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Use of Transposons in Cloning Poorly Selectable Genes of *Escherichia coli*: Cloning of *uvrA* and Adjacent Genes

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A transposon was introduced close to a poorly selectable gene. This gene could be cloned by using selection for the antibiotic resistance marker of the transposon.

A method has been developed to clone *Escherichia coli* genes for which no appropriate selection is available. The procedure involves the insertion of a transposon harboring an antibiotic resistance marker close to the gene that has to be cloned. Then antibiotic-resistant clones are selected, which might contain this gene.

The transposon can be introduced directly into the chromosome or into a multicopy plasmid containing genes which, in the chromosome, are adjacent to the gene concerned. In the latter case, described in this paper, this plasmid subsequently has to be integrated into the chromosome by homologous recombination to get the transposon close to the gene to be cloned. The advantage of using such a plasmid is that the transposon can be introduced near the wanted gene if no selection for insertion into a nearby gene is available. Moreover, because the adjacent genes are present both in the chromosome and on the plasmid, integration of the transposon into an essential gene may also occur.

The method was applied to the cloning of *uvrA*, a gene involved in excision repair of DNA (8, 13, 14), and adjacent genes. To obtain a transposon insertion close to the *uvrA* gene, we used plasmid pLC44-14 (from the Clarke-Carbon collection [4]), harboring a fragment of the *E. coli* chromosome with genes situated close to the *uvrA* gene (1, 10), and transposon Tn*1* located on RP4 (6), which contains a β-lactamase gene.

The procedure for the selection of a *uvrA* recombinant plasmid consisted of the three following experimental steps.

(i) Transposition of Tn*1* from RP4 to plasmid pLC44-14 was achieved by growing cells containing both plasmids at 30°C for 48 h in L-broth with 50 μg of ampicillin per ml. Then plasmid DNA was isolated (2) after amplification with chloramphenicol (5). The RecA strain JC1553 was transformed with the DNA preparation, and Ap^r colonies were selected. An analysis of 600 transformants revealed that 594 colonies were, in addition to Ap^r, also Tc^r and Km^r, which was indicative of the presence of RP4. The remaining six transformants exhibited an Ap^r Col^{imm} (Tc^s Km^s) phenotype and appeared to contain pLC44-14 to which Tn*1* had been transposed. Different Tn*1* insertion mutants of pLC44-14 were obtained. The plasmid with a Tn*1* insertion at the site nearest to the *uvrA* gene in the chromosome was used in the next experimental step.

(ii) Plasmid pLC44-14::Tn*1* was transferred to the PolA12 strain MM383 (M. Monk) at 32°C. Recombinants between the plasmid and chromosome were selected by growth at 40°C for 24 h in L-broth containing 50 μg of ampicillin per ml. At this temperature the plasmid cannot replicate in a PolA12 strain (9). Recombination might have yielded different end products (Fig. 1): the entire plasmid could have integrated into the chromosome by one crossover, or, alternatively, only a part of the plasmid could have been integrated by a two-crossover event. Integration of a part of the plasmid would have been possible only when Tn*1* was not located in an essential gene. After one crossover, two possible types of locations for Tn*1* could be expected, namely, close to *uvrA* (type IA) or rather distant from the *uvrA* gene (type IB). Finally, the procedure could lead also to transposition of Tn*1* to a random site in the chromosome. However, this event would have occurred only with very low frequency. To obtain the cells in which the *uvrA* gene was close to Tn*1*, phage P1 was grown on the mixture of Ap^r cells, and, subsequently, $UvrA^+$ Ap^r transductants were selected, using the UvrA strain AB1886 as a recipient. A transductant displaying the highest cotransducing frequency (88%) between Ap^r and *uvrA* was chosen for cloning the *uvrA* gene together with the β-lactamase gene.

(iii) Chromosomal DNA was isolated by the method of Miura (11) from the transductant mentioned above. A number of restriction endonucleases were used to obtain fragments,

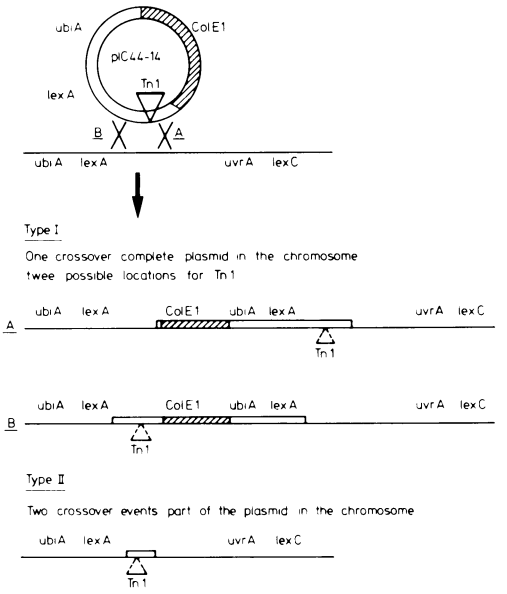

FIG. 1. *Options after recombinational events between plasmid pLC44-14::Tn1 and the E. coli chromosome. The locations of the different genes are according to Bachmann and Low (1).*

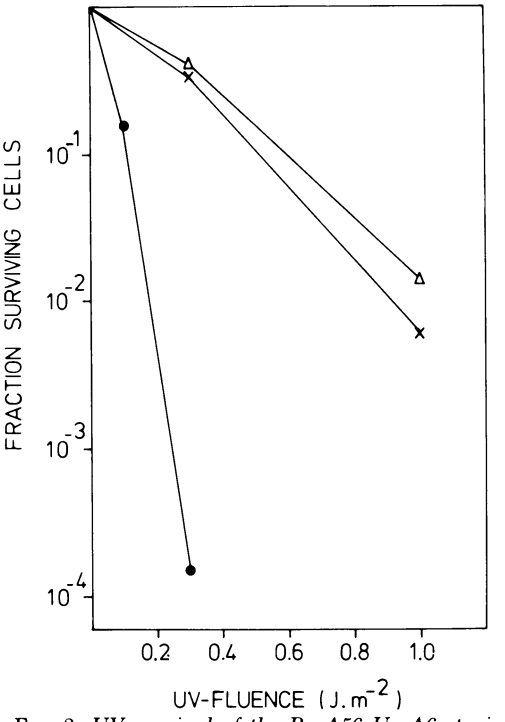

FIG. 2. *UV survival of the RecA56 UvrA6 strain CS4281 (our laboratory), containing either the uvrA plasmid pJA01* (×) *or pACYC184* (●). *For comparison, the survival of the RecA56 Uvr$^+$ strain JC5088* (△) *is included. Duplicate determinations varied less than 10%. To determine the fraction of viable cells after various UV doses, bacteria were grown in L-broth (supplemented with appropriate antibiotics) to a density of 2 × 10^8 cells per ml. Serial dilutions were plated on L-broth agar plates and incubated for 1 h at 37°C, preceding irradiation with various doses of UV light. After 16 to 20 h of incubation at 37°C, the surviving cells were counted.*

which were cloned into several plasmid vectors. These were transformed to the RecA (to prevent recombination events between the plasmid and chromosome) UvrA (to test for complementation) strain CS4281 (our laboratory). Selection was made for Ap^r colonies. Using the restriction endonuclease *SalI* and vector pACYC184 (3), 1 out of 50 Ap^r clones appeared to contain the *uvrA* gene (Fig. 2). Digestion of the recombinant plasmid, designated pJA01, with *SalI* revealed that the plasmid was the result of insertion of two *SalI* fragments into the *SalI* site of pACYC184. No complementation of *uvrB* or *uvrC* mutations by plasmid pJA01 was found. A physical map of plasmid pJA01 is presented in Fig. 3. The recombinant plasmid consisted of two *SalI* fragments, with lengths of 24.9 and 12.8 kilobases, ligated into vector pACYC184. The two *SalI* fragments are contiguous in the chromosome (unpublished data).

In a RecA UvrA background, complementation of the *uvrA* mutation by plasmid pJA01 was found up to the level of UV resistance of a RecA mutant (Fig. 2). However, due to the *recA* mutation of this strain, the complementation could be investigated only at low UV doses. To study the expression of the *uvrA* gene on pJA01 at higher UV doses also, the plasmid was transferred into a Rec$^+$ UvrA strain. It appeared that at UV doses of 10 to 60 J/m^2, the complementation of the *uvrA* mutation by plasmid pJA01 was incomplete. This might have been due to the presence of other genes on pJA01. The size of the *uvrA* plasmid suggested that besides *uvrA*, several adjacent genes might be present. From complementation studies, which will be presented elsewhere, we have concluded that pJA01 harbors the genetic information of at least four genes, i.e., *uvrA*, *lexA*, *ubiA*, and *lexC*.

In the course of this work, cloning of the *uvrA* gene was reported by Sancar and Rupp (12), who inserted a 9.4-kilobase fragment of the *E. coli* chromosome into vector pBR322. They showed that the recombinant plasmid, designated pDR2000, harbors, besides the *uvrA* gene, the *lexC* gene, whereas the *lexA* gene appeared to be absent.

The cloning method described in this paper will also allow cloning of mutant genes. By cloning *uvrA* alleles we will be able to compare the properties of *uvrA*$^+$ and mutant *uvrA* genes and

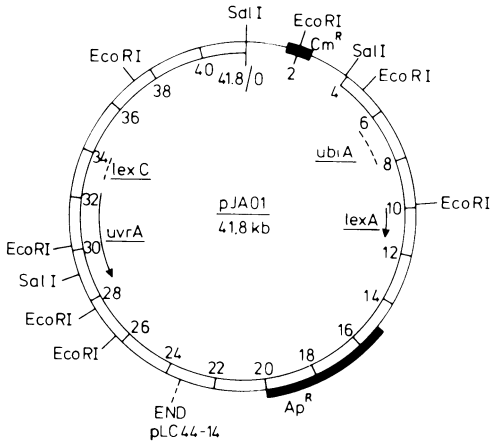

FIG. 3. *Physical map of plasmid pJA01. Incubation of DNA with restriction endonucleases was carried out for 1 h at 37°C. Enzymes were inactivated by heating the mixture for 5 min at 65°C, followed by quenching in ice. Samples to be analyzed were made 5% (wt/vol) Ficoll and submitted to electrophoresis on 0.7% agarose slab gels (7). The restriction endonucleases were obtained from Miles Laboratories, Inc. (Elkhart, Ind.). Restriction sites for the enzymes SalI and EcoRI are shown. The position of the lexA gene was taken from reference 10. The approximate locations of the other genes were derived from the E. coli K-12 map of Bachmann and Low (1). The direction of transcription of the uvrA gene is represented by the direction of the arrow (W. D. Rupp, personal communication).*

their products. Obviously, the method can be applied also for the cloning of other genes for which no simple, direct selection is available.

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