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Construction of an Hfr Strain Useful for Transferring *recA* Mutations Between *Escherichia coli* Strains

LASZLO N. CSONKA† AND ALVIN J. CLARK*

Department of Molecular Biology, University of California, Berkeley, California 94720

Strain JC10240 (Hfr PO45 *srlC300*::Tn*10* *recA56* *thr-300* *ilv-318* *rpsE300*) was constructed. On account of the close linkage of Tn*10* to *recA56*, the latter can be moved to other *Escherichia coli* (and closely related) strains in transductional or conjugational crosses selecting resistance to tetracycline.

We have constructed a strain of *Escherichia coli* K-12 which carries the transposon Tn*10* (and therefore is tetracycline resistant) inserted into the *srl* operon, in close proximity to *recA* (2). A host *E. coli* K-12 strain, AB1157 (*supE44* *thr-1* *leuB6* *proA2* *his-4* *argE3* *thi-1* *mtl-1* *ara-14* *lacY1* *galK2* *xyl-5* *rpsL31* *tsx-33*), was infected with phage λ NK55 (*b221* *cT857* cIII163::Tn*10* *0* am29) at a multiplicity of eight phage per cell, and the survivors were incubated at 42°C in the presence of tetracycline. Since NK55 cannot be maintained in the lysogenic state under these conditions, stable tetracycline-resistant survivors arise as a result of translocation of Tn*10* from the phage DNA to the bacterial chromosome (4). The recipient strain, AB1157, carrying the amber supressor *supE44* is permissive for the vegetative growth of NK55. To minimize the reinfection of tetracycline-resistant survivors, we added sodium citrate (10 mM, pH 7.0) to the growth medium to chelate Mg^{2+} ions required for phage adsorbtion.

Among 50,000 Tet^r colonies we found 4 that did not utilize sorbitol. Their growth in the presence of sorbitol indicated that they were mutant in either *srlA* or *srlC*, required for sorbitol uptake and phosphorylation, rather than in *srlD*, which determines D-glucitol-6-phosphate dehydrogenase. One of the four strains was named JC10236, and the mutation that it harbored was called *srl-300*::Tn*10*.

By P1 transduction, *srl-300*::Tn*10* was shown to be 6% linked to $cysC^+$ and 79% linked to $alaS^+$, near the site of other *srl* mutations (2). There was no detectable D-glucitol-6-phosphate dehydrogenase activity in extracts of JC10236 grown under conditions that normally induce the *srl* operon, as determined by the assay procedure of Lengeler and Lin (5). Heterozygotes carrying *srl-300*::Tn*10* on the chromosome and $srlD^+$ $srlA^+$ *srlC1* on a ColE1-derived plasmid (L. M. Csonka, A. Templin, and A. J. Clark, unpublished data) were unable to ferment D-glucitol, indicating the lack of complementation between *srl-300*::Tn*10* and the missense mutation *srlC1*. Previous deletion analysis showed that the gene order is *srlD* *srlA* *srlC*, with the direction of transcription being from *srlC* to *srlD* (3). Thus, we conclude that *srl-300*::Tn*10* is an insertion into *srlC* (or perhaps an unknown gene between *srlC* and the promoter), polar on *srlD*.

The *srlC300*::Tn*10* mutation was transduced from JC10236 into JC5088 (Hfr PO45 *recA56* *thr-300* *ilv-318* *rpsE300*), a derivative of Hfr KL16 (1) which transfers *srl* as an early marker (6). Tetracycline-resistant transductants appeared at 1% of the frequency observed with Rec^+ recipients. Of the transductants 25% which inherited *srlC300*::Tn*10* failed to inherit the $recA^+$ gene of the donor.

Two progeny were saved: JC10240, *recA56*, and JC10241, $recA^+$. With JC10240 it is possible to transfer the *recA56* mutation to a $recA^+$ recipient either by conjugation or by P1 transduction, selecting resistance to tetracycline (15 mg/liter). By either method, the linkage of *recA56* to the Tn*10* is high (>90%) and *recA56* transductants can be detected simply (3) by their extreme sensitivity to UV irradiation. To construct a *recA56* strain which is tetracycline sensitive, introduce *srlC300*::Tn*10* from JC10240 and then transduce the resultant strain to srl^+ *recA56* by a P1 lysate grown on a srl^+ $recA^+$ donor. Strain JC10240 has been used to introduce the *recA56* allele into a wide number of *E. coli* K-12 strains and even into *Klebsiella pneumoniae* (7). We have also constructed strains which carry *srlC300*::Tn*10* adjacent to a number of *recA* alleles, including the heat-sensitive *recA200* mutation, *recA430* (formerly *lexB30*), *zab-53*, and *recA441* (formerly *tif-1*).

† Present address: Plant Growth Laboratory/Department of Agronomy and Range Science, University of California at Davis, Davis, CA 95616.

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