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Simplified Method for Interruption of Conjugation in *Escherichia coli*

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A simplified procedure is described in which interruption of mating and recombinant selection both take place on plates containing selective medium plus nalidixic acid.

A convenient replica plating method has been described for locating a gene within one of fifteen segments of the *Escherichia coli* chromosome (4). To determine more precisely the position of a gene, an interrupted mating is usually carried out. Time of entry of a gene is normally determined by removing samples of a mating mixture at intervals of time, treating bacteria in some way to prevent further mating, and then plating a sample on selective medium that only allows growth of recombinant clones (3). Prevention of further mating is usually accomplished either by treating a mixture of T6-sensitive males and T6-resistant females with a high multiplicity of T6 phage to lyse the Hfr bacteria from without (3), or by vigorous mechanical agitation in order to physically separate parental bacteria (5).

We describe here a simpler procedure for determining the kinetics of mating and the order of entry of genes located on the proximal portion of an Hfr genome. Nalidixic acid has been used as a counterselection agent in conjugation experiments (e.g., reference 2). Also, it is known that this antibiotic immediately halts transfer of Hfr deoxyribonucleic acid (DNA) (1). We have used nalidixic acid to perform both functions in time-of-entry experiments. In this procedure, samples are removed from a mating mixture at intervals of time and are diluted in a medium that prevents further transfer of Hfr DNA; then without an intervening treatment step, samples are plated onto a selective medium for enumeration of recombinants.

In experiments to test this procedure, nalidixic acid-sensitive Hfr bacteria were mated with nalidixic acid-resistant F^- bacteria. (Nalidixic acid-resistant F^- mutants, after ultraviolet light mutagenesis, were selected on a rich medium containing 100 μg of nalidixic acid per ml). Parental bacteria were mated in minimal medium, and samples were removed at intervals and diluted in 0.01 M phosphate buffer, pH 7, containing 100 μg of nalidixic acid per ml to

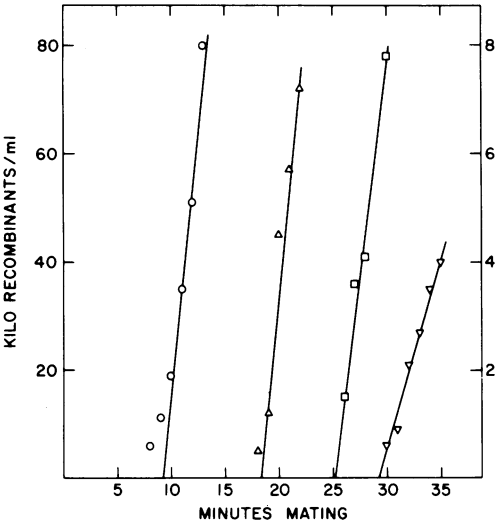

FIG. 1. *Hfr AB312 (thr, leu, thi) × F^- CSH57bN [(arg, xyl, ilv, met, his, ade, thi, leu, lac, trp, nalA [100]). Parental cultures were grown to exponential phase in appropriately supplemented M9 minimal medium. M9 salts contain, per liter, Na_2HPO_4, 7 g; KH_2PO_4, 7 g; NaCl, 5 g; NH_4Cl, 1 g; $MgSO_4 \cdot 7H_2O$, 0.25 g. Parental bacteria were mixed to give a mating mixture containing about 10^8 F^- per ml and 10^7 Hfr bacteria per ml. Mating was carried out without agitation at 37 C. At intervals of time, either 0.02- or 0.2-ml samples were taken into dilution tubes containing 2.0 ml of 0.01 M potassium phosphate buffer, pH 7, and 100 μg of nalidixic acid per ml and were mixed vigorously with a Vortex mixer. Samples (0.1 ml) were mixed with 2.5 ml of melted agar (2.5 ml of M9 salts, 0.7% agar, and 50 μg of nalidixic acid per ml) and were poured on selective plates. Selective plates contained M9 salts, 1.2% agar, 20 μg of nalidixic acid per ml, 20 μg of required amino acids per ml, 10 μg of adenine per ml, 2 μg of thiamine per ml, and 0.2% carbon source. As appropriate, a single amino acid was omitted or xylose was substituted for glucose as the carbon source. Left ordinate: (○) Arg^+ Nal^R recombinants. Right ordinate: (△) Xyl^+ Nal^R recombinants; (□) Ilv^+ Nal^R recombinants; (▽) Met^+ Nal^R recombinants.*

halt immediately the transfer of Hfr DNA. Samples were plated on selective minimal plates containing 20 μg of nalidixic acid per ml. Neither parental strain is capable of growth on the plates, but nalidixic acid-resistant F^- bacteria are able to integrate the transferred donor DNA, giving rise to nalidixic acid-resistant recombinants that are capable of growth on the selective medium.

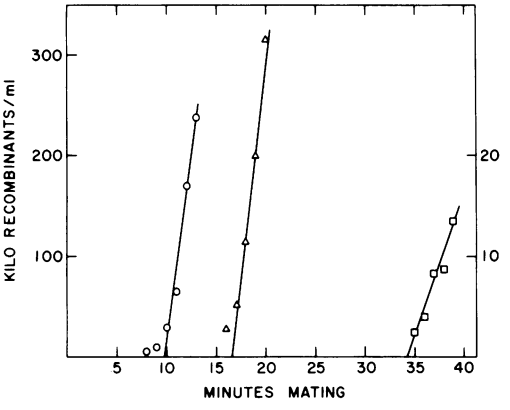

FIG. 2. *HfrH (thi, rel, λ⁻) × F⁻ CSH57bN (vide supra). Mating and selection of recombinants was carried out as described for Fig. 1. Left ordinate: (○) Leu⁺ Nal^R recombinants; (△) Lac⁺ Nal^R recombinants. Right ordinate: (□) Trp⁺ Nal^R recombinants.*

Examples of interrupted mating kinetics determined by this procedure for Hfr AB312 and HfrH are given in Fig. 1 and 2. In other experiments, Hfr P4 × 6 and Hfr KL226 were mated with an appropriate F^- strain, giving the results expected for these Hfr strains for the time of entry of the *pro* and *leu* genes. The method presumably has general application to all Hfr strains with the exception of those that transfer the wild-type allele of the *nalA* gene as an early marker.

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LITERATURE CITED

1. **Fisher, K. W., and M. B. Fisher.** 1968. Nalidixic acid inhibition of DNA transfer in *Escherichia coli* K12. Cold Spring Harbor Symp. Quant. Biol. 33:629-633.
2. **Guerry, P., J. Van Emden, and S. Falkow.** 1974. Molecular nature of two nonconjugative plasmids carrying drug resistance genes. J. Bacteriol. 117:619-630.
3. **Hayes, W.** 1957. The kinetics of the mating process in *Escherichia coli*. J. Gen. Microbiol. 16:97-119.
4. **Low, B.** 1973. Rapid mapping of conditional and auxotropic mutations in *Escherichia coli* K-12. J. Bacteriol. 113:798-812.
5. **Low, B., and T. H. Wood.** 1965. A quick and efficient method for interruption of bacterial conjugation. Genet. Res. 6:300-303.