., Fareed, G. C. & Richardson, C. C. (1968) *J. Biol. Chem.* **243**, 4543–4555.
12. Green, P. J. Betlach, M. C. & Boyer, M. W. (1974) in *DNA Replication* ed, Wickner, R. B. (Marcel Dekker, Inc., New York), pp. 87–133.
13. Demerec, M., Adelberg, E. A., Clark, A. J. & Hartman, P. E. (1966) *Genetics* **54**, 61–76.
14. Bachmann, B. J., Low, K. B., & Taylor, A. (1976) *Bacteriol. Rev.* **40**, 116–167.
15. Novick, R. P., Clowes, R. C., Cohen, S. N., Curtiss, R., Datta, N. & Falkow, S. (1976) *Bacteriol. Rev.* **40**, 168–189.
16. Clark, A. J. & Margulies, A. D.(1965) *Proc. Natl. Acad. Sci. USA* **53**, 451–459.
17. Willetts, N. S., Clark, A. J. & Low, K. B. (1969) *J. Bacteriol.* **97**, 244.
18. Kushner, S. R. (1974) *J. Bacteriol.* **120**, 1213–1218.
19. Weigle, J., Meselson, M. & Paigen, K. (1959) *J. Mol. Biol.* **1**, 379–386.
20. Kushner, S. R. (1974) *J. Bacteriol.* **120**, 1219–1222.
21. Wickner, W., Brutlag, D., Schekman, R. & Kornberg, A. (1972) *Proc. Natl. Acad. Sci. USA* **68**, 965–969.
22. Herschman, H. & Helinski, D. (1967) *J. Biol. Chem.* **242**, 5360–5368.
23. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
24. Cohen, S. N., Chang, A. C. Y. & Hsu, C. L. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2110–2114.
25. Davis, R. & Vapnek, D. (1976) *J. Bacteriol.* **125**, 1148–1155.
26. Tanaka, T. & Weisblum, B. (1975) *J. Bacteriol.* **121**, 354–362.
27. Halling, R. B., Goodman, H. M. & Boyer, H. W. (1974) *J. Virol.* **14**, 1235–1244.
28. Cosloy, S. D. & Oishi, M. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 84–87.
29. Lehman, I. R. & Nussbaum, A. L. (1964) *J. Biol. Chem.* **239**, 2628–2632.
30. Gross, J. & Gross, M. (1969) *Nature* **224**, 1166–1168.
31. Lehman, I. R. & Richardson, C. C. (1964) *J. Biol. Chem.* **239**, 233–241.
32. Setlow, P., Brutlag, D. & Kornberg, A. (1972) *J. Biol. Chem.* **247**, 224–231.
33. Brutlag, D. & Kornberg, A. (1972) *J. Biol. Chem.* **247**, 241–248.
34. Richardson, C. C. & Kornberg, A. (1964) *J. Biol. Chem.* **239**, 242–250.
35. Richardson, C. C., Lehman, I. R. & Kornberg, A. (1964) *J. Biol. Chem.* **239**, 251–258.
36. Kushner, S. R., Nagaishi, H. & Clark, A. J. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3593–3597.
37. Willetts, N. S. & Mount, D. W. (1969) *J. Bacteriol.* **100**, 923–934.
38. Goldmark, P. J. & Linn, S. (1972) *J. Biol. Chem.* **247**, 1849–1860.
39. Chase, J. W. & Richardson, C. C. (1974) *J. Biol. Chem.* **249**, 4545–4552.
40. Capaldo, F., Ramsey, G. & Barbour, S. D. (1974) *J. Bacteriol.* **118**, 242–249.
41. Bastarrachea, F. & Clark, A. J. (1968) *Genetics* **60**, 641–660.
42. Vapnek, D. & Rupp, W. D. (1970) *J. Mol. Biol.* **53**, 287–303.
43. Smith, H. O. & Nathans, D. (1973) *J. Mol. Biol.* **81**, 419–423.