Genetic Mapping

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INTRODUCTION

The importance of knowing the relative positions of elements of the genome is obvious, not only for the analysis of gene function and regulatory mechanisms but also for an understanding of the cell cycle and the organization and evolution of the chromosome. Fortunately, *Escherichia coli* and *Salmonella* spp. are well endowed with gene transfer and recombination mechanisms which greatly simplify the localization of new genes and other genetic elements on their single circular chromosomal linkage groups— i.e., their genetic maps. In many cases this localization by genetic means is the easiest and quickest route toward a subsequent physical identification and mutational and sequence analysis.

The purpose of this chapter is to recall these gene transfer and selection mechanisms as they relate to linkage analysis and to help the reader in choosing suitable approaches to rapid genetic localization of particular types of genes and mutations in *E. coli* and *Salmonella.* Some corollaries of these techniques, which are also cited, relate to the mutational inactivation of genes and the exchange of various allelic forms of these genes from one site on the chromosome (or bacteriophage or plasmid) to another, as aids to mapping, functional analysis, and further manipulation.

GENETIC MAPPING APPROACHES

Introduction

Genetic Markers. LaRossa (chapter 139, this volume) lists many useful selectable or scorable phenotypes for mutations which can be used, in addition to straightforward auxotrophic and conditional lethal phenotypes, for linkage analysis in crosses involving uncharacterized loci. In addition, the use of transposon pools (16, 85) or sets of random but defined transposon insertions (39, 87; chapters 109 and 141) gives the experimenter a wide choice of markers for use in conjugational and transductional mapping (see below), to relate new loci to ones already on the existing genetic maps (chapters 109 and 110).

Long- versus Short-Range Mapping. The total length of the *E. coli* chromosome is defined as 100 min (≈4,655 kb), referring to the approximate length of time it takes for an Hfr donor cell to transfer the entire linkage group to a recipient cell by conjugation at 37°C (3; chapter 109). The total length of the *Salmonella* chromosome, which behaves similarly in conjugation but is less well defined in terms of transfer time, is analogously defined as 100 centisomes (cs) (chapter 110). For a mutation of totally unknown location, a global mapping approach is useful to roughly map the mutant site. In particular, genetic linkage in Hfr crosses (or F-prime complementation, where possible) is usually the easiest approach, as long as the mutation has a selectable or scorable phenotype. Once a mutation is localized to within 5 or 10 min (or cs) of known markers on the chromosome, finer resolution can be obtained by generalized transduction or complementation using ordered clones, as mentioned below.

Conjugational Mapping

Gradient of Transfer. The frequency of transfer of any given marker from any particular Hfr strain to recipient cells is strongly dependent on the distance of the marker from the Hfr origin of transfer. The "gradient of transmission" refers to the approximately exponential decrease in recombinant frequency as a function of the distance from the origin. Jacob and Wollman (reference 34, p. 144–150) showed that this was due to a spontaneous random interruption of conjugational transfer (the cause of which is still not known), producing an exponential "gradient of transfer," followed by a more or less constant high probability of integration (34 [p. 126]) for a marker after it is transferred:

$$\text{recombinant frequency} \quad \alpha\, e^{-kd}$$

Here d is the distance (in minutes) from the Hfr point of origin to the marker and k_t is approximately 0.064 min^{-1} for *E. coli* (34 [p. 150], 102) (this varies somewhat from Hfr to Hfr). Due to the gradient of transfer, the frequency of recombinant formation for an "early" marker from the Hfr (see exception below for markers less than ~1 min from the point of origin) is nearly 1,000-fold greater than for a "late" Hfr marker. The roughly exponential dependence allows one to directly determine an approximate map region for a mutation in cases in which an allele from the Hfr can be directly selected in recombinants and in which its recombination frequency can be compared with other known markers on a curve such as that shown in Fig. 1. The availability of various Hfr strains with different points of origin (see chapters 127 and 128) usually enables one to find the general map region for a mutation within 3 to 10 min by this method. Experimental details for this type of analysis are given in references 17, 52, 62, and 63.

Rapid Mapping. Since the map locations of the points of origin of transfer for most of the commonly used Hfrs are well known (see chapters 127 and 128), the vast difference in recombination frequency for early versus late Hfr markers allows a rapid localization of new markers between Hfr points of origin based on simple comparative crosses using various Hfrs. In cases in which an Hfr marker can be directly selected after transfer and recombination into a recipient strain, the early-marker versus late-marker approach can be greatly simplified by replica plating ("print mating") (49). This rapid mapping is especially useful in mapping large numbers of mutations (23), including temperature-sensitive Mu*d*(*lac*) fusions (98).

Genetic Linkage in Hfr Crosses. When a mutant phenotype does not allow a clean direct selection of the corresponding Hfr allele in crosses, but scoring it indirectly is possible, linkage to other (selectable) Hfr markers can be carried out by replica plating random selected Hfr F^- recombinant colonies to test for coinheritance (linkage) of the unmapped mutant phenotype (42, 49; less conventional methods have also been reported [95, 103]). The nature of genetic linkage in Hfr crosses has been studied and discussed several times in the literature over the years, resulting in several models and varying biases in interpretation (18, 34, 45, 89, 96). However, none of these analyses is generally satisfactory, as shown by the considerable differences in the results obtained in various studies using different strains and conditions. For Hfr donor markers proximal to (i.e., transferred earlier than) the selected marker (but not within a few minutes of either the Hfr point of origin or the selected marker itself), it is clear that their inheritance can be observed in anywhere from 30% to 80% of the selected recombinant colonies (34, 45, 96). No doubt contributing to this variation is the fact that merozygotes formed after mating often give rise to impure colonies (especially after relatively short matings and when certain Hfrs are used). The progeny often segregate with time into two or more recombinant types, which indicates some replication of unstably maintained genetic material in some cells (7, 34 [p. 242], 46, 101) and which furthermore masks some recombination events because of the mixture of genotypes in the clones. Most of the published linkage results refer to experiments in which insufficient time was allowed for segregation and appearance of all possible recombinant types (56, 101). This will lead to misinterpretation for any analysis based simply on

the fraction of recombinants scored as receiving any one marker. In one of the few published studies in which an extensive analysis was carried out for a relatively long chromosomal interval, Jacob and Wollman (34 [p. 227]) showed that virtually all possible recombinant genotypes can be recovered, in a more or less random way. The results indicate a broad spectrum of recombination events in terms of lengths of Hfr genetic information incorporated, various endpoints, various multiple crossovers, etc. Fifteen percent of the recombinants, at most, may have inherited all of the Hfr genetic information (perhaps even less if we allow for possible undetected crossovers in regions of the chromosome not tested). This value contrasts with the "80% long chunk" hypothesis recently proposed (89). In fact, however, the linkages observed experimentally, even when adequate time for segregation is allowed, can vary widely from strain to strain. For example, Wilkins and Howard-Flanders (99), who studied a shorter chromosomal interval but did allow for segregation, reported 64 to 69% coinheritance of proximal Hfr markers, using an Hfr which has a different point of origin and direction of transfer compared to the one used by Jacob and Wollman (and others), who obtained the very contrasting linkage results (15%) cited above. Rather complete incorporation of Hfr markers seems to occur in the case of certain *rec* recipient strains in which the proportions of various types of recombination events are different from those observed with the *rec*$^+$ configuration (see chapter 119).

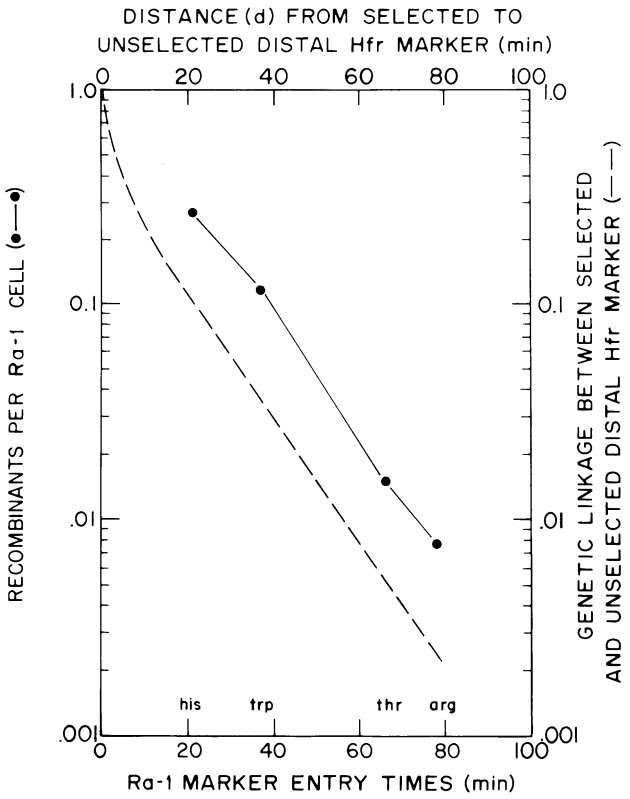

FIGURE 1 Typical transmission gradient (solid dots) for an Hfr (type Ra-1) x F$^-$ cross. Also plotted is a typical genetic linkage relationship (dashed line) for a distal Hfr marker when scored among colonies selected for an earlier Hfr marker in an Hfr x F$^-$ cross (see text) (modified from reference 48, with permission).

Genetic linkage for two markers less than a few minutes apart increases up to near 100%, depending on the separation. Jacob and Wollman estimated an approximate recombination frequency of 20% recombination (i.e., 20 recombination units) per minute (34 [p. 234]) in Hfr × F^- crosses. However, this was based on the early estimates of distance available at the time, namely 0.5 min for the *thr-leu* distance, which is now known to be 1.75 min. (chapter 109). Thus, a more useful revised linkage function based on the *thr-leu* and *leu-fhu* (=T1) (=1.9 min separation) recombination frequencies reported by Jacob and Wollman and others (18, 34 [p. 227], 45) is ~5% recombination per minute, for intervals less than a few minutes long.

This type of mapping function was obviously never intended to suggest absolute accuracy, nor does it apply to all situations. In particular, crosses between very close (intragenic) markers can show very large marker-specific effects in frequency (13, 34 [p. 230–231], 55, 67), which completely override any expectations from the 5% recombination per minute relation above. Nevertheless, in most cases this relation gives a very useful indication of distance in the 0.1 to 2 min range, and even when exact distances are uncertain, the relative ordering of mutations can be determined using reciprocal three-factor crosses (34 [p. 230–231]). If more than one close pair of markers are studied in the same cross, calculated intervals may be nonadditive due to negative interference effects, which can be considerable (51, 55). In summary, a marker situated proximal to a selected marker is tightly linked to it (>90%) only if the distance between markers is less than ~1 to 2 min. For very tightly linked markers, linkage can be decreased, and relative separation can be estimated by irradiating the merozygotes after transfer (97).

If the unselected marker is located distal to the selected one (i.e., if it is transferred later by the Hfr), the type of linkage referred to above is compounded by the gradient of transfer, and a linkage relation such as shown in Fig. 1 (dashed line) is observed.

For Hfr markers very close to the point of origin (less than ~1 min away) it is found that incorporation into recombinants after Hfr × F^- crosses can be very infrequent (22, 47, 73). This effect is not understood but likely has to do with the adjoining F factor DNA being nonhomologous to the F^- chromosome (thus decreasing the likelihood of pairing up to the point where homology begins) and/or the presence of the single-strand DNA end at the origin of transfer, which can facilitate the binding and activity of nucleases and other enzymes involved in recombination (see chapter 119).

In short, we have at present a very unclear picture of the contributions of various processes to genetic linkage in conjugation. Results such as those cited above, in practice, are subject to experimental conditions (102), marker-specific recombination effects (13, 55, 67), inhomogeneity of recombinant clones (7, 34 [p. 242], 46, 101), and strain differences (34, 45, 96). It is perhaps surprising that despite all these caveats, these approaches to mapping are very simple and informative and in many cases are the easiest first approach to mapping totally uncharacterized mutations.

Time of Entry. Once some idea of map position for a mutation is known, its location can usually be found to within ~1 to 2 min by time-of-entry ("blender") experiments (34). This is most easily carried out either with a mechanical shaker (52, 54, 62) or by using nalidixic acid to interrupt transfer (107). An example of the resolution obtainable with these methods is shown in Fig. 2. Various other time-of-entry curves obtained for different purposes are shown in references 8, 48, 50, 52, 54, 62, 63, and 104. Owing to variability sometimes observed in marker entry times due to strain differences, temperature, or distance from the point of origin (8, 48, 102), it is important to compare the time-of-entry curve of a new locus with that for at least one other (known) marker in the same experiment in order to get a meaningful comparison of chromosomal locations.

Time of Entry with Genetic Analysis. If two nearby markers are too close to be resolved by entry time, subsequent genetic analysis of the colonies obtained can clearly distinguish the order (97).

Complementation and Gene Dosage.

A global search by complementation or gene dosage can sometimes be used to find approximate map position in cases in which a mutant phenotype is recessive to wild type or can be scored by DNA-DNA or DNA-RNA hybridization or increased functional activity, as follows.

F-Prime and R-Prime Factors. A large set of F-prime factors is available to test for complementation (chapters 129 and 135). F-primes have also been used to locate rRNA genes (19) and tRNA genes (33). Selectable transposon markers can be easily introduced onto primes (63; chapter 129).

Genomic Libraries; Mapping a Cloned Gene. Libraries of cloned genes on plasmids (66; chapters 115 and 135) can be screened by complementation, gene dosage, or recombination. If a particular plasmid is found to carry the desired gene, but its map location is not known, it can be mapped (in the case of ColE1-derived plasmids) by selection for integration by homologous recombination in a conditional PolA$^-$ strain (19, 24, 25, 86) and subsequently can be mapped by the other methods in this chapter. Finer resolution of map position can often be obtained by complementation or recombination using specialized transducing phages (38; chapter 131) or deletion analysis (4, 16, 26, 32, 63, 82, 85; chapters 120, 135, 140, and 141).

Inserts which inactivate cloned genes and which have a selectable phenotype, such as an antibiotic resistance determinant, can be used to replace chromosomal genes by transformation of the cloned gene as a linear fragment into a *recD*$^-$ or *recBC*$^-$*sbcBC*$^-$ recipient (35, 77, 78, 100). Several hundred bases (preferably at least 500) of homology in the cloned fragment is necessary on either side of the insert in order to obtain homologous recombination into the recipient chromosome at a reasonable frequency. These and other approaches for mapping and exchange of alleles are outlined in Table 1.

Generalized Transduction

Mapping Functions for P1 and P22. For close-range mapping and gene ordering (intervals of less than ~2 min in *E. coli* and ~1 min (=1 centisome [cs]) in *Salmonella*), generalized transduction in *E. coli* with phage P1 and in *Salmonella* with phage P22 is usually the fastest genetic approach. This gene transfer process involves rare nonrandom pieces of chromosomal DNA carried and injected by phage heads into a recipient cell, followed by recombination into the recipient genome (60; chapter 130). Despite the nonrandom distribution of cut sites (10, 20, 40, 69, 72, 75, 81, 93) and the strong dependence of absolute transduction frequencies on marker, strain, position, and recombination (3, 15, 59, 61, 65), there is nevertheless usually an inverse correlation between distance between donor markers and cotransduction frequency. Wu (105) derived a relation between cotransduction frequency (*F*), marker separation (*d*), and effective length of transducing DNA (*L*), as follows:

$$F = (1 - d/l)^3$$

This function is plotted as a function of *d* in chapter 130. Shown on that same graph are curves using *L* = 2.1 (for *E. coli* transduction using bacteriophage P1) and 0.9 (for *Salmonella* transduction using bacteriophage P22). Aside from the inherent uncertainties involved in using this mapping function, mentioned above, the optimal values of *L* to use are not obvious; i.e., various end effects and the presence of Tn insertions may dictate a value of *L* which is significantly different from the normal length of the P1 or P22 genome (50, 80; chapter 130). Other curves which may be more applicable to crosses that involve large insertions such as Tn elements are discussed by Sanderson and Roth (80), and other mapping

functions are discussed in reference 50. Even with the uncertainties inherent in the use of transducing mapping functions, the sequence of markers can almost always be determined by using three-point crosses (15), and absolute distances of separation between markers can usually be determined to within 0.1 to 0.2 min. Noteworthy examples of transductional mapping can be found in the papers by Chelala and Margolin (10), Crawford and Preiss (15), Kelley (37), Signer et al. (84), and Stadler and Kariya (90).

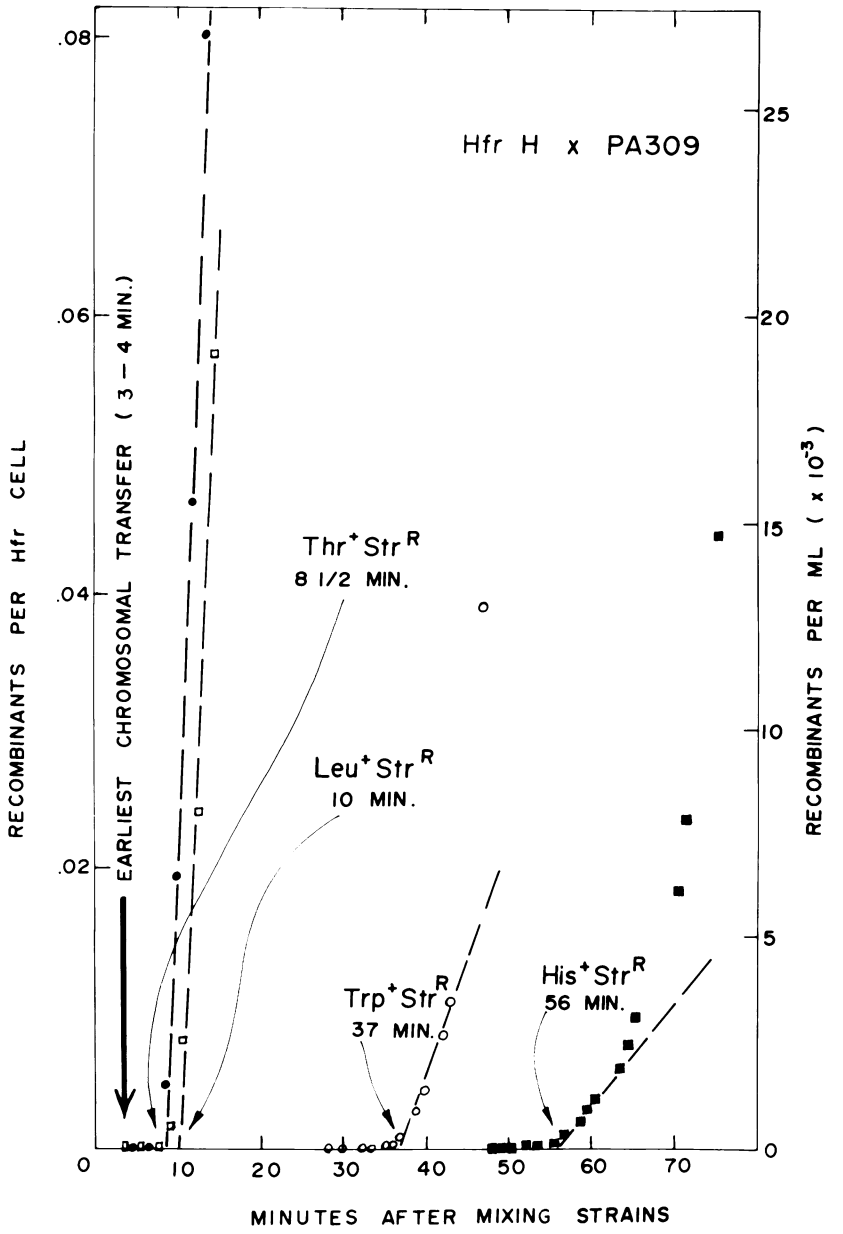

FIGURE 2 Time-of-entry ("blender") curves for a cross between an Hfr (Hayes type) and F⁻ (PA309). Experimental details are given in reference 52 (from reference 52, with permission).

In addition to P1 and P22, certain other bacteriophages are potentially useful for cotransductional mapping, including T4, which is capable of cotransduction over roughly twice the range of P1 (106; chapter 130).

Transformation

Transformation of chromosomal markers is not generally very practical for mapping purposes because strains which are wild type for *recB recC recD* yield virtually no haploid transformants (see chapter 119). However, in special cases cotransformation of linked markers has been observed in *E. coli* K-12, and in one study using a *recB*$^-$ *recC*$^-$ *sbcB*$^-$ strain (30) the cotransformation frequency, *f*, varied approximately as e^{-Kx}, where x = the distance between selected and unselected markers. In these experiments, the constant, *K*, was approximately 3.6 ± 0.3 min–1. Not enough is known about this system at present to know how generally useful it could be for mapping.

TABLE 1 Useful procedures for mapping and/or allele replacement

Procedure	Approximate range or resolution[a]	Comments	References[b]
Conjugation			
HFR × F$^-$ crosses:			
Uninterrupted	1–100 min	General strain construction	52, 74; A
Uninterrupted, transfer gradient	~3–10 min	Long-range mapping	52, 62, 63; †
Uninterrupted, transfer gradient, replica plating	5–20 min	Rapid mapping of many mutants, with selectable marker	23, 49, 98
Uninterrupted, linkage analysis	~2–30 min	Long-range mapping	34, 45, 96; †
Uninterrupted, linkage analysis, with irradiation	0.1–1 min	Short-range mapping (in special cases)	97
Interrupted	1–3 min	Mapping to within transducing range	52, 54, 62; †
Interrupted, with genetic analysis	0.1–1 min	Determination of gene order (in special cases)	97
Tn-directed points of origin	5–20 min	Rapid mid-range mapping of Tn insertion; "generalized conjugation"	11, 92; A
Transfer of chromosomal mutations to plasmids			70
F-prime × F$^-$ crosses	~1–15 min	Mapping by extent of F′ or by gene (or gene product) dosage	6, 52; B
Plus gene rearrangements (and homogenotization)		Transfer of alleles to chromosome or F′; "levitation"	11, 52; B
Transformation; allele replacement	0–1 min	Requires *recBC*$^-$ *sbcBC*$^-$ or *recD*$^-$ recipient	1, 30, 35, 78, 100
Transduction			
Generalized:	0–2 min (*E.c.*)	Mid-range mapping and allele replacement	63, 68, 74, 80; C
	0–1 min (*S.t.*)		
Tn*10* pools	0–100 min	Mapping by linkage to one of many sites	16, 52, 85
With known Tn insertions	0–100 min		39, 80, 87; D, E
Of integrated plasmids			57
With irradiation	0–100 min	Increases fraction of rare recombinants	C

Double transduction		Transfer of large plasmids	32, 83
"Transductional walking"			44
P22 with new hosts	0–1 min		64
Specialized	0.1–1 min	Mid-range mapping and cloning (depends on prophage locations)	A
Libraries	2–20 kb	Mapping and cloning	38; A, F
Libraries, recombination with chromosome	2–20kb		28
Lysates from family of prophages	0–7 min	Rapid mid-range mapping with set of Mu*d*-P22 lysogens	5
Using P22			31
Prophage induction	0.1–1 min	Deletion mapping and construction (depends on prophage and locations)	16, 85; A
Eduction	0.1–1 min	Deletion mapping and construction (special cases)	27
Complementation and/or recombination using plasmids or duplications	0.1–15 min		12; B, G, H
Allele replacement involving cloned genes			9, 26, 36, 43, 71, 76, 79, 88, 91
Useful markers for selection in allele replacement, deletion or essential genes			14, 21, 29, 41, 58, 94

[a]1 min ≈ 46 kb. *E.c., E. coli; S.t., Salmonella typhimurium* (*Salmonella enterica* serovar Typhimurium).
[b]A, chapter 135, this book; B, chapter 129; C, chapter 130; D, chapter 140; E, chapter 141; F, chapter 109; G, chapter 115; H, chapter 120. †, See the text.

REVIEW OF APPROACHES

Table 1 is an outline of the procedures mentioned in this chapter and others. These procedures comprise most of the current tools available for genetic approaches to mapping and allele replacement in *E. coli* and *Salmonella.* Other citations are given in references 2 and 53.

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LITERATURE CITED

1. **Arps, P. J., and M. E. Winkler.** 1987. Structural analysis of the *Escherichia coli* K-12 *hisT* operon by using a kanamycin resistance cassette. *J. Bacteriol.* **169:**1061–1070.
2. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. *Microbiol. Rev.* **44:**1–56.
3. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. *Bacteriol. Rev.* **40:**116–167.
4. **Beckwith, J. R., A. Galizzi, and G. R. Smith.** 1985. The tools of bacterial genetics, p. 1–23. *In* J. Scaife, D. Leach, and A. Galizzi (ed.), *Genetics of Bacteria.* Academic Press, Inc., New York.
5. **Benson, N. R., and B. S. Goldman.** 1992. Rapid mapping in *Salmonella typhimurium* with Mu*d*-P22 prophages. *J. Bacteriol.* **174:**1673–1681.
6. **Bergquist, P. L.** 1987. Incompatibility, p. 37–78. *In* K. G. Hardy (ed.), *Plasmids: a Practical Approach.* I. R. L. Press, Oxford.
7. **Bresler, S. E., V. A. Lanzov, and A. A. Blinkova.** 1967. Mechanism of genetic recombination during bacterial conjugation of *Escherichia coli* K12. I. Heterogeneity of the progeny of conjugated cells.

Genetics **56:**105–116.
8. **Broda, P., and J. F. Collins.** 1974. Gross map distances and Hfr transfer times in *Escherichia coli* K-12. *J. Bacteriol.* **117:**747–752.
9. **Chattoraj, D. K., K. Cordes, M. L. Berman, and A. Das.** 1984. Mutagenesis and mutation transfer induced by ultraviolet light in plasmid-cloned DNA. *Gene* **27:**213–222.
10. **Chelala, C. A., and P. Margolin.** 1974. Effects of deletions on cotransduction linkage in *Salmonella typhimurium:* evidence that bacterial chromosome deletions affect the formation of transducing DNA fragments. *Mol. Gen. Genet.* **131:**97–112.
11. **Chumley, F. G., R. Menzel, and J. R. Roth.** 1980. Hfr formation directed by Tn*10. Genetics* **91:**639–655.
12. **Clarke, L., and J. Carbon.** 1976. A colony bank containing synthetic *ColE1* hybrid plasmids representative of the entire *E. coli* genome. *Cell* **9:**91–99.
13. **Coulondre, C., and J. H. Miller.** 1977. Genetic studies of the *lac* repressor. III. Additional correlation of mutational sites with specific amino acid residues. *J. Mol. Biol.* **117:**525–575.
14. **Craine, B. L.** 1982. Novel selection for tetracycline- or chloramphenicol-sensitive *Escherichia coli. J. Bacteriol.* **151:**487–490.
15. **Crawford, I. P., and J. Preiss.** 1972. Distribution of closely linked markers following intragenic recombination in *Escherichia coli. J. Mol. Biol.* **71:**717–733.
16. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. *Advanced Bacterial Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **de Haan, P. G., W. P. M. Hoekstra, C. Verhoef, and H. S. Felix.** 1969. Recombination in *Escherichia coli.* III. Mapping by the gradient of transmission. *Mutat. Res.* **8:**505–512.
18. **de Haan, P. G., and C. Verhoef.** 1966. Genetic recombination in *Escherichia coli.* II. Calculation of incorporation frequency and relative map distances by recombinant analysis. *Mutat. Res.* **3:**111–117.
19. **Ellwood, M., and M. Nomura.** 1982. Chromosomal locations of the genes for rDNA in *Escherichia coli* K-12. *J. Bacteriol.* **149:**458–468.
20. **Enomoto, N.** 1967. Composition of chromosome fragments participating in phage P22-mediated transduction of *Salmonella typhimurium. Virology* **33:**474–482.
21. **Feutrier, J., M. Lepelletier, M.-C. Pascal, and M. Chippaux.** 1982. Tn1- insertions directed in the *pyr*D-*ser*C region and improved mapping of *pep*N in *Escherichia coli* K-12. *Mol. Gen. Genet.* **185:**518–519.
22. **Glansdorff, N.** 1967. Pseudoinversions in the chromosome of *Escherichia coli* K-12. *Genetics* **55:**49–61.
23. **Godson, G. N.** 1973. Isolation and partial characterization of temperature-sensitive mutants in ten loci that affect cell membrane synthesis in *Escherichia coli:* isolation and genetic sorting. *J. Bacteriol.* **113:**813–824.
24. **Greener, A., and C. Hill.** 1980. Identification of a novel genetic element in *Escherichia coli* K-12. *J. Bacteriol.* **144:**312–321.
25. **Gutterson, N. I., and D. E. Koshland, Jr.** 1983. Replacement and amplification of bacterial genes with sequences altered *in vitro. Proc. Natl. Acad. Sci. USA* **80:**4894–4898.
26. **Hamilton, C. M., M. Aldea, B. K. Washburn, P. Babitzke, and S. R. Kushner.** 1989. New method for generating deletions and gene replacements in *Escherichia coli. J. Bacteriol.* **171:**4617–4622.
27. **Hector, M. L.** 1984. Use of phenotypic suppression for direct selection of loss of aminoglycoside antibiotic resistance determinants. *Mol. Gen. Genet.* **196:**254–257.
28. **Henry, M. F., and J. E. Cronan, Jr.** 1991. Direct and general selection for lysogens of *Escherichia coli* by phage λ recombinant clones. *J. Bacteriol.* **173:**3724–3731.
29. **Hill, S. A., and J. W. Little.** 1988. Allele replacement in *Escherichia coli* by use of a selectable marker for resistance to spectinomycin: replacement of the *lexA* gene. *J. Bacteriol.* **170:**5913–5915.
30. **Hoekstra, W. P. M., P. G. de Haan, J. E. N. Bergmans, and E. M. Zuidweg.** 1976. Transformation

in *E. coli* K12: relation of linkage to distance between markers. *Mol. Gen. Genet.* **145:**109–110.
31. **Hoppe, I., and J. Roth.** 1974. Specialized transducing phages derived from *Salmonella* phage P22. *Genetics* **76:**633–654.
32. **Hughes, K. T., and J. R. Roth.** 1985. Directed formation of deletions and duplications using Mu*d*(Ap, *lac*). *Genetics* **109:**263–282.
33. **Ikemura, T., and H. Ozeki.** 1977. Gross map location of *Escherichia coli* transfer RNA genes. *J. Mol. Biol.* **117:**419–446.
34. **Jacob, F., and E. L. Wollman.** 1961. *Sexuality and the Genetics of Bacteria.* Academic Press, Inc., New York.
35. **Jasin, M., and P. Schimmel.** 1984. Deletion of an essential gene in *Escherichia coli* by site-specific recombination with linear DNA fragments. *J. Bacteriol.* **159:**783–786.
36. **Joyce, C. M., and N. D. F. Grindley.** 1984. Method for determining whether a gene of *Escherichia coli* is essential: application to the *polA* gene. *J. Bacteriol.* **158:**636–643.
37. **Kelley, W. S.** 1980. Mapping of the *polA* locus of *Escherichia coli* K12: genetic fine structure of the cistron. *Genetics* **95:**15–38.
38. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. *Cell* **50:**495–508.
39. **Kukral, A. M., K. L. Strauch, R. A. Maurer, and C. G. Miller.** 1987. Genetic analysis in *Salmonella typhimurium* with a small collection of randomly spaced insertions of transposon Tn*10*Δ16Δ17. *J. Bacteriol.* **169:**1787–1793.
40. **Langley, D., and J. R. Guest.** 1974. Biochemical and genetic characteristics of deletion and other mutant strains of *Salmonella typhimurium* LT2 lacking α-keto acid dehydrogenase complex activities. *J. Gen. Microbiol.* **82:**319–335.
41. **Lawes, M., and S. Maloy.** 1995. Mu*d*SacI, a transposon with strong selectable and counterselectable markers: use for rapid mapping of chromosomal mutations in *Salmonella typhimurium. J. Bacteriol.* **177:**1383–1387.
42. **Lederberg, J., and E. M. Lederberg.** 1952. Replica plating and indirect selection of bacterial mutants. *J. Bacteriol.* **63:**399–406.
43. **Lee, C. A., and M. H. Saier, Jr.** 1983. Use of cloned *mtl* genes of *Escherichia coli* to introduce *mtl* deletion mutations into the chromosome. *J. Bacteriol.* **153:**685–692.
44. **Lin, R.-J., M. Capage, and C. W. Hill.** 1984. A repetitive DNA sequence, *rhs*, responsible for duplications within the *Escherichia coli* K-12 chromosome. *J. Mol. Biol.* **177:**1–18.
45. **Lloyd, R. G., and A. Thomas.** 1984. A molecular model for conjugational recombination in *Escherichia coli* K12. *Mol. Gen. Genet.* 197:328–336.
46. **Lotan, D., E. Yagil, and M. Bracha.** 1972. Bacterial conjugation: an analysis of mixed recombinant clones. *Genetics* **72:**381–391.
47. **Low, B.** 1965. Low recombination frequency for markers very near the origin in conjugation in *E. coli. Genet. Res.* **6:**469–473.
48. **Low, B.** 1967. Inversion of transfer modes and sex factor-chromosome interactions in conjugation in *Escherichia coli. J. Bacteriol.* **93:**98–106.
49. **Low, B.** 1973. Rapid mapping of conditional and auxotrophic mutants of *Escherichia coli* K-12. *J. Bacteriol.* **113:**798–812.
50. **Low, K. B.** 1987. Mapping techniques and determination of chromosome size, p. 1184–1189. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology.* American Society for Microbiology, Washington, D.C.
51. **Low, K. B.** 1988. Genetic recombination: a brief overview, p. 13. *In* K. B. Low (ed.), *The Recombination of Genetic Material.* Adademic Press, Inc., New York.
52. **Low, K. B.** 1991. Conjugational methods for mapping with Hfr and F-prime strains. *Methods*

Enzymol. **204:**43–62.
53. **Low, K. B., and R. D. Porter.** 1978. Modes of gene transfer and recombination in bacteria. *Annu. Rev. Genet.* **12:**249–287.
54. **Low, K. B., and T. H. Wood.** 1965. A quick and efficient method for interruption of bacterial conjugation. *Genet. Res.* **6:**300–303.
55. **Maccacaro, G. A., and W. Hayes.** 1961. Pairing interaction as a basis for negative interference. *Genet. Res.* **2:**406–413.
56. **Mahajan, S. K., and A. R. Datta.** 1979. Mechanisms of recombination by the RecBC and the RecF pathways following conjugation in *Escherichia coli* K12. *Mol. Gen. Genet.* **169:**67–78.
57. **Mahan, M. J., J. M. Slauch, and J. J. Mekalanos.** 1993. Bacteriophage P22 transduction of integrated plasmids: single-step cloning of *Salmonella typhimurium* gene fusions. *J. Bacteriol.* **175:**7086–7091.
58. **Maloy, S. R., and W. D. Nunn.** 1981. Selection for loss of tetracycline resistance by *Escherichia coli. J. Bacteriol.* **145:**1110–1112.
59. **Masters, M.** 1977. The frequency of P1 transduction of the genes of *Escherichia coli* as a function of chromosomal position: preferential transduction of the origin of replication. *Mol. Gen. Genet.* **155:**197–202.
60. **Masters, M.** 1985. Generalized transduction, p. 197–215. *In* J. Scaife, D. Leach, and A. Galizzi (ed.), *Genetics of Bacteria.* Academic Press, Inc., New York.
61. **Masters, M., B. J. Newman, and C. M. Henry.** 1984. Reduction of marker discrimination in transductional recombination. *Mol. Gen. Genet.* 196:85–90.
62. **Miller, J. H.** 1972. *Experiments in Molecular Genetics,* p. 436–438 and 451–454. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
63. **Miller, J. H.** 1992. *A Short Course in Bacterial Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
64. **Neal, B. L., P. K. Brown, and P. R. Reeves.** 1993. Use of *Salmonella* phage P22 for transduction in *Escherichia coli. J. Bacteriol.* **175:**7115–7118.
65. **Newman, B. J., and M. Masters.** 1980. The variation in frequency with which markers are transduced by phage P1 is primarily a result of discrimination during recombination. *Mol. Gen. Genet.* **180:**585–589.
66. **Nishimura, A., K. Okiyama, Y. Kohara, and K. Horiuchi.** 1992. Correlation of a subset of the pLC plasmids to the physical map of *Escherichia coli* K-12. *Microbiol. Rev.* **56:**137–151.
67. **Norkin, L. C.** 1970. Marker-specific effects in genetic recombination. *J. Mol. Biol.* **51:**633–655.
68. **O'Connell, M. P.** 1984. Genetic transfer in prokaryotes: transformation, transduction, and conjugation, p. 2–13. *In* A. Pühler and K. N. Timmis (ed.), *Advanced Molecular Genetics.* Spring-Verlag KG, Berlin.
69. **Ozeki, H.** 1959. Chromosome fragments participating in transduction in *Salmonella typhimurium. Genetics* **44:**457–470.
70. **Park, C., and G. L. Hazelbauer.** 1986. Transfer of chromosomal mutations to plasmids via Hfr-mediated conduction. *J. Bacteriol.* **165:**312–314.
71. **Parker, B., and M. G. Marinus.** 1988. A simple and rapid method to obtain substitution mutations in *Escherichia coli:* isolation of a *dam* deletion/insertion mutation. *Gene* **73:**531–535.
72. **Pearce, U., and B. A. D. Stocker.** 1965. Variation in composition of chromosome fragments transduced by phage P22. *Virology* **27:**290–296.
73. **Pittard, J., and E. M. Walker.** 1967. Conjugation in *Escherichia coli.* Recombination events in terminal regions of transferred deoxyribonucleic acid. *J. Bacteriol.* **94:**1656–1663.
74. **Provence, D. L., and R. Curtiss III.** 1994. Gene transfer in gram-negative bacteria, p. 317–345. *In* P. Gerhardt, R. G. E. Murray, W. A. Wood, and N. R. Krieg (ed.), *Methods for General and Molecular Bacteriology.* American Society for Microbiology, Washington, D.C.

75. **Roth, J. R., and P. E. Hartman.** 1965. Heterogeneity in P22 transducing particles. *Virology* **27:**297–307.
76. **Russel, M., and P. Model.** 1984. Replacement of the *fip* gene of *Escherichia coli* by an inactive gene cloned on a plasmid. *J. Bacteriol.* **159:**1034–1039.
77. **Russell, C. B., and F. W. Dahlquist.** 1989. Exchange of chromosomal and plasmid alleles in *Escherichia coli* by selection for loss of a dominant antibiotic sensitivity marker. *J. Bacteriol.* **171:**2614–2618.
78. **Russell, C. B., D. S. Thaler, and F. W. Dahlquist.** 1989. Chromosomal transformation of *Escherichia coli recD* strains with linearized plasmids. *J. Bacteriol.* **171:**2609–2613.
79. **Saarilahti, H. T., and E. T. Palva.** 1985. *In vivo* transfer of chromosomal mutations onto multicopy plasmids utilizing *polA* strains: cloning of an *ompR2* mutation in *Escherichia coli* K12. *FEMS Microbiol. Lett.* **26:**27–33.
80. **Sanderson, K. E., and J. R. Roth.** 1988. Linkage map of *Salmonella typhimurium,* edition VII. *Microbiol. Rev.* **52:**485–532.
81. **Sandri, R. M., and H. Berger.** 1980. Structure of abortively transduced DNA. *Virology* **106:**30–40.
82. **Schmeissner, U., D. Ganem, and J. H. Miller.** 1977. Genetic studies of the *lac* repressor. II. Fine structure deletion map of the *lacI* gene, and its correlation with the physical map. *J. Mol. Biol.* **109:**303–326.
83. **Segall, A. M., and J. R. Roth.** 1994. Approaches to half-tetrad analysis in bacteria: recombination between repeated, inverse-order chromosomal sequences. *Genetics* **136:**27–39.
84. **Signer, E. R., J. R. Beckwith, and S. Brenner.** 1965. Mapping of suppressor loci in *Escherichia coli. J. Mol. Biol.* **14:**153–166.
85. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. *Experiments with Gene Fusions.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
86. **Silver, P., and W. Wickner.** 1983. Genetic mapping of the *Escherichia coli* leader (signal) peptidase (*lep*): a new approach for determining the map position of a cloned gene. *J. Bacteriol.* **154:**569–572.
87. **Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel, W. Dove, K. J. Jaacks, A. D. Grossman, J. W. Erickson, and C. A. Gross.** 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli. Microbiol. Rev.* **53:**1–24.
88. **Slater, S., and R. Maurer.** 1993. Simple phagemid-based system for generating allele replacements in *Escherichia coli. J. Bacteriol.* **175:**4260–4262.
89. **Smith, G. R.** 1991. Conjugational recombination in *E. coli:* myths and mechanisms. *Cell* **64:**19–27.
90. **Stadler, D., and B. Kariya.** 1973. Marker effects in the genetic transduction of tryptophan mutants of *E. coli. Genetics* **75:**423–429.
91. **Steed, P. M., and B. L. Wanner.** 1993. Use of the *rep* technique for allele replacement to construct mutants with deletions of the *pstSCAB-phoU* operon: evidence of a new role for the PhoU protein in the phosphate regulon. *J. Bacteriol.* **175:**6797–6809.
92. **Stibitz, S., and N. H. Carbonetti.** 1994. Hfr mapping of mutations in *Bordetella pertussis* that define a genetic locus involved in virulence gene regulation. *J. Bacteriol.* **176:**7260–7266.
93. **Susskind, M. M., and D. Botstein.** 1978. Molecular genetics of bacteriophage P22. *Microbiol. Rev.* **42:**385–413.
94. **Takiff, H. E., T. Baker, T. Copeland, S.-M. Chen, and D. L. Court.** 1992. Locating essential *Escherichia coli* genes by using mini-Tn*10* transposons: the *pdxJ* operon. *J. Bacteriol.* **174:**1544–1553.
95. **Thwaites, W.** 1968. Device for the comparison of replica plates. *Appl. Microbiol.* **16:**956–957.
96. **Verhoef, C., and P. G. de Haan.** 1966. Genetic recombination in *Escherichia coli.* I. Relation between linkage of unselected markers and map distance. *Mutat. Res.* **3:**101–110.
97. **Wann, M., S. K. Mahajan, and T. H. Wood.** 1970. Genetic mapping in *Escherichia coli* K-12 by radiation-induced crossing-over. *J. Bacteriol.* **103:**601–606.

98. **Wanner, B. L., and R. McSharry.** 1982. Phosphate-controlled gene expression in *Escherichia coli* K-12 using Mu*dl*-directed *lacZ* fusions. *J. Mol. Biol.* **158:**347–363.
99. **Wilkins, B. M., and P. Howard-Flanders.** 1968. The genetic properties of DNA transferred from ultraviolet-irradiated Hfr cells of *Escherichia coli* K-12 during mating. *Genetics* **60:**243–255.
100. **Winans, S. C., S. J. Elledge, J. H. Krueger, and G. C. Walker.** 1985. Site-directed insertion and deletion mutagenesis with fragments in *Escherichia coli. J. Bacteriol.* **161:**1219–1221.
101. **Wood, T. H.** 1967. Genetic recombination in *Escherichia coli:* clone heterogeneity and the kinetics of segregation. *Science* **157:**319–321.
102. **Wood, T. H.** 1968. Effects of temperature, agitation, and donor strain on chromosome transfer in *Escherichia coli* K-12. *J. Bacteriol.* **96:**2077–2084.
103. **Wood, T. H., and S. K. Mahajan.** 1970. A rapid and efficient replica plating technique. *Genet. Res.* **15:**335–338.
104. **Wood, W. H., and J. B. Egan.** 1972. Integration site of non-inducible coliphage 186. *J. Bacteriol.* **111:**303–307.
105. **Wu, T. T.** 1966. A model for three-point analysis of random general transduction. *Genetics* **54:**405–410.
106. **Young, K. K. Y., and G. Edlin.** 1983. Physical and genetical analysis of bacteriophage T4 generalized transduction. *Mol. Gen. Genet.* 192:241–246.
107. **Zipkas, D., and M. Riley.** 1976. Simplified method for interruption of conjugation in *Escherichia coli. J. Bacteriol.* **126:**559–560.