JOURNAL OF BACTERIOLOGY, May 1987, p. 1787–1793
0021-9193/87/051787-07$02.00/0
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Vol. 169, No. 5

# Genetic Analysis in *Salmonella typhimurium* with a Small Collection of Randomly Spaced Insertions of Transposon Tn*10*Δ16Δ17

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Received 6 October 1986/Accepted 29 January 1987

**We report the isolation of a group of 279 *Salmonella typhimurium* strains carrying randomly spaced insertions of the minitransposon Tn*10*Δ16Δ17 and describe the use of these strains to facilitate genetic analysis. The insertions were isolated initially in individual recombinant λ clones from a genomic library. Individual insertions were then moved into the *S. typhimurium* chromosome, where the distribution of insertion sites relative to standard genetic markers was analyzed in a series of transductional crosses. Since a different, randomly chosen clone was used to generate each insertion, the distribution of insertion positions should have been as random as the cloning events leading to the formation of the library. In agreement with this expectation, most *S. typhimurium* markers tested were cotransducible with one or more of these Tn*10*Δ16Δ17 insertions. We expect that most new mutations will be quickly classified and mapped by determination of the pattern of cotransduction with this set of insertions. This use is illustrated by the analysis of a group of *lac* operon fusions regulated by anaerobiosis. We also describe several other applications that should make this collection a useful new tool in *S. typhimurium* genetics.**

Recently, we described (11) a convenient system for insertion mutagenesis of λ bacteriophage by using a 3-kilobase derivative of transposon Tn*10* (Tn*10*Δ16Δ17 [6]). This system allows the efficient isolation of many independent insertions into a cloned segment of DNA carried in a λ vector. When the cloned DNA originates from *Salmonella typhimurium* or *Escherichia coli*, insertions in the cloned segment can be easily moved to the homologous site in the bacterial chromosome by recombination.

Here, we show that this two-step procedure for placing Tn*10*Δ16Δ17 insertions in bacterial chromosomes can be exploited to create a small collection of strains bearing Tn*10*Δ16Δ17 insertions distributed in highly random fashion around the *S. typhimurium* chromosome. Each such strain is derived by the two-step procedure from a different λ-*S. typhimurium* in vitro recombinant chosen at random from a genomic library. Thus the overall distribution of insertion sites is governed by the randomness of molecular cloning events rather than by the insertion specificity of the transposable element, and the tendency of Tn*10* to insert at hot spots is avoided.

Using this procedure, we have generated a collection of 279 strains, each carrying an independently derived Tn*10*Δ16Δ17 insertion. These strains have been characterized by testing the insertions individually for transductional linkage to standard genetic markers. Our results show that the vast majority of *S. typhimurium* genetic loci can be linked to at least one insertion in the collection. Further, we illustrate how this collection of strains can be used to achieve initial classification of uncharacterized mutant collections. In many cases, this classification will identify a λ clone carrying the gene of interest. Finally, we suggest other applications made possible by the interplay of transposition and molecular cloning.

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## MATERIALS AND METHODS

**Bacterial strains.** The bacterial strains used in this work are listed in Table 1. In addition to the strains listed, we used other *S. typhimurium* strains bearing a wide variety of mutations for transductional crosses (see Tables 2 and 3). Most of the loci listed in these tables are compiled in the most recent *S. typhimurium* linkage map (14). Exceptions include *oxd* and *oxr* loci (15), *dna-204*, *dna-599*, *dna-603*, and *dna-610* (from the same experiments described in reference 11 but not characterized in detail), *dnaZ* (4), *corA* (cobalt resistance; 7a), *dal* (16; subsequently mapped to min 91), and *pepM* (C. G. Miller, K. L. Strauch, A. M. Kukral, J. L. Miller, P. T. Wingfield, G. Mazzei, R. Werlan, P. Graber, and N. R. Movva, Proc. Natl. Acad. Sci. USA, in press).

General bacteriological and genetic procedures and media were described previously (2). The collection of bacterial strains described in this paper is available from the Salmonella Genetic Stock Center, Department of Biology, University of Calgary, Calgary, Alberta, Canada. The related λ clones are available from the authors. Users of these strains are asked to send new mapping data to R. Maurer.

**Isolation of strains containing Tn*10*Δ16Δ17.** *Sau*3AI partial-digest fragments (15 to 20 kilobases) of DNA of strain DB9005 were ligated with *Bam*HI-digested λ1059 DNA (9, 11). The ligated DNA was packaged in vitro (8), and a dilution of the packaged mixture was plated on a lawn of strain RM42. This host permits plaque formation only by phages carrying foreign DNA in place of the central fragment of λ1059. Thus, every plaque formed on strain RM42 represented the progeny of an independent λ-*S. typhimurium* recombinant. A total of 316 well-isolated plaques were then gridded onto two bacterial lawns, first on strain RM11 to form a source for a permanent phage stock and then on strain RM22. In strain RM22, Tn*10*Δ16Δ17 undergoes frequent transposition. Lambda phage propagated on this host acquire Tn*10*Δ16Δ17 insertions at a frequency of approximately $10^{-4}$ (11). The unique identity of each phage line was maintained in all subsequent steps.

TABLE 1. Bacterial strains

| Strain (synonym) | Genotype | Reference |
|---|---|---|
| DB9005[a] | $F^-$ *thyA deo* (fels-free, plasmid free) | 11 |
| RM5[b] (TS736) | F'112(?) Δ*malB trpB2 ilv-452 metA22 metE551 rpsL120 flaA66 xyl-404 galE496 H1-b nml H2-e,n,x* (Fels-2)$^-$ *hsdL6* ($r^-$ $m^+$) *hsdSA29* ($r^-$ $m^+$); cold-sensitive growth | 5, 11, 13 |
| RM11[c] (DB5564) | $F^-$ *thr leu supE lacY* $T1^r$ $T5^r$ $\phi 80^r$ | 11 |
| RM22[c] (RB132) | $F^-$ Δ*lac gal rpsL zxx*::Tn*10*Δ4HH104(pNK217) | 11 |
| RM42[c] (Q359) | $F^-$ *hsdR supE* $\phi 80^r$ (P2) | 9 |

[a] *S. typhimurium* LT2.
[b] *E. coli* K-12–*S. typhimurium* LT2 λ-sensitive hybrid derived from CL4419 (5).
[c] *E. coli* K-12.

To amplify selectively the λ::Tn*10*Δ16Δ17 phages formed on the RM22 plate, the plug of agar containing the phage spot was suspended in 0.5 ml of $SMO^+$ (20 mM Tris [pH 7.5], 10 mM NaCl, 10 mM $MgSO_4$, 0.01% gelatin) and treated with chloroform to form a lysate. A drop of this lysate was spotted on LB tetracycline (7 μg/ml) agar seeded with $5 \times 10^8$ cells of strain RM11 in drug-free top agar. On such a plate, the cells, which are tetracycline sensitive, form a faint lawn in which only phage that have acquired an insertion of Tn*10*Δ16Δ17 can form a plaque.

Thus amplified, the λ::Tn*10*Δ16Δ17 were eluted into $SMO^+$. This new lysate was spotted onto a λ tetracycline (10 μg/ml) agar plate seeded (by spreading) with 0.1 ml of overnight culture of strain RM5, a *S. typhimurium-E. coli* hybrid strain that can adsorb λ (13). Tetracycline-resistant survivors of this infection are not lysogenic for λ and contain the Tn*10*Δ16Δ17 element as a chromosomal insertion formed by recombination between the chromosome and homologous sequences in the λ insert (11). One tetracycline-resistant colony from each spot was purified and retained. The entire procedure was completed successfully in 279 cases (88%).

The insertions were given allele numbers 3001 to 3316 to correspond to their parental λ phages, λAK1 to λAK316. When we failed to isolate a chromosomal insertion for a particular λ, we omitted that allele number (see Table 3, footnote *a*, for a list). For simplicity, in the tables we have omitted the three-letter designations which would convey the map position of the insertions (e.g., *zad-3131* for an insertion at min 3, or *zxx-3011* for an unmapped insertion [1]).

**P22 lysates and rapid transduction experiments.** P22 lysates were prepared by mixing together, in a 5-ml screw-cap tube, 2 ml of LB broth containing 0.05% (wt/vol) galactose, 0.5 ml of overnight culture of the tetracycline-resistant bacterial strain, and $10^7$ PFU of P22HT*int*12/4. The lysates were incubated overnight at 37°C with aeration and then sterilized with chloroform. A few lysates chosen at random had titers of $2 \times 10^9$ to $5 \times 10^9$ PFU/ml. For transduction experiments, lysates were used either undiluted or at a 10-fold dilution.

For long-term storage as well as convenient dispensing of P22 lysates, we used micro-fraction collector racks (Gilson Medical Electronics). The individual wells hold about 1.7 ml of lysate, which can be kept sterile with chloroform. During storage, the plates were sealed with adhesive-backed Mylar sealers (Flow Laboratories). A multipronged inoculator was used to transfer 28 lysates at a time to transduction plates.

For rapid identification of Tn*10*Δ16Δ17 insertions linked to genetic markers, the following procedure was used. Recipient cells were grown in LB broth to mid-log phase, pelleted, and resuspended at a density of approximately $5 \times 10^9$ cells per ml in fresh broth. Concentrated cells (0.1 ml) were spread on the surface of an LB agar plate containing 10 μg of tetracycline per ml. Next, the P22 lysates to be tested were spotted onto the plates. After overnight incubation, each P22 lysate produced a spot of growth consisting of 50 to 500 tetracycline-resistant transductants. In a second step, these transductants were replica plated to selective media. On the selective plates, linked insertions produced a response that varied from a few colonies to confluent growth. Unlinked insertions generally yielded zero or one colony per spot. (The occasional colony arising in the latter situation presumably reflects transduction of a revertant, reversion of a transductant, or transduction by two independent particles.) Strains carrying β-galactosidase-expressing *lac* operon fusions were tested in a single step by using tetracycline agar plates containing the chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) or by using MacConkey lactose tetracycline (10 μg/ml) plates. Linkage was confirmed and quantitated by carrying out individual whole-plate transductions with each insertion that had been tentatively identified as linked in the spot transductions.

**Rescue of Tn*10*Δ16Δ17 onto λ phage from a bacterial chromosome.** Rescue of Tn*10*Δ16Δ17 onto λ phage from a bacterial chromosome was achieved by homologous recombination as described previously (11).

## RESULTS

**Choice of Tn*10*Δ16Δ17.** The intended experimental steps imposed certain limitations that dictated careful choice of a transposon. Several features of Tn*10*Δ16Δ17 made it particularly suitable for our purposes. First, its size (2.95 kilobases) allowed it to be readily accommodated in λ phage genomes. Second, it confers a drug resistance which is selectable in λ phage during lytic growth and is also selectable in bacteria. It was also helpful that the drug, at low concentrations, is bacteriostatic in sensitive bacteria. At such concentrations of the drug, cellular resistance can be expressed upon infection by a λ::Tn*10*Δ16Δ17 phage or a P22 transducing particle. Multiple transductions to tetracycline resistance can be carried out on a single drug plate without the inconvenience of preincubation in the absence of drug. In contrast, we could not identify concentrations of either kanamycin or chloramphenicol that permitted direct transduction to resistance. The third important feature of this element is experimental control of its transposition. The element does not encode its own transposition functions, but in a suitable host in which transposition functions are provided by a second Tn*10*, Tn*10*Δ16Δ17 transposes readily. In other strains in which no transposition functions are provided, this element does not transpose. In these strains, transfer of the insertion from one replicon to another occurs exclusively by homologous recombination. This feature is important both in moving the insertions back and forth between λ and bacterial chromosomes, and in using the insertions as stable genetic markers in linkage studies.

**Random distribution of Tn*10*Δ16Δ17 insertions.** Insertions of Tn*10*Δ16Δ17 in the *S. typhimurium* chromosome were obtained by transposition into individual λ clones followed by homologous recombination between the mutant phage and the bacterial chromosome. The genomic library used as a source of clones contains 15- to 20-kilobase *Sau*3AI partial-digest fragments of *S. typhimurium* DNA. Since

*Sau*3AI partial digestion produces nearly random fragmentation of the genome, every chromosomal region should be present in the genomic library at nearly the same frequency. The successful isolation of clones bearing widely dispersed *dna* (DNA replication) genes from the same library used here suggests that *S. typhimurium* genes are indeed well represented (11). Since we retained only one chromosomal insertion corresponding to each initial λ clone, the distribution of chromosomal insertion sites should reflect the random character of the library. The procedure used to isolate Tn*10*Δ16Δ17 insertions in the λ clones allowed sensitive detection of the transposition products. Insertions into the cloned DNA could be obtained, therefore, even if the cloned sequence were a relatively poor target for Tn*10* transposition. The vast majority of clones originally picked gave rise to an insertion-containing phage. Nearly all of the dropouts resulted from failure to move the insertion into the chromosome (as discussed below). We believe therefore that we have avoided the problem of hot spotting—the insertion of Tn*10* at relatively few preferred sites—inherent in other methods for generating populations of "random" insertions.

The randomness of insertion sites was assessed by screening our collection for insertions linked to a variety of known loci in P22-mediated transduction. These tests involved spot transductions, in which P22 lysates grown on each strain of the collection were spotted on lawns of the chosen, tetracycline-sensitive recipient on tetracycline plates. By using the procedure described in Materials and Methods, it was possible to carrry out 28 transductions per petri dish with direct selection for tetracycline resistance. The entire collection could be screened for linkage to a particular marker by using 10 plates.

To distinguish genuine joint transduction of tetracycline resistance and the unselected marker, indicative of linkage, from false cotransduction mediated by other mechanisms such as double transduction (involving contributions by two distinct transducing particles) or transduction to tetracycline resistance accompanied by reversion of the unselected marker, all suspected cases of cotransduction were verified in a low-multiplicity transduction carried out by standard protocols. Our experience suggests that a cotransduction frequency greater than about 5% will produce an unmistakable signal in the spot test. On the basis of this result, we believe we can reliably detect cotransduction of individual Tn*10*Δ16Δ17 insertions with any markers falling within a 1-min interval (0.5 min to each side of the insertion).

The results of the survey are shown in Tables 2 and 3. Table 2 shows the genetic loci and the linked insertions that were found. Table 3 shows the Tn*10*Δ16Δ17 insertions individually, any genetic markers that have been linked to each one, and the cotransduction frequency. The salient points are as follows. First, the vast majority of tested markers were linked to at least one insertion. Second, the linkages that were observed were consistent with the known genetic map of *S. typhimurium*. Unlinked markers were associated with different Tn*10*Δ16Δ17 insertions. Conversely, markers that exhibited individual linkage to a common insertion invariably were markers known to be closely linked (e.g., the *pepA argI pyrB* group). In some cases, linked markers did not share common linkage to all the nearby Tn*10*s. For example, *tyrA* and *pheA* are linked in common to two Tn*10*s, but a third Tn*10* (no. 3055) is linked only to *tyrA*. This observation, which was verified in a low-multiplicity transduction, can be explained if the map order is Tn*10-tyrA-pheA*. This and other similar examples are noted in Tables 2 and 3.

**Characterization of unclassified mutations.** The advantage of working with a small collection of transposon insertions lies in the preservation of the identity of individual insertions. Thus, mutual linkage of two mutations can be established by their individual linkage to a common insertion. Conversely, mutations can be shown to be unlinked by their individual linkage to mutually exclusive sets of insertions. The use of the collection to sort *lac* operon fusions generated with Mu d1(*lac* Ap) illustrates these points. A group of approximately 30 independently isolated Mu d-*lac* fusions to promoters regulated by anaerobiosis were isolated (15; C. Miller, unpublished data). Each of these strains produces fisheye (red-centered) colonies on MacConkey lactose plates. Each of these fusions was tested for cotransduction with each member of the Tn*10*Δ16Δ17 collection by spot transductions on MacConkey lactose tetracycline medium. Insertions linked to the *lac* fusions were easily identified as spots of transductants containing both fisheye and white colonies. The results of these transductions allowed the placement of each anaerobically regulated fusion into a unique class on the basis of its pattern of linkage to one or more Tn*10*Δ16Δ17 insertions (Table 4). Mutations (*oxr*) that prevent the induction by anaerobiosis can also be easily classified in a similar way. These mutations change the appearance of a strain carrying an *oxd* fusion from fisheye to pink on MacConkey lactose agar. In this case, Tn*10*Δ16Δ17 insertions linked to the *oxr* mutation are identified as spots of transductants that contain both fisheye and pink colonies. Obviously, these procedures can be used to classify any set of *lacZ* fusions and mutations which regulate their expression.

**Identification of Tn*10*Δ16Δ17 insertions linked to unselectable alleles.** The techniques described above can also be adapted to the mapping of mutations when the corresponding wild-type allele is not selectable or readily scorable. This is illustrated by the mapping of a mutation (*pepM100*) that allows a multiply peptidase-deficient *S. typhimurium* strain to use the peptide Met-Gly-Gly (MGG) as a source of methionine. As above, the P22 lysates were used to transduce the *pepM100* strain to tetracycline resistance. In this case, however, the transductant spots were chloroformed in situ and used directly (by replica plating) as a source of P22 to transduce a *met pepM*$^+$ strain to tetracycline resistance. These transductants were then replica plated to score for growth on MGG. A linked Tn*10*Δ16Δ17 (no. 3149) was identified, and its linkage was subsequently validated by low-multiplicity transduction. This procedure depends on the formation of new transducing particles by the growth of phage on some of the original transductants. In particular, the pertinent transductants are those that remain *pepM100* while acquiring a Tn*10*Δ16Δ17 near the *pepM* locus. P22 phage mulitplying on such transductants then produce particles that cotransduce the second-stage recipients to tetracycline resistance and growth on MGG.

**Use of Tn*10*Δ16Δ17 insertions in directed Hfr formation.** In many cases (Table 4), the exact map position of a new mutation will be known as soon as its pattern of linkage to the Tn*10*Δ16Δ17 insertion set has been determined. When the map position of the linked insertions is unknown, the insertion can be useful for determining the map position. Chumley and Roth (1) have described a procedure in which Tn*10* is used as a region of homology to direct the integration of F′::Tn*10* factors, thus forming Hfrs with origins of transfer at the site of the Tn*10*. We have found that Tn*10*Δ16Δ17 also serves as a target for integration of the F′ factors described by Chumley and Roth (1), forming Hfrs that can be

TABLE 2. Linkage groups[a]

| Approx map position | Standard marker | Linked insertion |
|---|---|---|
| 0 | *thrB13* | 3116 |
| 3 | *leuBCD485* | 3131, 3137 |
| 3.5 | *pepM100* | 3149 |
| 6 | *dnaE305* | 3262 |
| 7 | *proAB25*, *pepD3* | 3139, 3150, 3214, 3215 |
| 8 | *proC134* | 3029, 3030, 3170 |
| 11 | *purE11* | 3217, 3260 |
| 18 | *galE496*,[b] *oxd-8* | 3020 |
| 19 | *oxd-12*, *aroA9* | 3142 |
| 21 | *pyrD13*, *pepN90* | 3232 |
| 23 | *pyrC7* | 3176 |
| 25 | *pepT7*::Mu d1 (*lac*) | 3041, 3140, 3233 |
| 25 | *oxd-18* | 3041, 3140, 3233 |
| 25 | *oxd-6* | 3032, 3140, 3233 |
| 26–32 | *dcp-1* | 3314 |
| 29–32 | *oxrA1* | 3127, 3258, 3261 |
| 36 | *dadB1* | 3037, 3063, 3234 |
| 42 | *his-644* | 3255 |
| 44 | *metG419* | 3061 |
| 46 | *oxd-3*, *oxd-9*, *oxd-10*, *oxd-13*, *oxd-14*, *oxd-16* | 3118, 3198, 3291 |
| 47 | *purF145* | 3138 |
| 50 | *cysA533* | 3271 |
| 55.5 | *tyrA19* | 3028, 3055, 3181, 3222 |
| 55.5 | *pheA15* | 3181, 3222 |
| 61.5 | *thyA6* | 3121, 3122, 3146 |
| 62 | *lysA*::Tn*10*[c,d] | 3098, 3132, 3143, 3179, 3231 |
| 62.5 | *pepP1* | 3085, 3209 |
| 62.5 | *serA13* | 3085, 3159, 3209 |
| 64 | *metC88* | 3017, 3213, 3246 |
| 64 | *oxd-5* | 3017, 3076, 3134, 3189, 3213, 3246 |
| 69 | *argG72*, *dna-610* | 3163, 3177 |
| 71 | *aroE150*, *oxrB8* | 3124, 3195, 3301 |
| 73 | *aroB74* | 3081, 3173 |
| 78 | *xylA26* | 3040 |
| 79 | *oxrE11* | 3312 |
| 79.5 | *pyrE27*[d] | 3312 |
| 80 | *gltC*[d] | 3294 |
| 80.5 | *mgtB11*::Mu dJ | 3048, 3104, 3123, 3125, 3205, 3295 |
| 81 | *dnaA727* | 3119, 3120, 3130 |
| 81 | Tn*10*Δ16Δ17 Kan[re] | 3068, 3119, 3120, 3130 |
| 82 | *apeR49*::Tn*5* | 3068, 3119, 3120, 3130, 3167, 3241, 3248 |
| 83 | *ilvC401*[d] | 3145, 3265 |
| 84 | *corA27* | 3161, 3162, 3235 |
| 84 | *metE551*[b] | 3024, 3161, 3162, 3235 |
| 84 | *pepQ1* | 3024, 3145, 3161 |
| 85 | *polA2*[d] | 3024 |
| 96 | *purA87* | 3290 |
| 98 | *pyrB64*, *argI547*, *pepA16* | 3103, 3160, 3196, 3200, 3252, 3253 |
| 99.5 | *serB42* | 3042, 3112, 3116, 3240 |
| ? | *dnaZ731* | 3154, 3166 |
| ? | *dna-204* | 3091 |
| ? | *dna-599* | 3210, 3266 |
| ? | *dna-603* | 3026, 3211 |
| ? | *oxd-4*, *oxd-11*, *oxd-17* | 3011, 3023, 3093, 3144 |
| ? | *oxd-15* | 3018, 3251 |
| ? | *oxd-2* | 3012, 3084 |

[a] Insertions linked to the wild-type allele (except as noted below) of each standard marker are listed. When more than one marker or more than one insertion are shown for a given entry, each such marker is linked to each such insertion. Markers tested for which we could find no evidence of a linked insertion were *dal-1*, *argH88*, *oxd-7*, *tre-3*, and *rha-461*. Potentially linked insertions not listed as linked in this table, and for which no transductional data are listed in Table 3, gave no evidence of linkage in spot tests and were not tested further. However, in these cases, a low percentage of linkage has not been ruled out (e.g., *pepP1* and insertion 3159).

[b] The insertion was linked to the mutant allele.

[c] For mapping linkage to *lysA*::Tn*10*, prototrophic recombinants were selected, and any of these that proved tetracycline resistant were scored as acquiring the nearby Tn*10*Δ16Δ17. As a control, the insertion *zhb-3195*::Tn*10*Δ16Δ17, unlinked to *lysA*, gave no tetracycline-resistant prototrophic recombinants when used as a donor in a parallel cross.

[d] These markers were not tested in spot tests. Therefore, their possible linkage to nearby insertions other than those listed in this table, or specifically mentioned as "not linked" in Table 3, is unknown.

[e] This insertion, present in the recipient strain, confers kanamycin resistance and is linked to *dnaA*.

used to map the insertion site. This procedure is illustrated by the mapping of *oxd-3*::Mu d. First the pattern of linkage of *oxd-3*::Mu d to the set of Tn*10*Δ16Δ17 insertions was determined as described above. Next, one of the linked insertions, no. 3291, was used as a target for F′::Tn*10* integration. Then the resulting Hfrs were crossed with a set of auxotrophs, prototrophic recombinants were selected, and the number of recombinants was scored. The results indicate that the transfer origin was between *his* (42 map units) and *cysC* (60 map units).

A second set of crosses to other mutations in this interval refined this location to the region between *his* and *purF* (47 map units). Finally, transductional crosses between the *oxd-3*::Mu d strain and a series of strains carrying mutations in this region showed that *oxd-3* is cotransducible with *hisP* (27%) and *ompC* (38%). The map position of *oxd-3* (and the other members of its class [Table 4]) is therefore 46 map units.

## DISCUSSION

The application of transposons to genetic mapping and other problems in bacteria is by now familiar (10). Typically, a pool of several thousand independent insertions is screened for an insertion linked to a mutation of interest. Such an insertion can then be mapped, often more easily than the original mutation, by transductional or conjugational crosses. Subsequently, the linked insertion can simplify other tasks such as derivation of additional mutations of the same gene, strain construction, or construction of defined duplications containing the gene in question.

Even more information could be garnered if pools of insertions could be screened while preserving the individual identity of each insertion. The large number of insertions required has been the major barrier to this kind of analysis. Indeed, in traditionally prepared pools containing several thousand insertions, it is sometimes difficult to find an

TABLE 3. Individual insertions and their linkage to nearby markers[a]

| Insertion no. | Approx map position | Nearby markers (% cotransduction frequency) |
|---|---|---|
| 3011 | ? | oxd-4 (50%), oxd-11 (99%), oxd-17 (72%) |
| 3012 | ? | oxd-2 (59%) |
| 3017 | 64 | metC (60%), oxd-5 (2%) |
| 3018 | ? | oxd-15 (36%) |
| 3020 | 18 | oxd-8 (6%), galE (10%) |
| 3023 | ? | oxd-4 (8%), oxd-11 (34%), oxd-17 (70%) |
| 3024 | 84–85 | pepQ (65%), metE (2%), polA (6%) |
| 3026 | ? | dna-603 (63%) |
| 3028 | 55.5 | tyrA (6%) |
| 3029 | 8 | proC (12%) |
| 3030 | 8 | proC (28%) |
| 3032 | 25 | oxd-6 (75%) |
| 3037 | 36 | dadB (90%) |
| 3040 | 78 | xyl (4%) |
| 3041 | 25 | pepT (41%), oxd-18 (46%), oxd-6 (not linked) |
| 3042 | 99.5 | serB (99%) |
| 3048 | 80.5 | mgtB (95%) |
| 3055 | 55.5 | tyrA (2%), phe (not linked) |
| 3061 | 44 | metG (6%) |
| 3063 | 36 | dadB (90%) |
| 3068 | 81–82 | Tn10Δ16Δ17 Kan$^r$ (4%), apeR (50%) |
| 3076 | 64 | oxd-5 (10%), metC (not linked) |
| 3081 | 73 | aroB (70%) |
| 3084 | ? | oxd-2 (71%) |
| 3085 | 62.5 | serA (36%), pepP (78%) |
| 3091 | ? | dna-204 (30%) |
| 3093 | ? | oxd-4 (86%), oxd-11 (32%), oxd-17 (46%) |
| 3098 | 62 | lysA (26%), thyA (ND)[b] |
| 3103 | 98 | pepA (46%), argI (82%), pyrB (22%) |
| 3104 | 80–80.5 | mgtB (11%), pyrE (not linked) |
| 3112 | 99.5 | serB (78%) |
| 3116 | 99.5–100/0 | thrB (8%), serB (25%) |
| 3118 | 46 | oxd-3 (100%), oxd-9 (100%), oxd-10 (100%), oxd-13 (100%), oxd-14 (100%), oxd-16 (100%) |
| 3119 | 81–82 | Tn10Δ16Δ17 Kan$^r$ (94%), dnaA (56%), apeR (22%) |
| 3120 | 81–82 | Tn10Δ16Δ17 Kan$^r$ (100%), dnaA (68%), apeR (24%) |
| 3121 | 61.5 | thyA (12%) |
| 3122 | 61.5 | thyA (15%) |
| 3123 | 80.5 | mgtB (95%), pyrE (not linked), apeR (not linked) |
| 3124 | 71 | aroE (69%), oxrB (2%) |
| 3125 | 80.5 | mgtB (95%) |
| 3127 | 29–32 | oxrA (2%) |
| 3130 | 81–82 | Tn10Δ16Δ17 Kan$^r$ (36%), dnaA (90%), apeR (7%) |
| 3131 | 3 | leuBCD (24%) |
| 3132 | 62 | lysA (20%) |
| 3134 | 64 | oxd-5 (7%), metC (not linked) |
| 3137 | 3 | leuBCD (9%) |
| 3138 | 47 | purF (14%) |
| 3139 | 7 | pepD (ND), proAB (6%) |
| 3140 | 25 | oxd-6 (85%), oxd-18 (2%), pepT (1%) |
| 3142 | 19 | oxd-12 (23%), aroA (2%) |
| 3143 | 62 | lysA (3%) |
| 3144 | ? | oxd-4 (36%), oxd-11 (100%), oxd-17 (88%) |
| 3145[c] | 83–84 | pepQ (89%), ilvC (33%), metE (0–1%), corA (not linked) |
| 3146 | 61.5 | thyA (18%) |
| 3149 | 3.5 | pepM (42%) |
| 3150 | 7 | pepD (ND), proAB (82%) |
| 3154 | ? | dnaZ (99%) |
| 3159 | 62 | serA (64%) |
| 3160 | 98 | pepA (46%), argI (88%), pyrB (8%) |
| 3161 | 84 | metE (30%), pepQ (4%), corA (8%), ilvC (not linked) |
| 3162 | 84 | metE (75%), corA (25%), ilvC (not linked), pepQ (not linked) |
| 3163 | 69 | argG (49%), dna-610 (85%) |
| 3166 | ? | dnaZ (12%) |
| 3167 | 82 | apeR (12%) |
| 3170 | 8 | proC (26%) |
| 3173 | 73 | aroB (86%) |
| 3176 | 23 | pyrC (30%) |
| 3177 | 69 | argG (34%), dna-610 (88%) |
| 3179 | 62 | lysA (6%) |
| 3181 | 55.5 | pheA (38%), tyrA (42%) |
| 3189 | 64 | oxd-5 (20%), metC (not linked) |
| 3195 | 71 | aroE (84%), oxrB (5%) |
| 3196 | 98 | pepA (38%), argI (72%), pyrB (22%) |
| 3198 | 46 | oxd-3 (50%), oxd-9 (48%), oxd-10 (41%), oxd-13 (37%), oxd-14 (40%), oxd-16 (24%) |
| 3200 | 98 | pepA (15%), argI (30%), pyrB (21%) |
| 3205 | 80–80.5 | mgtB (36%), gltC (not linked), dnaA (not linked), pyrE (not linked), apeR (not linked) |
| 3209 | 62.5 | serA (16%), pepP (10%) |
| 3210 | ? | dna-599 (85%) |
| 3211 | ? | dna-603 (6%) |
| 3213 | 64 | metC (82%), oxd-5 (30%) |
| 3214 | 7 | pepD (4%), proAB (5%) |
| 3215 | 7 | pepD (2%), proAB (7%) |
| 3217 | 11 | purE (14%) |
| 3222 | 55.5 | pheA (54%), tyrA (44%) |
| 3231 | 62 | lysA (12%) |
| 3232 | 21 | pyrD (32%), pepN (20%) |
| 3233 | 25 | pepT (20%), oxd-6 (17%), oxd-18 (23%) |
| 3234 | 36 | dadB (40%) |
| 3235 | 84 | metE (7%), corA (37%), pepQ (not linked) |
| 3240 | 99.5 | serB (86%) |
| 3241 | 82 | apeR (85%) |
| 3246 | 64 | metC (30%), oxd-5 (2%) |
| 3248 | 82 | apeR (40%), dnaA (not linked) |
| 3251 | ? | oxd-15 (74%) |
| 3252 | 98 | pepA (70%), argI (52%), pyrB (12%) |
| 3253 | 98 | pepA (ND), argI (ND), pyrB (ND) |
| 3255 | 42 | his (59%) |
| 3258 | 29–32 | oxrA (20%) |
| 3260 | 11 | purE (7%) |
| 3261 | 29–32 | oxrA (2%) |
| 3262 | 6 | dnaE (54%) |
| 3265 | 83 | ilvC (9%) |
| 3266 | ? | dna-599 (24%) |
| 3271 | 50 | cysA (99%) |
| 3290 | 96 | purA (88%) |
| 3291 | 46 | oxd-3 (68%), oxd-9 (67%), oxd-10 (65%), oxd-13 (57%), oxd-14 (64%), oxd-16 (60%) |
| 3294 | 80 | gltC (5%), pyrE (not linked), mgtB (not linked), ilvC (not linked) |
| 3295 | 80 | mgtB (4%), ilvC (not linked), pyrE (not linked), apeR (not linked) |
| 3301 | 71 | aroE (20%), oxrB (2%) |
| 3306 | 80.5 | mgtB (10%) |
| 3312 | 79 | oxrE (40%), pyrE (20%), apeR (not linked) |
| 3314 | 26–32 | dcp (6%) |

[a] All insertions that have been linked to a standard marker are shown. Absence of linkage to nearby markers, confirmed in standard transductions, is also indicated (not linked). Refer to Table 2 and its footnotes for allele designations for the standard markers and comments about some of the crosses. Insertions exist for all numbers, 3001 to 3316 inclusive, except for the following dropouts (see Materials and Methods for further explanation): 3002, 3003, 3004, 3008, 3010, 3014, 3015, 3016, 3025, 3053, 3056, 3078, 3094, 3101, 3113, 3114, 3128, 3133, 3135, 3141, 3174, 3224, 3225, 3226, 3227, 3236, 3250, 3268, 3274, 3276, 3277, 3285, 3286, 3303, 3308, 3311, 3313.

[b] ND, Apparently linked in spot test, but quantitative linkage value not determined.

[c] The linkage data for this insertion appear to be in conflict with the accepted map order *ilvC-metE-pepQ*. No such discrepancy is evident for other insertions in this vicinity. We are investigating possible explanations.

TABLE 4. Classification of anaerobically regulated *lacZ* fusions[a]

| Class | Fusion | Map position (map units) | Linked insertion |
|---|---|---|---|
| 1 | *pepT7*, *oxd-18* | 25 | 3041, 3233 |
| 2 | *oxd-2* | ? | 3012, 3084 |
| 3 | *oxd-3*, *oxd-9*, *oxd-10*, *oxd-13*, *oxd-14*, *oxd-16* | 46 | 3118, 3198, 3291 |
| 4 | *oxd-4* | ? | 3011, 3023, 3093, 3144 |
| 5 | *oxd-5* | 64 | 3017, 3076, 3134, 3189, 3213, 3246 |
| 6 | *oxd-6* | 25 | 3032, 3140, 3233 |
| 7 | *oxd-7* | 93 | None found |
| 8 | *oxd-8* | 18 | 3020 |
| 9 | *oxd-11*, *oxd-17* | ? | 3011, 3023, 3093, 3144 |
| 10 | *oxd-12* | 19 | 3142 |

[a] Each fusion was independently formed by using a phage Mu derivative. The groupings shown were determined by the unique characteristic linkage pattern to the Tn*10*Δ16Δ17 insertions. Groups 4 and 9 are believed to be different on the basis of quantitative linkage values (Table 3).

insertion linked to a particular gene because of the propensity of Tn*10* to insert at hot spots (7). The approach taken here does not alter the insertion specificity of Tn*10*, but the problem of hot spots is nonetheless avoided by retaining for the collection only one insertion corresponding to each phage clone. Thus, a collection of insertions has been obtained which is small enough to enable individual analysis with a high probability of a successful outcome.

What is the probability of success? In these experiments, a bacterial strain carrying an insertion of Tn*10*Δ16Δ17 was obtained for 88% of the phages initially chosen. Two alternative hypotheses might explain the failure to isolate an insertion from some phage clones. In one case, the failures are random; in the other case, the failures represent a specific fraction of the chromosome that is inaccessible to Tn*10*Δ16Δ17 insertion by the method used. Taking as a guide the estimate that a particular insertion marks 1 min of the 100-min *S. typhimurium* chromosome, it is possible to calculate the theoretical coverage of the chromosome according to the alternative hypotheses. In the first case, the 279 insertions would be distributed among 100 min of chromosome, giving 2.79 insertions per min. The probability that a gene is unlinked to any of the insertions is the same as the probability that the 1-min interval surrounding the gene is devoid of insertions. This probability is $e^{-2.79}$ or about 6%. Therefore, 94% of the chromosome would be covered. In the other case, the 279 insertions would be distributed among 88 min of the chromosome, giving 3.17 insertions per minute. Of the 88 min, the fraction not covered would be $e^{-3.17}$ or about 4% (=3.5 min). In this case, therefore, about 84.5% of the chromosome would be covered. In either case, the expected coverage is much higher than has previously been possible from collections of the size used here.

There is one reason for thinking that some or all of the phages from which no insertion derivative was isolated could derive their bacterial insert from a particular region of the chromosome. The strain used as a source of DNA for the λ library is an unalloyed *S. typhimurium* strain, whereas the strain used as host of the Tn*10*Δ16Δ17 insertions is an *S. typhimurium-E. coli* hybrid, used because of its ability to adsorb λ phage. The hybrid strain may contain a substitution of *E. coli* DNA containing *lamB* and an unknown amount of adjacent material in place of the corresponding genes of *S. typhimurium* (11). Such a replacement would block recombinational transfer of Tn*10*Δ16Δ17 from a λ phage into this region of the chromosome, since there is enough sequence nonhomology between *E. coli* and *S. typhimurium* to severely depress recombination. Southern hybridization experiments show that for one locus in this vicinity, *dnaB* (min 90), the *S. typhimurium-E. coli* hybrid strain carries only the *E. coli* gene (L. Kean and R. Maurer, unpublished data). It is noteworthy that we failed to find insertions linked to *argH* (min 88), *dal* (min 91), or *oxd-7*, another locus in this vicinity (near *mel* at min 93). *dnaB* was not tested in linkage studies. It thus appears that our collection does not contain insertions in the region of *E. coli* substitution and will not be useful in identifying new mutations in this relatively small area around min 90. No evidence of additional gaps in chromosome coverage has emerged to date. Note, however, that the majority of insertions remain to be mapped.

**General use of the insertion collection in genetic analysis.** This insertion collection should be particularly useful in analyzing groups of newly isolated mutations that share a common phenotype. When the pattern of linkage of each new mutation to the set of Tn*10*Δ16Δ17 insertions is determined, the minimum number of loci represented in the set of new mutations is immediately known. The mutations will have been quickly and easily placed into classes on the basis of map position. If a new mutation is linked to insertions with known map positions, the precise map position of the new mutation will be immediately known. (As further information accumulates, we expect that most new *S. typhimurium* mutations will be precisely mapped simply by determining linkage to the insertions in this collection). If a new mutation is linked to an unmapped insertion, the procedure of Chumley and Roth (1) can be used to find the map position.

One consideration to be borne in mind when using the collection for this purpose is the expected presence in the individual strains of the parental markers of strain RM5. Conceivably, in a few of the 279 strains, a marker could have been lost during the transfer of Tn*10*Δ16Δ17 from the λ context into the chromosome, but we have not confirmed an instance of this to date. However, we have noted several instances of insertions located close to an RM5 marker (*metE551* or *galE496*), revealed by cotransduction of a prototrophic recipient to tetracycline resistance and $Met^-$ or $Gal^-$ (Tables 2 and 3). The 279 strains have not been systematically examined for the retention of RM5 markers or for linkage of an insertion to an RM5 marker. In general, this concern has not impeded our use of the collection in the ways we have described. Should a nearby RM5 marker prove problematic for a given insertion, a straightforward solution is to construct a substitute strain carrying that insertion in a more suitable background.

**Additional uses of Tn*10*Δ16Δ17.** The system that is described here suggests itself for additional uses that include and go beyond the uses for transposons described previously. Several of the applications described below have been developed in our laboratories in a variety of experiments.

**(i) Facilitated molecular cloning of wild-type genes.** We have retained the particular recombinant phage used to generate each of the chromosomal Tn*10*Δ16Δ17 insertions. Combined, the 279 phages carry approximately 60% of the *S. typhimurium* genome (based on the average amount of DNA per clone and allowing statistically for overlaps). Therefore, there is an excellent probability that the phage used to generate a particular insertion will also carry the wild-type gene that corresponds to a mutation linked to that insertion.

If not, a one-step chromosomal walk should produce the desired clone.

**(ii) Facilitated cloning of mutant genes.** In principle, a chromosomal mutation can be transferred by recombination into a λ clone bearing the corresponding nonmutant DNA. This circumvents the necessity of making a genomic libarary and isolating a specific clone for each mutation to be studied. However, the marker rescue frequency in such crosses between λ and bacteria is so low that selection or a very sensitive screen for the desired recombinant is necessary. Tn*10*Δ16Δ17 provides a convenient, closely linked marker that is selectable in λ. Moreover, when the mutant phenotype cannot be verified directly in λ, the linked Tn*10*Δ16Δ17 facilitates the placement of the mutation back into the chromosome for verification (11).

**(iii) Isolation of overlapping clones.** A similar recombinational approach can be used to isolate overlapping clones for any particular region from a partial-digest or random-shear genomic library. The library is passaged through a strain bearing a particular Tn*10*Δ16Δ17 insertion, and tetracycline-resistant progeny phages are selected. Such phages must share the site of the Tn*10*Δ16Δ17 insertion but can vary in the extent of flanking material. Characterization of the clones then leads to a correlated physical and genetic map of the region.

**(iv) Unequal crossing over.** Unequal crossing over between two copies of a transposon has been used to generate defined duplications and other kinds of chromosomal rearrangements. The relatively simple restriction map of Tn*10*Δ16Δ17 (6) opens the possibility of constructing unequal crossovers in vitro and then transferring the rearranged DNA back into the chromosome. Subcloning suitable pieces of DNA into plasmids can greatly aid this process. In principle, one ligates together two pieces of DNA, each of which has one end at a unique restriction site within Tn*10*Δ16Δ17. If different Tn*10*Δ16Δ17 insertions are used, the result is a rearranged piece of DNA. For example, we have used this technique to construct a small internal deletion of the *dnaQ* gene which is exactly replaced by Tn*10*Δ16Δ17. This deletion mutation is selectable in *S. typhimurium* (by virtue of the associated drug resistance) and has the additional feature that it does not revert to true wild type even if Tn*10*Δ16Δ17 undergoes precise excision. Such a construction would have been difficult in vivo.

**(v) Determination of the null phenotype of a gene.** In many situations, it is desirable to know the consequences of complete loss of a gene. Base- substitution mutations are notoriously unreliable as null mutations. By using the methods described here, it is possible to isolate insertions in a cloned gene of interest. Such insertions may be identified either by loss of any convenient complementation property of the gene or by physical mapping. Then the insertions can be transferred into the chromosome and the null phenotype can be assessed. Of course it is necessary to analyze genomic DNA from the transductants (i.e., by Southern analysis) to make sure that there is no duplication of the gene associated with the transduction. The frequency with which chromosomal transductants are obtained may also be useful as an indicator that the gene is or is not essential (11, 12, 16).

**Extension to other organisms.** The collection as described here is directly useful only in *S. typhimurium*. With a few modifications, a similar set of insertions would be derived for *E. coli*, which is the natural host for phage λ. Recently, a technique has been described that extends that potential host range of λ to other bacterial species (3). Such bacteria should thus become amenable to the kind of analysis described here.

## ACKNOWLEDGMENTS

We thank Betsey Daub and David Botstein for assistance in testing linkage to *dadB* and *dal*.

This work was supported by Public Health Service grants AI10333 (to C.G.M.) and AI19942 (to R.A.M.) from National Institutes of Health.

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