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Rapid Mapping of Conditional and Auxotrophic Mutations in *Escherichia coli* K-12

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The approximate genetic map locations of auxotrophic and conditional lethal mutations of *Escherichia coli* can be rapidly determined with replica plating techniques. A set of patches of 15 streptomycin-sensitive (Str^S) Hfr strains with points of origin distributed around the map is replica plated onto a recombinant-selective plate with a lawn of Str^R cells which carry an unmapped mutation. The map interval defined by the Hfr points of origin which are closest to the mutant locus is seen by the presence or absence of heavy patches of recombinants produced by transfer of early wild-type genes from the Hfrs. An alternative method is to replicate patches of different mutant strains (100 per plate) onto Hfr lawns; in this case more than 1,000 different mutants can be mapped in a single experiment in a few days. In this way, many types of mutations with similar phenotypes can be grouped as to approximate location on the genetic map. For ordering mutations within groups, the same replica plating methods can be used to cross F-prime derivatives of mutants with other mutants of the same group. Relative merits of these and other mapping methods of *E. coli* are discussed.

Escherichia coli is involved in many studies of fundamental molecular biological problems largely because it is so genetically tractable. This bacterium is at present the only whole organism which lends itself to the following combination of genetic techniques: (i) efficient mutagenesis on a random basis (for a review, see reference 46) or for any desired specific region of the chromosome (1, 26; R. Celis, *personal communication*); (ii) genetic mapping at the level of the chromosome (29), the gene (7), and the codon (23, 76); (iii) dominance and complementation studies with a full range of episomal elements for the construction of stable merodiploids (8, 29, 38, 39; B. Low, *unpublished results*); (iv) directed transposition, deletion, and fusion of genetic elements (6, 46, 47); and (v) many-fold amplification of specific genes and gene products, predictably for almost any desired chromosomal region, for use in purified in vitro systems (7, 22, 27, 54, 59, 60; W.-J. Schrenk, R. Weisberg, and K. Shimada, *personal communication*).

The purpose of the material described in this paper is to allow easy and rapid mapping to be carried out on any mutants of *E. coli* K-12 for which the wild-type phenotype can be selected and the mutant prevented from growing on semi-solid growth media. In addition to simplifying the identification of various auxotrophic mutants commonly encountered, the strains and methods described herein will allow, hopefully, the isolation of rare mutants which are only obtainable after many mutants with a similar phenotype are screened. Conditional lethal mutations represent one such phenotype, and an example of the use of some of these mapping procedures is seen in the accompanying paper of Godson (20) who separated a large number of temperature-sensitive mutants defective in phospholipid synthesis into groups based on map position of the mutation and into subgroups based on recombination frequencies between mutations within a group. Godson's paper also shows results of liquid matings and complementation tests used to corroborate the preliminary mapping data. For convenience in assessing the most suitable approach to specific mapping problems, this paper concludes with a brief mention of various cognate genetic techniques which are used in present day analyses with *E. coli*.

MATERIALS AND METHODS

Strains. *E. coli* K-12 strains are listed in Table 1 which shows all their known mutant alleles. The points of origin for the Hfr strains are shown in Fig. 1 as arrowheads on the genetic map, situated between

TABLE 1. *E. coli K-12 strains*

Strain	Genotype[a]	Stability[b] of integrated F	Source or derivation
Hfr H (AB259)	Hfr *thi-1 rel-1* λ^-	S	E. A. Adelberg
Hfr 6	Hfr *metB1 rel-1 mut-2 mtl-8 mal-20* $\lambda^R\lambda^-$	U	E. McFall. Derivative of W3807 (P.H.A. Sneath)
KL 99	Hfr *thi-1 rel-1 lac-42* λ^-	U	AB259 → F^+ KL20, then treated with UV
PK 191	Hfr Δ(*proB-lac*) X 111 *sup-56* λ^-	VS	P. Kahn via E. Kort. Non-colicinogenic derivative of PK19 (31)
KL 14	Hfr *thi-1 rel-1* λ^-	VS	AB259 → F^+ KL20, then treated with UV
KL 25	Hfr *supE42* λ^-	U	W1485 from C Yanofsky, treated with UV
Ra-2	Hfr *supE42 mal-28* $\lambda^R\lambda^-$	VU	(37)
KL 209	Hfr *sup-53 malB16* $\lambda^R\lambda^-$	U	Hfr J4 (= P10) recombinant with KL224
KL 228	Hfr *thi-1 leu-6 sup-54 lacY1* or *Z4 gal-6* λ^-	U	Hfr AB313 from A. L. Taylor (66) cured of λ and crossed with JC355. Gift of E. Birge
KL 16	Hfr *thi-1 rel-1* λ^-	S	AB259 → F^+ KL20, then treated with UV
KL 983	Hfr *xyl-7 lac Y1* or *Z4 mglP1*λ^-	U	F^+ KL23 treated with UV → Hfr KL98; KL98 *thyA* × Hfr 7-4 → KL98-2, then crossed with Hfr B7
KL 96	Hfr *thi-1 rel-1* λ^-	U	AB259 → F^+ KL20, then treated with UV
KL 208	Hfr λ^-	U	Hfr B7 from P. Broda (10) recombinant with KL224.
KL 226	Hfr *rel-1 tonA22* $T2^R\lambda^-$	VS	Hfr Cavalli (K-10) from A. Garen, cured of λ
BW 113	Hfr *metB1* λ^-	S	Hfr P4X (AB247) from E. A. Adelberg (2) cured of λ. Gift of B. M. Wilkins
F15/KL 129	F15 (*thy*$^+$)/KL129 (*thyA38 aroB11 metB1 leu-6 argG6 recA1 tonA2 sup-55 gal-6 lacY1* or *Z4 xyl-7 mtl-2 malA1* $\lambda^R\lambda^-$)		F15 factor (28) from A. J. Clark strain JC5488
KLF3/KL217	KLF3 (= F103) (*his*$^+$)/KL217 (*his-1 thr-21 pyrD34 trp-1 metB1 arg-34 gal-6 recA1 nalA12* λ^-)		KLF3 factor derived from KL96 (38)
F-*lac*/KL131	F-*lac*$^+$ (=F42/KL131 (*lacY1* or *Z4 aroB11 metB1 leu-6 argG6 recA1 tonA2 sup-55 gal-6 xyl-7 mtl-2 malA1* $\lambda^R\lambda^-$)		F-*lac* factor from GY854 of R. Devoret
AB 2991	F311 (*ilvE*$^+$*D*$^+$*A*$^+$$O_A$*113*)/AB2987 (*ilvE12 argG12 his-42 recA1*)		D. E. Duggan (45)
JC 1552	F^- *metB1 leu-6 his-1 argG6 trp-31 lacY1* or *Z4 gal-6 tonA2 xyl-7 mtl-2 malA1 sup-55 str-104* $\lambda^R\lambda^-$		A. J. Clark
KL 141	F^- *pyrE41 argG6 thyA37* (*thi-1?*) *malA1 strA1 rbs-1 gadR2 gadS1 gltS-7* $\lambda^R\lambda^-$		*thyA* derivative of AT722 from A. L. Taylor
AB 1133	F^- *thi-1 thr-1 leu-6 proA2 argE3 his-4 lacY1 galK2 xyl-5 mtl-1 ara-14 str-31 sup-37* λ^-		A. L. Taylor
AB 1157	F^- same as AB1133 but *tsx-33*		E. A. Adelberg
PLS73	F^- *pls-73 thi-1 his-1 pyrD34 thyA25 str-118 glpD3 glpR*^{c}*2 phoA8*		G. N. Godson (20)
KL 231	F^-*thy-35 str-120 leuS31*		(40)

[a] Genetic symbols are as given by Taylor (65). All loci are assumed to be wild type except those listed here.

[b] Refers to relative stability *vs* reversion to F^+ upon subculture or storage at temperatures above 0 C. Any newly purified culture of Ra-2 contains a few percent F^+ cells. All the other Hfr strains listed are more stable than this. VS, Very stable; S, stable; U, unstable; VU, very unstable.

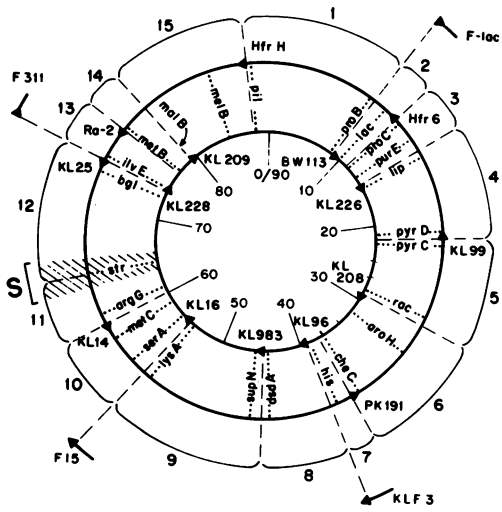

FIG. 1. *Genetic map of E. coli K-12 showing Hfr points of origin. Modified from the map of Taylor (65). The Hfr strains listed in Table 1 have points of origin shown by the arrowheads on the two heavy concentric circles. The outer brackets numbered from 1 to 15 denote map intervals which are bounded by the Hfr points of origin. The outer four short arcs indicate the chromosomal regions and parental Hfr points of origin corresponding to the four F-prime factors listed in Table 1.*

the first and last known markers transferred during mating. (For example, Hfr KL16 transfers *lysA* very early and *serA* very late.) Hfrs KL99, KL14, KL25, KL98, and KL96 were isolated from their respective F^+ strains (Table 1) as follows. An exponentially growing broth culture (2×10^8 to 3×10^8 cells/ml) was chilled to 0 C, diluted 1:10 into 56/2 buffer, and ultraviolet (UV) irradiated to 1 to 10% survival. Cells were then plated at various dilutions onto the surface of LB plates, and the colonies (200–1,000 per plate, several plates for each culture) were grown overnight at 37 C. The following day the colonies were replica-plated directly onto recombinant-selective plates which had been spread with $\sim 10^8$ cells of an auxotrophic Str^R F^- strain such as JC1552. After 1 to 2 days of incubation at 37 C, the positions of rare patches of recombinants (2–3 per 1,000 original colonies) were matched with the corresponding colonies on the original LB plates. These original clones were purified, and the resulting new Hfr strains were tested by various print-matings and interrupted matings to determine the new Hfr points of origin.

Hfr strains B7 and J4, from which KL208 and KL209 were derived (Table 1) were found to produce slow lysis of strain AB1157 when thickly grown patches were printed onto a lawn of AB1157 cells on LB Str plates (Pin-Fang Lin, *personal communication*). By using each of these strains as a recipient in 60-min interrupted matings with KL224 (a *lys*$^-$ Cavalli-type Hfr), recombinants were obtained (KL208 and KL209) which had the original B7 and J4 points of origin but no longer showed the slow lysis effect.

All strains are routinely stored by adding sterile 80% glycerol to 5-ml overnight cultures (final glycerol concentration = 30%) and keeping these cell suspensions in a freezer at −10 to −15 C. This concentration of glycerol prevents the suspensions from freezing solid, and efficient recovery of viable cells is obtained for more than 3 years.

Media. Luria broth (LB) (42) without glucose was used for matings, for routine growth of most strains, and for selection of temperature-resistant recombinants. Minimal medium 56 (48) was modified as follows: 0.037 M KH_2PO_4, 0.06 M Na_2HPO_4, 0.02% $MgSO_4 \cdot 7H_2O$, 0.2% $(NH_4)_2SO_4$, 0.001% Ca $(NO_3)_2$, 0.00005% $FeSo_4 \cdot 7H_2O$. This was used at one-half strength (denoted 56/2) and was for the selection of recombinants for nutritional markers. Sugars were used at a final concentration of 0.3%, amino acids and nucleosides were 50 μg/ml, and vitamins were 0.1 μg/ml. Sterile stocks of all these solutions (20% for sugars, 1% for others) were kept at room temperature in calibrated dropping bottles (Scientific Glass no. 583998) which obviated the need for any pipettes when preparing recombinant-selective media. Agar in plates was used at a concentration of 1.5%. Batches of agar were routinely melted in a microwave oven (Westinghouse model KM520) which requires only 2 to 5 min instead of the usual 30 min needed when steam is used.

Sterile streptomycin was prepared by adding streptomycin sulfate (Eli Lilly Co.) to sterile water (final concentration 2%) and storing this at 4 C. Streptomycin was used at a final concentration of 100 μg/ml.

Preparation of master grids. Colonies were "gridded," onto master plates in an array of 100/plate with the use of a template such as that shown in Fig. 2. Inoculation usually was carried out either with sterile toothpicks, or, more often, with a ¾ inch (ca. 1.9 cm) piece of 0.005 inch (ca. 0.013 cm) diameter 90% platinum–10% iridium wire (Sigmund Cohn, Co., Mount Vernon, N.Y.). The wire has a tiny fold at its tip for smoother operation and is held in a small needle holder (Will Scientific, no. 20371). After inoculation of each patch, the wire tip was passed through a tiny flame for quick sterilization.

Replica plating. The replica-plating method ("printing", "replicating") of Lederberg and Lederberg (34) was modified as follows. A sterile piece of black Italian velvet, which has a close nap, was held on a wooden or Lucite cylinder which is 3.25 inches (ca. 8.26 cm) in diameter, 3 inches (ca. 7.62 cm) high, and has slightly rounded edges. The metal retaining ring, or sleeve, has an inside diameter which is 5/32 inch (ca. 0.40 cm) larger than the cylinder. After replication, velvets were autoclaved but not washed, thus preserving the nap for many reuses.

Streptomycin spraying. Delayed application of streptomycin to print-mating plates was carried out with a common throat atomizer which contains sterile 2% streptomycin. This was held 1 ft (ca. 30 cm) away from a print-mating plate which was leaning against a wall, and several strong sprays were applied. The small droplets which covered the plate

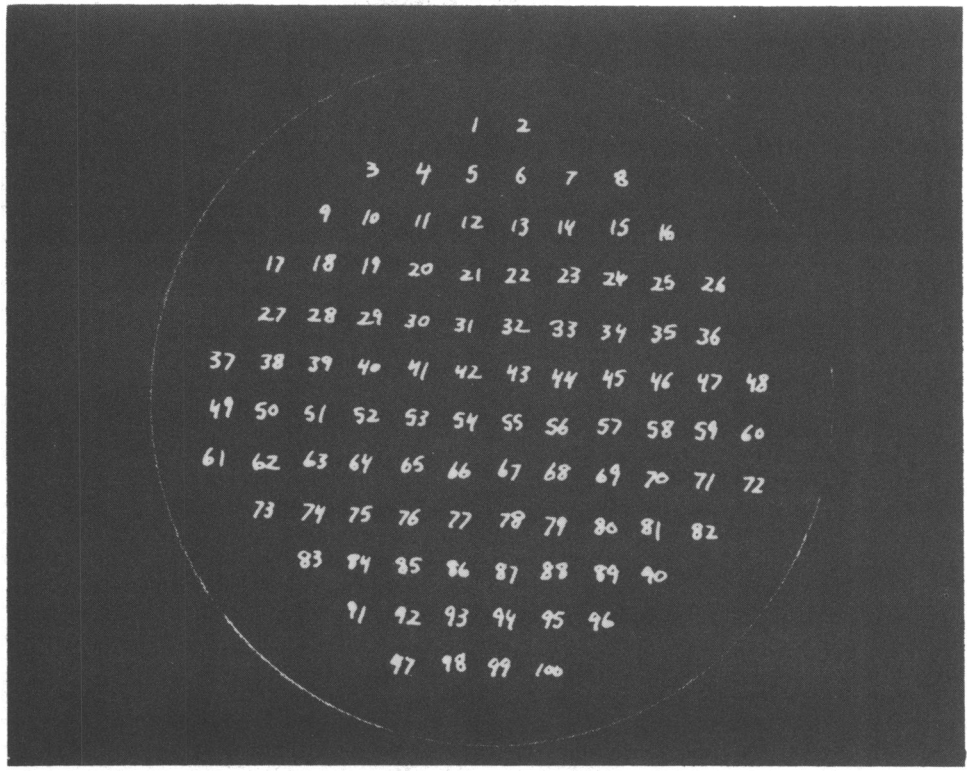

FIG. 2. *Template used for gridding. This size template may be trimmed to fit inside an inverted petri dish top which can then be used to hold new plates for gridding.*

were usually absorbed in less than 1 min. Slightly dry plates should be used to avoid smearing.

Interruption of liquid matings. A vibratory shaker (41) was used to interrupt liquid matings. A modern version of this apparatus is depicted in Miller's new volume (46), and parts can be purchased from the Cold Spring Harbor Laboratory in conjunction with the kit of strains which Miller describes.

Purification of Hfr strains. Table 1 gives an indication of which Hfr strains are the least stable, i.e, accumulate F^+ segregants with the highest rate. To obtain "pure" isolates of Hfr strains from highly reverted cultures, cells were plated out onto LB plates, and colonies were obtained after overnight growth. They were then gridded onto a fresh LB plate. With some Hfr strains, the Hfr colonies were slightly smaller than the corresponding F^+ revertant colonies. This grid was incubated at 37 C for 6 to 10 hr so that patches were visible and still growing rapidly. The patches were then replicated onto a recombinant-selective plate which had been spread with a lawn of 10^8 to 5×10^8 cells of a suitable F^- strain, such as JC1552 or KL141. The F^- strain and plating medium should be chosen so that some early marker from the Hfr is needed to form the desired recombinants e.g., *his*$^+$ when KL983 is mated with JC1552 on plates lacking histidine and containing streptomycin to prevent growth of the donor. After 1 to 1.5 days of incubation, strong patches of recombinants were produced corresponding to the Hfr clones on the original grid. Fresh overnight cultures of these strains may be kept in a freezer (see above) for long periods without significant reversion to F^+, so that good stock cultures can be readily available.

Isolation of Str^R mutants. To facilitate the isolation of Str^R mutants, which arise at a relatively low rate from Str^S strains, the following method is useful. Spread one to two drops of 2% streptomycin on only one-half of each of several LB plates. After the streptomycin solution has dried, spread a few drops of a thick, overnight culture of a Str^S strain on each plate. After 2 to 3 days of incubation, small Str^R colonies may be found, usually in the middle of the plates where the streptomycin diffuses slowly away from where it was first applied. Possible Str^R mutants should be checked to make sure they are not Str^D (streptomycin dependent).

Curing strains of lambda. The Kaiser and Masuda methods (32) were modified as follows. A drop of phage λb2imm21 (10^9/ml) was put onto a lawn of $\lambda^+\lambda^S$ cells (approximately 10^8/plate) on a fresh LB plate. After 4 to 6 hr of incubation at 37 C, partial lysis was evident, and surviving cells were streaked from this region onto LB. The resulting colonies were tested for λ immunity or λ production, and λ^- ones (usually 10–50% of those colonies checked) were repurified.

UV irradiation. Irradiation with UV was carried

out with a Sylvania G15T8 germicidal lamp which was 50 cm distant from a petri dish in which cell suspensions were held. The dose rate was adjusted to 10 ergs/mm²-sec with the use of a Latarjet dosimeter.

RESULTS AND DISCUSSION

Mapping procedures. I. Rapid mapping by print-matings with a set of Hfr strains. The methods described in this section were designed to enable rapid assignment of a mutation's approximate map position to one of the possible map intervals defined by the points of origin of Hfr strains. These methods can be used to map any mutation for which some plating condition can be used to prevent the mutant, but not the wild type, from growing. This includes auxotrophic and conditional (temperature-sensitive, cold-sensitive, etc.) mutants, as well as mutants which are sensitive to metabolic inhibitors or radiation. In the following scheme, a set of 15 selected Hfrs is used, and the map intervals to which mutations are assigned have an average map length of 6 min (equivalent to approximately 7% of the complete map). Parts A and B, below, describe two different versions of this mapping scheme, the choice depending on whether one has just a few (less than about 20) mutants to map at a time (method A), or whether one has hundreds or thousands of mutants to deal with (method B). With method A, the results are somewhat more quickly scored than with method B. For mapping large numbers of mutants, however, method B requires far fewer print-mating plates (15 plates for each 100 mutants) than does method A (1 plate for each mutant). Before describing the details of these procedures, their common features will be discussed.

The most important aspect of the conjugation system utilized here is the gradient of transfer. This denotes the dependence of recombination frequency on the natural and spontaneous cessation of marker transfer from Hfr to recipient cell some time after transfer has begun at the point of origin of the Hfr. Since the probability of cessation of transfer appears to be a constant per unit of mating time for any given mating pair, the chance that any given Hfr marker will be transferred in any large, randomly chosen sample of mating pairs decreases exponentially with its distance from the origin (17, 29). As a result, Hfr markers very far from the origin (in terms of order of transfer) appear in recombinants with less than 0.1% of the frequency observed for early markers, and markers at intermediate positions have correspondingly intermediate levels of transfer. This characteristic of the gradient of transfer makes it a convenient measure of approximate map position as determined from appropriate liquid matings (17). For present purposes, this natural gradient of transfer is important because the mutants and Hfr strains are mated by the simple process of replica plating (34, and Materials and Methods), and the proximity of a mutation's map position to the point of origin of an Hfr is seen very clearly by the presence or absence of a heavy patch of recombinants which result from entry of wild-type alleles from the Hfr into the mutant cells.

For a number of compelling reasons, the counterselection of the Hfr cells in these "print-matings" is carried out by using streptomycin. Hence, the mutant strains to be analyzed by this method need one essential genetic marker—*str*⁻—producing streptomycin resistance. Another attribute which is desirable but not essential is that the mutant strains be F^-. Strains which contain F in some form, however, are excellent recipients as long as they are grown well into stationary phase (33, 67), and this condition is easily incorporated into the mapping system reported here. However, if dominance and complementation studies are needed as well, it is far easier to work with mutants which are F^- rather than Hfr, F-prime, or F^+ (see section III below).

The reasons that streptomycin counterselection is highly recommended are the following. (i) Streptomycin is bacteriocidal. This prevents the Hfr cells, which are in intimate contact with mutant cells on recombinant-selective plates, from being involved in cross-feeding which might introduce spurious effects. (ii) The level of streptomycin which can be tolerated by the usual Str^R mutants is very high (~1,000 μg/ml) compared to the level which kills Str^S cells (10 μg/ml) (3). Therefore, the application of streptomycin (see Materials and Methods and below) does not demand that any special pains be taken in achieving a uniform level of the antibiotic. (iii) It is relatively easy to assemble a group of suitable Str^S Hfr strains from this and other laboratories (see below, and Table 1). By the same token, many F^- strains in common use which have desirable genetic backgrounds for the screening of most mutant phenotypes are already Str^R. When needed, it is an easy matter to isolate a Str^R derivative of some special strain which is otherwise ready to use in a mutant search (see Materials and Methods).

The bacteria used in these procedures are rapidly growing (37 C) Hfr cells (on an Hfr master plate [method A] or in liquid culture, [method B]) and recipient cells which are

generally grown to late log or stationary phase (overnight culture [method A] or overnight grid master [method B]), whichever is most convenient. These conditions, particularly for the Hfr cells, result in healthy F-pili and high fertility (15).

The Hfr strains listed in Table 1 are all *str*$^+$ and represent a variety of points of origin in both directions of transfer. In addition, all of these Hfr strains are nonlysogenic for phage λ (see Materials and Methods) so that zygotic induction in λ$^-$ recipients is avoided. They are all highly fertile males, although some of them segregate F$^+$ revertants at a noticeable rate (see Materials and Methods). Also considered important in the choice of these strains is their predominantly wild genotype—very few auxotrophic markers or suppressor mutations are present in these strains, so that in general one need not be concerned with growth factor requirements inherited from the donors.

Method A. This method (for mapping up to 20 mutants at a time) involves the replication of a set of rapidly growing patches of Hfr cells, all on one plate, onto a lawn of the mutant strain which has been spread onto a recombinant-selective plate. An especially convenient way of arranging the Hfr strains on the master plate is shown in Fig. 3A. This photo shows patches of the Hfr strains placed in two concentric circles, in rough correspondance to the positions of the respective points of origin as shown on the genetic map (Fig. 1). Thus, the Hfrs which transfer markers in a clockwise sequence are on the outer circle, and those with counterclockwise polarity are on the inner circle. In this way, the Hfrs which transfer the wild-type gene to any given mutant with high frequency (and consequently produced many recombinants) are conveniently grouped for easy inspection (see Fig. 3B-3E and discussion below). In carrying out this method, the following routine is suggested.

(A1) Inoculate small patches of the Hfr strains onto a plate of LB agar or similar rich medium, using the arrangement shown in Fig. 3A. Incubate until growth is clearly visible (10–20 hr at 37 C). This master plate can be refrigerated and saved for several weeks for use in inoculating fresh Hfr masters by replica plating.

(A2) For each of the mutant strains to be mapped, grow a fresh overnight culture in 3 to 5 ml of LB (or other special medium if needed). These cultures may be grown in 15-ml tubes on a rotating drum or similar device. If the mutant cells are not F$^-$, it is important to provide aeration during overnight incubation in the rotator by using growth tubes whose caps do not seal tightly. If recombinant selection is to be done on minimal medium, centrifuge the culture and suspend the cells in the original volume of buffer such as 56/2.

(A3) Using the Hfr master made in step A1, replicate Hfr cells onto LB plates to obtain fresh Hfr masters. Each fresh Hfr master will be usable for mapping three or four different mutants.

(A4) Incubate the fresh Hfr master plate(s) at 37 C for 4 to 6 hr. The Hfr cells will then be rapidly dividing, and the patches will be visible.

(A5) Flood a slightly dry recombinant-selective plate (to which streptomycin has not yet been added) with mutant cells by pouring 3 to 5 ml of the overnight culture onto the surface and then pouring off the excess. Absorb the large remaining drop with a tissue at the edge of the plate and let the surface dry by leaving the lid off for a few minutes. (Note: conventional spreading with a glass spreader is not satisfactory because of the nonuniform layer thus produced.)

(A6) Use a fresh Hfr master (step 4) to replicate Hfr cells onto the mutant lawn.

(A7) Incubate this print-mating plate at 37 C for 30 min to permit chromosomal transfer. If the mutant cells are very temperature sensitive for growth and are killed by this short exposure to 37 C, the print-mating can be carried out at a lower temperature such as 34 or 30 C. In this case, more time (45–60 min) should be allowed for mating because chromosomal transfer velocity decreases with decreasing temperature (74).

(A8) Spray the surface of the print-mating plate with streptomycin (see Materials and Methods). The 30-min delay in the application of streptomycin allows a high efficiency of transfer of early markers from the (streptomycin-sensitive) Hfr cells and tends to accentuate the transfer gradient by diminishing the transfer of very late Hfr markers. If the selection for recombinants involves treating the cells in a way that is likely to affect adversely mating and recombinant formation, such as is the case with exposure to radiation (12, 30), this treatment should be delayed until after the 30-min pre-streptomycin mating period, or longer if necessary.

(A9) Incubate the plate until recombinant patches are seen. This normally takes less than 1 day at 37 or 42 C (for mapping auxotrophic or heat-sensitive mutants), and 1.5 to 2 days at 25 to 30 C (for mapping cold-sensitive mutants).

(A10) Determine the map interval in which the mutation lies by seeing which Hfrs produce

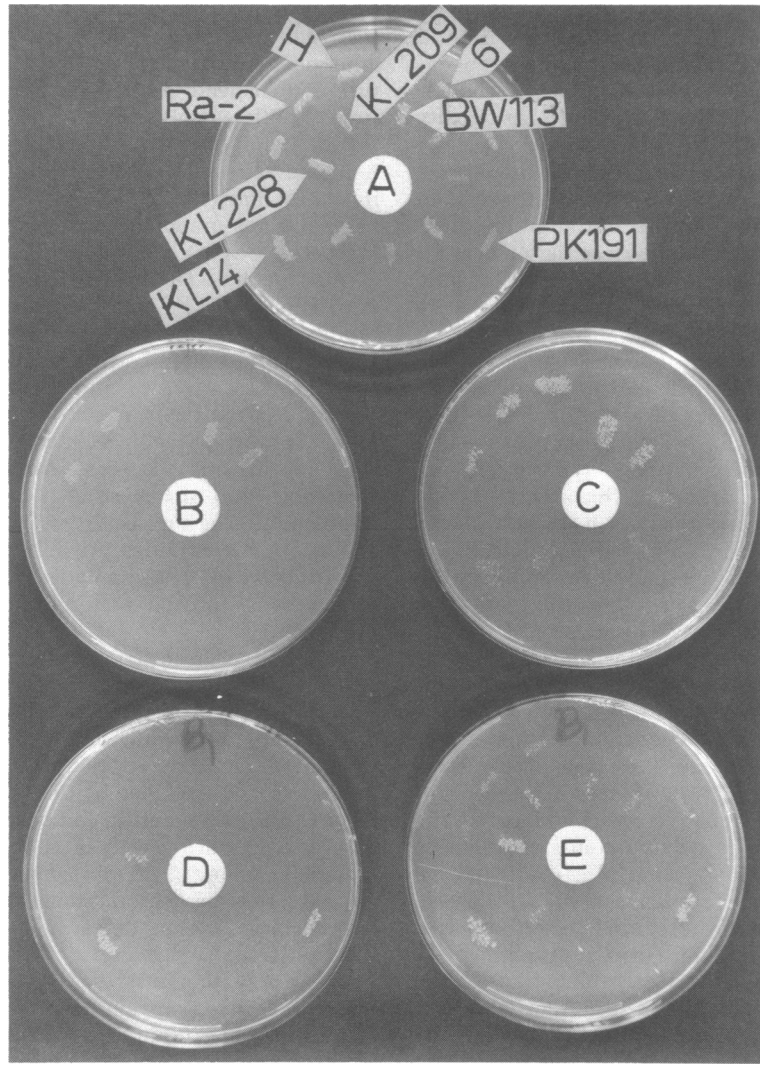

FIG. 3. *Hfr master and four print-mating plates using mapping method A. A, Master plate showing patches of Hfr cells grown on LB agar. The positions of the patches correspond to the map positions shown in Fig. 1. The Hfrs discussed in the examples in section A10 of the text are labeled specifically. B, Print-mating plate of LB agar, with lawn of strain PLS73. Mating took place for 45 min at 35 C followed by streptomycin spray and overnight incubation at 42 C. C, Print-mating plate of minimal medium lacking proline, with a lawn of strain AB1157. Mating took place at 37 C for 30 min, followed by streptomycin spray and overnight incubation at 37 C. D, Print-mating plate of minimal medium lacking arginine, with a lawn of strain KL141. Mating conditions were as in C. E, Print-mating plate of minimal medium lacking uracil, with a lawn of strain KL141. Mating conditions were as in C.*

the heaviest recombinant patches, i.e, transfer the wild type early (Fig. 1). There is usually a sharp demarcation among the Hfrs of one polarity, between the one which transfers the wild-type gene earliest (→ heaviest recombinant patch) and that which transfers the wild-type gene very late (→ very few, if any recombinants). In Fig. 3B to 3E, representative examples of print-mating plates are shown. To assign a mutation to 1 of the 15 map intervals shown in Fig. 1, it is usually necessary to determine the "earliest" and "latest" Hfrs for the clockwise set of Hfrs as well as for the counterclockwise set. For example, the print-mating plate pictured in Fig. 3C shows that among the clockwise Hfrs, HfrH transferred the necessary wild-type gene to this particular mutant early (i.e., with high frequency), and

Hfr6 transferred it late. From this, we assign the mutation's map location to either interval 1 or 2. The counterclockwise Hfr BW113 transferred the same gene early, whereas KL209 transferred it late. This indicates that the locus lies either in interval 1 or 15. The combined results from both Hfr sets, therefore, localize the mutation to interval 1 (in this example, the mutation was in the *proA* gene, which maps at min 7). A similar analysis of Fig. 3B leads to the assignment of the unknown locus in strain PLS73 to interval 15. The mutation in this example is a temperature-sensitive *pls* mutation, *pls-73* (20). In this case, the Hfrs which did not transfer the wild-type allele early (KL209, HfrH) provide a more accurate gene localization (i.e., to interval 15) than do the Hfrs which transferred it earliest (Ra-2, BW113).

It should be noted that the boundary between intervals 11 and 12 is not an Hfr point of origin but instead is the *str* locus. The reason that this is feasible is that when a recipient cell which is mutant at some unknown locus "X" receives the str^+ locus from an Hfr prior to receiving the wild-type "X"$^+$ allele, it will usually be killed when streptomycin is applied. This is because str^+/str^- merozygotes are, for some time after mating, phenotypically streptomycin sensitive (4, 9) and hence do not grow under the conditions of these print-matings. In contrast, an Hfr strain which transfers X^+earlier than it does str^+ will often, due to the gradient of transfer, donate chromosomal fragments which carry X^+ but do not extend as far as the *str* locus. Examples of the print-matings obtained using markers in intervals 11 and 12 are pictured in Fig. 3D and 3E. In the print-mating of Fig. 3D, the $argG^+$ allele (interval 11) was transferred to an $argG^-$ strain. Hfrs KL14 and PK191, which transferred $argG^+$ proximally to the str^+ locus, produced heavier patches of recombinants than did KL228, which transfers str^+ proximally to $argG^+$. The reverse situation is seen in Fig. 3E, which shows a plate on which $pyrE^+$ (str^-) recombinants were selected. The *pyrE* locus lies at min 72 (65), in interval 12. It should also be noted that if the selected wild-type donor allele lies extremely close to the *str* locus, i.e., in the region S shown in Fig. 1, the number of recombinants observed will be somewhat low due to the limited probability for chromosomal breakage during transfer (~ 6%/min [29, 74]) and for recombination within small intervals (20 recombination units per min [29, 36, 49]). The efficiency of transfer for early markers with the print-mating method, however, is so high that even for most mutants in region S (Fig. 1), assignment of the map location can at least be made to the combined 11-12 interval.

Method B. The difference between this method (for mapping 20-1,000 mutants at a time) and the previous one is that whereas method A involves Hfr master plates and (mutant) recipient lawns, method B uses master plates which contain up to 100 different mutant patches per plate, and these are printed onto Hfr lawns. This allows one experimenter to print and score up to approximately 1,000 mutants in a few days. The procedure involves the following steps.

(B1) On LB plates (or on other special growth medium if required), make master grids (see Materials and Methods) of up to 100 different mutants per plate. Incubate these grids 4 to 6 hr and then replicate each onto four more plates of the same growth medium. Incubate all of these master plates overnight. Each of these masters can be used for three or four print-mating replications, using different Hfr lawns.

(B2) After allowing overnight incubation of the master plates, prepare fresh cultures of each of the 15 Hfr strains by growing them in LB at 37 C to exponential phase at a concentration of 3×10^8 to 4×10^8 per ml. The cultures should then be chilled on ice, centrifuged, and suspended in one-half the volume of 56/2 buffer. (The cells can be used directly in the LB medium if the broth will not interfere with recombinant selection, e.g., when temperature sensitive mutations are being mapped.) Since 2 to 3 ml of Hfr suspension are needed to flood each print-mating plate, one needs to start with a number of milliliters of broth culture of each Hfr strain equal to approximately five times the number of mutant grids being printed. Somewhat less volume will suffice if excess Hfr cells are poured from one flooded plate onto some other which needs the same kind of Hfr lawn.

(B3) Flood a set of 15 recombinant-selective plates, each with cells of a different Hfr strain. The flooding procedure is as described in step A5, above, where mutant cells are used as lawns.

(B4) Using a set of master grids derived from one of the original grids of mutants, replicate the patches of mutant cells onto a set of Hfr-flooded plates, using a fresh velvet for each printing. As mentioned above, each grid master can be used to inoculate up to four separate velvets for these print-matings. One recombinant-selective plate which has not been flooded with Hfr cells should also be printed to see

whether any mutant patches grow or have a leaky phenotype.

(B5) Incubate the print-mating plates for 30 min at 37 C (see step A7 above).

(B6) Spray the plates with streptomycin (see step A8).

(B7) Incubate the plates as described in step A9 above.

(B8) Score the mapping results, using the same rationale as used in step A10, method A. When scoring a grid of 100 mutants, it is convenient to tabulate the results in a series of columns which refer to the Hfrs of one polarity (e.g., clockwise) in sequential order, followed by columns for the other set of Hfrs. By noting where strong or weak (or nonexisting) patches of recombinants are obtained, one can easily find the "earliest" and "latest" points of origin for each Hfr set, for any given mutant. An illustration of results from this print-mating procedure is shown in Fig. 4A to 4D. Figure 4A shows a master grid with three groups of patches: no. 1 to 20 are all patches of strain KL231, no. 31 to 50 are all patches of strain PLS73, and no. 61 to 80 are a random assortment of patches of these two strains, similar to what might be actually observed for a new group of uncharacterized mutants. Figures 4B to 4D show the recombinant patches obtained when this master plate is print-mated with Hfr strains KL208, H, and KL226, respectively, and temperature-resistant Str^R recombinants are selected. KL231 produces heavy patches of temperature-resistant (*serS*$^+$) recombinants when mated with Hfrs KL208 and H, but not KL226, as expected from the position of *serS*$^+$ in region 4 (40). In contrast, temperature-resistant recombinants of strain PLS73 are formed from the print-mating with KL226 but not with KL208 or HfrH, in agreement with the result seen in Fig. 3B, where strain PLS73 was print-mated using method A. It should be

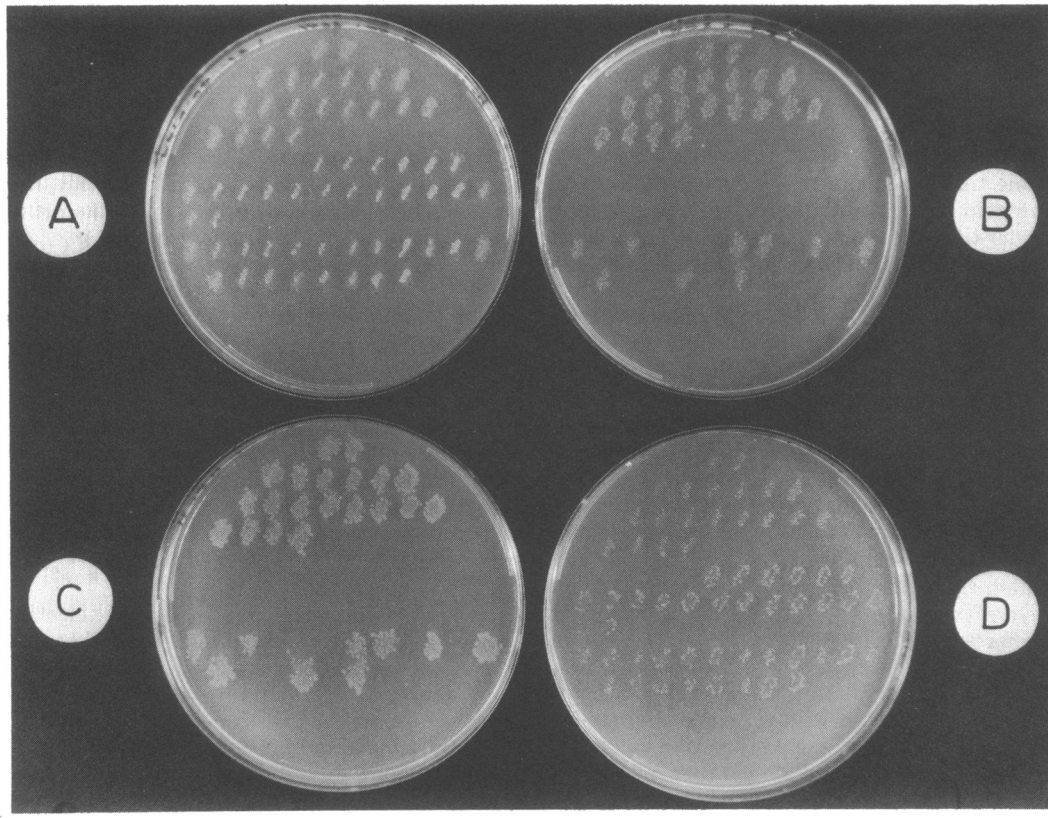

FIG. 4. *Grid of mutant strains and three print-mating plates using mapping method B. A, The patches in the upper group (positions 1–20) are all of strain KL231, on LB agar. The middle patches (no. 31–50) are all of strain PLS73. The bottom group is a mixture of patches of these two strains. B, Print-mating plate of LB agar using lawn of Hfr KL208. Mating took place at 35 C for 45 min, followed by streptomycin spray and overnight incubation at 42 C. C, Same as B, except that the HfrH was used as the lawn. D, Same as B, except that Hfr KL226 was used as the lawn.*

emphasized that mapping by method B requires that an entire set of different Hfr lawns be used, so that relative numbers of recombinants from Hfr to Hfr can be observed. In this way, other factors which influence recombinant formation which differ from mutant to mutant (such as the degree of temperature sensitivity of growth) will not interfere with interpretation of the mapping results.

Mapping procedures. II. Mutant sub-grouping by chromosome mobilization. If one has a number of mutants which have similar phenotypes and whose pertienent mutations all map in one region of the chromosome (e.g., one of the 15 map intervals shown in Fig. 1), it is sometimes desirable to determine quickly whether or not the mutations map very close to each other (e.g., within 10 to 20 genes of each other). This can usually be accomplished by converting one or more of the mutants into an F-prime male which will act as a moderately fertile donor for the region of the chromosome in which its mutation lies. (This process of chromosome mobilization by an F-prime factor involves cross-over events between episome and chromosome [51, 56], and is very conveniently used to move genes from one strain to another [43, 46]).

By using the other mutants of the groups as recipients according to the procedures of method B described above, it is usually possible to see which mutations map very close to the one carried by the F-prime strain. An outline of this procedure is as follows.

(i) Choice of an F-prime factor. In Fig. 1, the chromosomal map regions for four F-prime elements are shown: F15, KLF3, F-*lac*, and F311 (see also Table 1). These are examples of short F-prime factors which carry chromosomal material from widely separated map regions. These four particular F-prime factors all promote chromosomal transfer in the same direction (counterclockwise). Thus for any given chromosomal interval of interest, it is possible to choose one of the four F-prime factors which will promote transfer of that interval less than one-third the way around the map from the F-prime origin of transfer. The transfer of chromosomal markers from an F-prime donor is normally at least 10% as efficient as from the corresponding Hfr strain (51), and the use of an appropriate F-prime factor just described is sufficient to allow good efficiency of transfer in print-matings for any chromosomal region under study.

(ii) Conversion of a mutant strain to F-prime maleness. Most F^- strains can be convêrted readily to F-prime merodiploids by mating them for 2 hr with an F-prime donor. Broth cultures growing exponentially at 37 C (or as close to that temperature as is feasible) are recommended, and cells at a concentration of 2×10^8 to 3×10^8 cells per ml should be mixed in a 10 F-prime to 1 F^- cell ratio in order to maximize episome transfer per F^- cell. After plating the cells onto an appropriate medium which allows only the recipient cells to grow, the resulting colonies are picked and checked for maleness either by spot testing with a male-specific phage (MS-2, QB, F1, etc.) or by trying then as donors on a grid in print-matings as described in section I. method A, above. Usually greater than 5.0% of the recipient clones from the initial F-prime liquid mating experiment will be found to carry the desired F-prime factor.

If the particular mutant used is not easily sexduced (converted to F-prime maleness), methods of direct selection are available for any of the four F-prime elements mentioned above. F15 carries *thy*$^+$, and the recipients, if not already *thy*$^-$, can be easily made so by selection using trimethoprim (46, 62). Similarly, KLF3 carries *his*$^+$, and *his*$^-$ mutations are introducible by P2 eduction (46, 64). Lac$^-$ mutants for the selection of F-*lac*$^+$ can be isolated after simple mutagenesis (46), and F311 carries *ilvO*$_A^-$ which confers valine resistance (10^{-2} M) on strains which receive the episome (45, 55).

(iii) Mutant $\times$ mutant matings using an F-prime donor. After one mutant of a group has been made into an F-prime donor as described above, it can be mated with all the other mutants of the group by using the F-prime strain as a donor lawn with the print-mating procedure given above (section I, method B). In the present case, only one copy of each grid of mutants is necessary to print-mate with a given F-prime lawn on one recombinant-selective plate. The lawn can be prepared just as with an Hfr in steps B2 and B3. The streptomycin step is not relevant here since both donor and recipient strains are presumably Str^R. (In addition, for mutants of the same phenotype the recombinant-selective plating condition is the same for donors and recipients.)

If the mutants in the group represent loci which are separated by 0.5 min of chromosomal length or more, patches of recombinants should be seen easily in these print matings. The cross-over frequency characteristic of *E. coli* conjugation (approximately 20 recombination units per minute [29]) becomes a limiting factor in these print-matings only for intervals

of less than a few tenths of a minute on the map, or roughly 10 average-size genes in length (65). Two mutations which are in different but very closely linked genes (i.e., less than 10 to 20 genes apart) may not give rise to significant numbers of recombinants by the print-mating procedure, and complementation tests and P1 transduction (see section III) may be necessary as a final analysis. If the results of certain F-prime print-matings are in doubt, better resolution is also obtainable from liquid matings using the same strains. If the mutants have normal fertility and the required crossovers occur less than one quarter of the way around the chromosome from the F-prime point of origin, a 90-min uninterrupted mating carried out with exponentially growing cultures at 2×10^8 to 3×10^8 cells/ml (one F-prime to two F^- cell ratio) will usually produce fewer than 10^4 intragenic recombinants per ml (B. Low, *unpublished data*) in contrast to more than 10^4 recombinants per ml for markers separated by 1 min or more on the genetic map. In the accompanying paper of Godson (20), Tables 6, 9, and 12 show data of this kind, which illustrate recombination frequencies obtained from intergenic recombination as opposed to crosses involving mutations which Godson shows are most likely in the same complementation group. Godson's recombination frequencies were obtained from matings carried out at 34 C for 60 min and therefore are somewhat lower in value than comparable ones from 90-min matings at 37 C (74).

It should be emphasized that recombination frequencies obtained from the methods described in this section are simply indications of whether or not two mutations are more or less than ~0.5 min (10–20 genes) apart on the map. Low recombination frequencies may or may not indicate that two mutations are in the same gene, and genetic complementation tests or functional analysis, or both, is necessary before a definite answer can be had.

Mapping procedures. III. Complementation and other mapping techniques. When different mutations are found to be very close to each other on the genetic map, one usually wants to know whether or not they are in the same gene and what their positions are relative to each other and relative to nearby chromosomal markers. This section gives a brief outline of methods which are used for these detailed genetic analyses and some comments on other useful genetic localization methods.

(i) Mapping and complementation using stable merodiploids. The available ensemble of F-prime factors presently at hand for merodiploid work (mapping, dominance, and complementation studies) has grown to represent almost every region of the *E. coli* chromosome (8, 38, 39; Low, *unpublished results*). For mutations whose phenotypes are recessive in heterozygotes, the introduction of F-prime factors and selection for wild phenotype can often be used to localize map positions to within parts of intervals defined by Hfr points of origin. It is most useful if the donor strains used to introduce specific F-prime factors are *recA*$^-$, inasmuch as the *recA*$^-$ allele allows efficient F-prime transfer but prevents chromosome mobilization (except at an extremely low level [13]), and ensures that only the well-defined map interval represented by the F-prime is transferred (38, 71). Similar well-defined transfer is obtained from primary F-prime strains, which carry chromosomal deletions for the genes on the episome (8, 52, 57). Even for mutations with a dominant phenotype, localization to the chromosomal region on an episome is possible when *recA*$^-$ or primary F-prime donors are crossed with a mutant, and wild-type recombinants are observed (sometimes with low frequency) rather than merodiploids.

If several recessive mutations are localized in a region carried by an F-prime, complementation studies can be carried out using the following procedure which is a familiar generalization from the studies of Jacob et al. (see [29] p. 252).

(a) Mate the F-prime strain with one of the mutant strains, thereby obtaining a wild-type (+)/mutant (−) merodiploid. (Use a mutant which is Rec$^+$. See section II, ii for mating conditions.)

(b) From this merodiploid, obtain a −/− segregant. This is done by plating out the merodiploid culture onto the surface of plates which allow −/− (and usually +/−) colonies to grow. These colonies can be replicated directly onto plates which distinguish between phenotypes. (This replication isn't needed if the original colonies are distinguishable by morphology or *p*H indicator, etc. See e.g. reference 7, chapter 1). Normally −/− segregants are found among +/− cells at a frequency of 0.1 to 1% (46, 73; Low, *unpublished data*). Check the −/− strain to make sure that it is a donor (see section II, ii, above).

(c) Mate the −/− merodiploid with the other strain(s) whose mutation maps close to the first. When liquid matings are used, as in section II, ii, the number of wild-type colonies obtained (if any) gives some indication of whether or not the chromosomal and episomal

mutations are being complemented. (A low number of wild-type colonies, e.g., less than 1% per recipient cell, suggests intragenic recombination rather than simple sexduction and complementation. See Godson [20], Tables 4, 7, 10, and 14.)

(d) To prove complementation, the two mutant alleles must be identified in a putative +−/−+ merodiploid. The mutation on the chromosome is easily checked by obtaining an F^- (i.e., cured) segregant (5, 25, 63) and observing the mutant phenotype. The episomal mutation can be checked by mating the F-prime factor back into the original strain in which it acquired the mutation by recombination (step ii, above). If F-prime recombinants are obtained which have the −/− phenotype, the mutation on the F-prime must have been complemented in the +−/−+ merodiploid. As mentioned before, the use of *recA*$^-$ hosts in these merodiploid studies is advisable (in particular in steps c and d above, with large unstable F-prime factors) so that recombination and rearrangement of episomal and chromosomal alleles is effectively avoided.

Another way of proving the existence of recessive alleles in meroheterodiploids is to transduce the recessive alleles into other (haploid) strains. The generalized transducing phage P1 (see below) incorporates episomal as well as chromosomal deoxyribonucleic acid fragments so that transduction of some marker which is known to be closely linked to the mutation will reveal some cotransductants which carry the recessive (−) allele. This procedure presupposes a knowledge of a convenient closely linked marker in order to effect the transduction.

It should be emphasized strongly that whereas the existence of complementation as detected in +−/−+ merodiploids having wild-type (++) phenotype is strong evidence for different complementation groups, failure to obtain cells with wild-type phenotype, on the other hand, does not constitute proof that the two original mutations were in the same complementation group. Occasionally in the search for a "−/−" merodiploid from a +/− strain, a merodiploid is isolated in which the F-prime factor carries a deletion of the region of the mutated gene (4). Since such a deletion might extend through many adjacent genes, transfer of such an episome to another strain and failure to observe complementation might simply mean that the deletion overlaps the mutation in the second mutant strain. If wild-type recombinants are produced at low frequency from a merodiploid, in which episome and chromosome are presumed to carry different mutations in the same complementation group, the presence of a deletion as described above is ruled out. However, one should still bear in mind the less likely possibility that an episomal deletion might still be present and happen to terminate in the same gene as the "noncomplementing" mutation on the chromosome, in such a way that recombination to produce wild type was still possible.

In Godson's accompanying paper (20), several complementation groups are delineated, and low-frequency recombination in noncomplementing merodiploids is also shown (see reference 20, Tables 4, 7, 10, and 14).

(ii) Linkage and gene ordering by P1 transduction. P1 transduction is widely used and is a standard method for establishing relative positions of closely linked genes on the genetic map (35, 75). It is usable for relating gene positions for markers separated by less than 1 to 2 min of map length. However, the resolution of extremely close mutations (in neighboring or very close nucleotide base pairs) is more easily done with conjugational crosses (23, 76) whose high efficiency permits the selection of lower frequency cross-over events than are obtainable by transduction.

(iii) Interrupted matings: uses and limitations. The sequential donation of genes from Hfr and F-prime males to recipient cells has long been exploited in gene localization experiments which involve artificial interruption of transfer (24, 29, 41, 68). Some of the problems associated with mating interruption procedures have already been described (41, 68). Aside from the procedural aspects of carrying out mating interruptions, there is an inherent limitation to the resolution of mapping by mating interruption, due to the unsynchronized transfer of genes from any given culture of donors. This can be seen from a high-resolution interrupted mating experiment such as is shown in Fig. 5. In this experiment, the early kinetics of transfer of *thr*$^+$ and *pro*$^+$ from Hfr H to a *thr*$^-$ *pro*$^-$ *str*$^-$ F^- strain were measured. Rapidly growing broth cultures at 37 C were mixed without any initial chilling or other manipulations, and mating pairs were interrupted with a vibratory shaker (see Materials and Methods). As can be seen from Fig. 5, the distribution of transfer times for the donor *thr*$^+$ and *pro*$^+$ genes, among the population of mating pairs in the culture, is not sharp. On the contrary, the recombinant curves have a gradual and accelerated increasing slope for several minutes until a fairly linear portion of curve is reached. Since in practice it is usually not

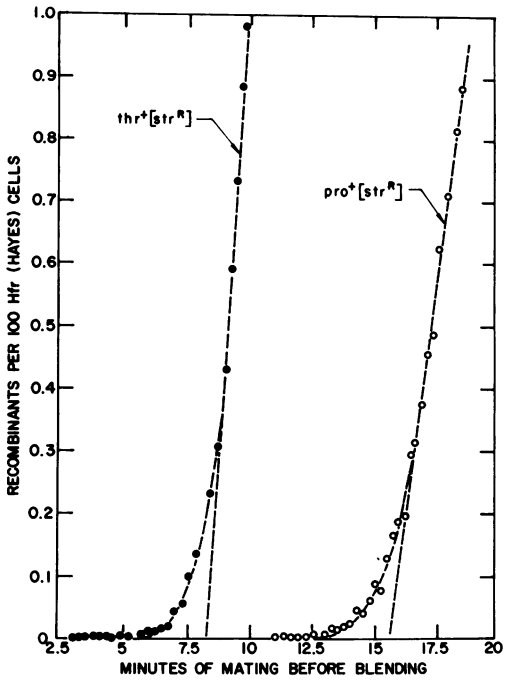

FIG. 5. *Interrupted mating, Hfr H × F⁻ AB1133. The two strains were grown exponentially at 37 C in LB to a concentration of 10^8 to 2 × 10^8 cells/ml. They were then immediately mixed in a 1 Hfr to 200 F⁻ cell ratio (total volume = 20 ml in a 125-ml flask) and shaken gently at 37 C. Samples (0.1 ml) were withdrawn. After interruption in 0.8% agar (41), the cell-agar mixture was poured onto a recombinant-selective plate to solidify.*

convenient to interrupt samples of mating pairs as frequently as in the experiment of Fig. 5, an experimenter is usually confronted with just a few points along this type of curve, from which an "entry time" is extrapolated. (Points for much later times are sometimes also shown on interrupted mating curves, and these usually bias the extrapolation one way or another.)

The assignment of an entry time to an interrupted mating curve is at present a somewhat ill-defined procedure and involves drawing a line through some early portion of the curve and extrapolating this line to the abscissa. Obviously, if the steeper and more linear portions of the curves are extrapolated, as shown in Fig. 5, to give entry times of ~8.2 min and ~15.5 min for *thr* and *pro*, respectively, quite different values are obtained than the corresponding ones from the earliest portions of the curves where they begin to rise significantly above background, i.e., 6 to 7 min for *thr* and 13–14 mins for *pro*. Interrupted mating results given in the literature usually vary between these two extremes as to the criteria used for assignment. The earlier extreme of entry time would seem to be the more characteristic of the distance of a marker from the donor point of origin (plus an added 3 to 5 min which is apparently the minimal time necessary between cell contact and initiation of chromosome transfer [36]), because it probably represents the contribution of mating pairs in the culture which initiated transfer without extra delays that may involve slow pair formation, cell age, or other physiological factors.

As a result of all these considerations, it is usually feasible to assign entry times for early markers only to within ±1 min, for a given interrupted Hfr × F⁻ cross. For late markers (more than 25–30 min from the point of origin), interrupted mating curves show even more gradual increases in numbers of recombinants per unit of time, and entry time determination is less precise. Markers extremely close (less than ~1 min) to the origin of transfer also are sometimes difficult to order by interrupted matings because of low probabilities of incorporation into recombinants (19, 36, 53). As Taylor and Thoman point out (68), the most fruitful use of interrupted mating curves is in the comparison of entry times for two or more markers in the same experiment. With this kind of experiment, the early events of effective pair formation and initiation of transfer do not strongly affect the relative entry times for two markers under comparison. For two very close markers (less than 1–2 min apart), the relative order of transfer can usually be verified by genetic analysis of some of the early recombinants for each marker. The recombinants for the first of the two markers to be transferred will include an increasing fraction, with increasing time before interruption, which have received the second donor marker. In contrast, all samples of recombinants for the second marker contain an approximately constant fraction which have incorporated the earlier marker. (Examples of this type of analysis are given in references 11 and 70.)

In summary, interrupted mating experiments can be very useful for the approximate determination of relative map positions, but resolution of markers separated by less than 1 min of map length is difficult and often not possible.

(iv) Other mapping methods. A few other mapping techniques are available for *E. coli* and can provide much useful information in cases where the genetic configuration permits. Short-range deletion mapping can be carried out for genes situated close to a locus whose deletion produces some easily selectable phe-

notype, such as resistance to a bacteriophage or to an antimetabolite (14, 46, 58, 61). Transductional shortening of F-prime factors has been used to order genes which are close to the F-factor on an F-prime episome (45, 50). The genetic linkage between selected and unselected markers in bacterial crosses can be used to estimate map distances (18, 29, 69), and a method has been reported for increasing the resolving power of recombination frequency as a measure of distance between close markers (70). This procedure utilizes the recombinogenic effect of radiation to decrease the observed linkage between markers (72). It should be kept in mind that correlations between recombination frequencies and absolute map distances are only approximate and are subject to the ever present perturbations due to marker specific effects (49, 75) and negative interference (44) which at present are not fully understood.

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