A Collection of Strains Containing Genetically Linked Alternating Antibiotic Resistance Elements for Genetic Mapping of Escherichia coli

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INTRODUCTION

Escherichia coli and the closely related enteric bacterium Salmonella typhimurium are two of the most completely characterized organisms in biology and continue to be widely studied to gain further understanding of basic biological processes. The early development of excellent genetic methodologies, such as conjugation (13, 16, 25, 40, 65, 66) and generalized transduction (29, 41, 66), for these organisms contributed significantly to their choice as a focus of investigation. These discoveries allowed for the construction of a genetic map of E. coli as early as 1964 (56). This map identified the position of approximately 100 genes. Today, over 1,000 genes have been identified and mapped to the E. coli chromosome (2), which has been divided into 100 min, each consisting of approximately 47 kilobase pairs of DNA (2, 37, 44). This rapid increase in information about the organization of the genome has been facilitated by continued genetic advances including the development of specialized transducing bacteriophages (62), the utilization of transposons (3–6, 26, 27, 35, 36), the construction of hybrid transposons (5, 61), and the development of an in vivo cloning technique involving mini-Mu (22, 23). The accumulated data resulting from these advances have resulted in various catalogs of information including the genetic maps of E. coli (2) and S. typhimurium (53), a physical map and ordered phage clone bank of E. coli (37), the gene-protein index of E. coli (51), and the various catalogs of genomic insertions (5) and Hfr and F' strains (42, 43). With the availability of such information and technologies, the use of E. coli and S. typhimurium species as model systems in the study of basic biological problems cannot be matched.

Despite the wealth of information about E. coli, it is estimated that less than half of the total number of genes have been identified to date. Additional genes are being identified at an accelerating rate. The use of classical mapping techniques to locate mutations which may identify new genes can be cumbersome and time-consuming. The development of fast and simple mapping procedures will facilitate the identification and characterization of previously unknown genes. Kleckner et al. (36) were the first to suggest...
the use of transposons conferring resistance to various antibiotics as a selectable marker in matings or transductions obviates the need to develop conditions under which the actual mutation must be selected. We have devised a rapid mapping strategy which takes advantage of transposons located at defined positions in the E. coli genome to be used as selectable markers in Hfr mating and P1 transductions.

We have constructed a coordinated set of strains containing either the transposon Tn10 (encoding tetracycline resistance) or Tn10kan (encoding kanamycin resistance) to facilitate the mapping of mutations in E. coli. The development of this mapping kit involved the construction of 182 isogenic strains, each of which contains either a Tn10 or a Tn10kan at a known position on the chromosome. The set contains antibiotic resistance elements around the entire chromosome that are linked by P1 transduction. Most positions are represented by both Tn10 and Tn10kan insertions. These strains can be used to rapidly map mutations to within a 1-min region of the chromosome by using a two-step mapping procedure. In the first step, the mutation is mapped to a 5- to 15-min region of the chromosome by using overlapping Hfr strains containing either Tn10 or Tn10kan as the selectable marker. In the second step, P1 transduction is used to place the mutation between pairs of antibiotic resistance elements located about 1 min apart. We discuss the construction of this mapping kit and its various uses in mapping mutations, facilitating strain construction, and other genetic manipulations.

**MATERIALS AND METHODS**

**Bacterial Strains, Phages, and Growth Conditions**

All bacterial strains used were derivatives of E. coli K-12, and are described in Table 1. P1 vir was used for P1 transductions. λ1104 (gyrB7-hisNdisG9424:cin10kan, c1857 Pam80 rnm) (61), λNKS61 (λh221 Oam29 c1857 Tn10) (20), and λNKS67 (λh221 c1857 Oam29 Pam80 recT1) (20) were obtained from N. Kleckner. T4GT7 (T4antE51 antC77 rNB5069 ort) (64) was obtained from C. Georgopoulos. The λ clone bank of Kohara et al. (37), containing ordered E. coli fragments cloned into λ EMBL4 [λ shbl λ1'9189 < polylinker (Spa-EcorIII) intr-29 nin44 c1857 trpe polylinker (EcorI-Sall) > KH54 chlC sr14A' nin5 sr14A'] or λK001 [λ shbl λ1'9189 int (linker) sr14A ninL. 45 χshdII λ4(bio) (linker) Δshblλ13-6hbl4]KH54 sr14A' chlC nr15 sr14A' shdIIα)], was obtained from F. Blattner.

Bacterial cultures were grown in LB medium (47) and supplemented with tetracycline (10 μg/ml), kanamycin (30 μg/ml), chloramphenicol (15 μg/ml), or ampicillin (50 μg/ml) when indicated. For solid support medium, 1.5% Bacto-Agar (Difco Laboratories) was included. Cultures used for λ infections were grown in N2Y medium (46) supplemented with 0.2% maltose. For the scoring of auxotrophic markers, M9 minimal plates (47) supplemented with the appropriate amino acids at 20 μg/ml were used. Sugar utilization phenotypes were scored on MacConkey agar plates (45), supplemented with the appropriate sugar at 1.0%.

**Genetic Manipulations**

P1 transductions were performed as described by Miller (47), and transductions with phage T4GT7 were done as described by Young and Edlin (64). Random insertion pools with Tn10, Tn10kan, or Tn5 were constructed as described by Kleckner et al. (36). Hfr mating strains were performed as described by Miller (47), with the following modifications. Recipient and donor cells were mixed and incubated for 35 min at 37°C. Mating mixtures were diluted 10-fold into fresh LB (or M9 salts for matings involving KL228 or KL14) and then vortexed vigorously for 2 min to disrupt mating aggregates. Serial dilutions were plated on selective plates and incubated overnight at 37°C. When selection was for Kan', cells were grown for 1 h in 10 ml of LB broth (or M9 glucose plus leucine for matings involving KL228 or KL14) following disruption of the mating pairs, to allow expression of the Kan' gene prior to plating on selective plates.

**Construction of the Mapping Strains**

We have constructed a series of isogenic mapping strains containing Tn10, Tn10kan, Tn5, or Tn9 at specific sites in the genome. The source of each of these insertions is indicated in Table 1. All insertions obtained from other laboratories were first transduced into MG1655. MG1655 is λ' and does not contain any known nonsense suppressors. These phenotypes are required for the conversion of Tn10 to Tn10kan as described below. In addition, placing the insertions in a common wild-type genetic background eliminated potential strain-specific effects, such as inversions and small deletions, which could affect mapping results.

**Conversion of Tn10 to Tn10kan.** To obtain insertions conferring different antibiotic resistances at the same location in the chromosome, we and others (7) have used recombination to replace the antibiotic resistance gene of a transposon resident in the chromosome with the antibiotic resistance gene from an incoming transposon, generating a transposon with a different antibiotic resistance element at the same location as the original element. In this case, a Tn10 transposon on the chromosome was replaced by an incoming Tn10kan transposon carried on a defective λ phage, λ1104. Reciprocal recombination between the incoming phage and the chromosome occurs within the IS10 elements which flank both antibiotic resistance genes of Tn10 and Tn10kan. This generates a Tn10kan element at the same location in the chromosome as the initial Tn10. Because λ1104 can neither replicate nor stably integrate in MG1655, the other product of this recombination event, λ1104 Tet', is diluted out of the population. Thus, this process produces a Kan' Tet' recombinant.

MG1655 derivatives containing the Tn10 to be converted were infected with λ1104 at a multiplicity of infection of 1.0, plated on LB-kanamycin plates to select for Kan' cells, and scored for Tet'. As described above, the cells derived from the desired recombination event will be Kan' Tet'. In contrast, cells in which the Tn10kan moved to the chromosome by transposition would be Kan' Tet'. Kan' Tet' candidates were obtained with a frequency between 1 × 10^-4 and 1 × 10^-5 per input phage, which is similar to the reported transposition frequency of 1 × 10^-5 for Tn10 (61).

P1 transductions between each Kan' Tet' candidate and its parental Tn10 strain were used to show that the Tn10kan transposon was located at the position as the original Tn10. P1 vir grown on the parental Tn10 strains was used as the donor to transduce the Kan' Tet' candidates to Tet'. Between 100 and 200 Tet' transductants were then scored for their Kan' phenotype. If at least a Kan' Tet' transductants were Kan', the Tn10kan was considered to be 100% linked to the original Tn10 and placed in the mapping set. Between 10 and 90% of the Kan' Tet' candidates obtained for any one conversion attempt showed the expected 100% linkage to...
| Line | Insertion position | Strain | Genotype | Source or construction |
|------|--------------------|--------|----------|------------------------|
| **Hfr strains** | | | | |
| 1 | 00.00 | NK5148 | rpsL lacZU118 thr-34::Tn10 | N. Kleckner |
| 2 | 00.00 | CAG18442 | MG1655 thr-34::Tn10 | N. Kleckner |
| 3 | 00.00 | CAG18425 | MG1655 thr-3091::Tn10kan | N. Kleckner |
| 4 | 00.75 | NK6034 | rpsL lacZU118 car-96::Tn10 | N. Kleckner |
| 5 | 00.75 | CAG12093 | MG1655 car-96::Tn10 | N. Kleckner |
| 6 | 00.75 | CAG18620 | MG1655 car-3092::Tn10kan | N. Kleckner |
| 7 | 02.00 | FMJ201 | zac-3051::Tn10 | D. Clark, formerly zac::Tn10 |
| 8 | 02.00 | CAG12095 | MG1655 zac-3051::Tn10 | D. Clark, formerly zac::Tn10 |
| 9 | 02.00 | CAG12131 | MG1655 zac-3093::Tn10kan | D. Clark, formerly zac::Tn10 |
| 10 | 03.50 | SJ16 | panD2 metB1 relA1 spoT1 gyrA216 yh-1 zac-220::Tn10 | C. O. Rock (30, 31) |
| 11 | 03.50 | CAG12025 | MG1655 Zac-220::Tn10 | C. O. Rock (30, 31) |
| 12 | 03.50 | CAG12105 | MG1655 Zac-3094::Tn10kan | C. O. Rock (30, 31) |
| 13 | 04.75 | JW353 | thr-1 leuB6 thyA6 met-89 thi-1 deoC1 lacY1 rpsL67 tonA1 supE44 Zac-502::Tn10 | B. Bachmann |
| 14 | 04.75 | CAG18436 | MG1655 Zac-502::Tn10 | B. Bachmann |
| 15 | 04.75 | CAG18580 | MG1655 Zac-3095::Tn10kan | B. Bachmann |
| 16 | 06.25 | CAG1681 | proAB81::Tn10 | Lab collection |
| 17 | 06.25 | CAG18447 | MG1655 proAB81::Tn10 | Lab collection |
| 18 | 06.25 | CAG18515 | MG1655 proAB9096::Tn10kan | Lab collection |
| 19 | 06.75 | CAG18633 | MG1655 Zac-3198::Tn10kan | Lab collection |
| 20 | 07.75 | RS1071 | leuB6 trp-31 hisG1 argG6 metB1 gal-6 rafA1 xyl-7 mtl-2 rpsL104 tonA2 supE44 Zac-281::Tn10 | B. Bachmann |
| 21 | 07.75 | CAG12080 | MG1655 Zac-281::Tn10 | B. Bachmann |
| 22 | 08.00 | CAG1538 | lacZU118 rpsL lacX42::Tn10 | Lab collection |
| 23 | 08.00 | CAG18439 | MG1655 lacZU118 lacX42::Tn10 | Lab collection |
| 24 | 08.00 | CAG18420 | MG1655 lacZU118 lacX4098::Tn10 | Lab collection |

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| Line | Insertion position | Strain | Genotype | Source or construction |
|------|--------------------|--------|----------|------------------------|
| 25   | 09.00              | CAG8091| galK rpsL proC zbi-3053::Tn10 | Lab collection |
| 26   | 09.00              | CAG18091| MG1655 proC zbi-3053::Tn10 | P1(CAG8091) x MG1655 |
| 27   | 09.00              | CAG18091| MG1655 proC zbi-3053::Tn10 | Tn10kan conversion of CAG18091 |
| 28   | 09.50              | P2719  | araD ompr relA rpsL naqT7 thiA ΔargF-lacY169 thiA txx-247::Tn10 | P. Reeves (48) |
| 29   | 09.50              | CAG12148| MG1655 txx-247::Tn10 | P1(P2719) x MG1655 |
| 30   | 09.50              | CAG18143| MG1655 txx-3100::Tn10kan | Tn10kan conversion of CAG12148 |
| 31   | 10.50              | SG20253| zbi-3053::Tn10 | S. Gottesmann (60) |
| 32   | 10.50              | CAG12017| MG1655 zbi-3053::Tn10 | P1(SG20253) x MG1655 |
| 33   | 10.50              | CAG12107| MG1655 zbi-3100::Tn10kan | Tn10kan conversion of CAG12017 |
| 34   | 11.50              | ML45   | zbi-3053::Tn10 | J. Croman (50), formerly zbi-::Tn10 |
| 35   | 11.50              | CAG12154| MG1655 zbi-3053::Tn10 | P1(M145) x MG1655 |
| 36   | 12.25              | NK6051 | relA spoT1 thi-1 ΔargF-lacY169 parE79::Tn10 | B. Bachmann |
| 37   | 12.25              | CAG12171| MG1655 parE79::Tn10 | P1(NK6051) x MG1655 |
| 38   | 12.25              | CAG18566| MG1655 parE200::Tn10kan | Tn10kan conversion of CAG12171 |
| 39   | 13.25              | CAG12021| MG1655 zbi-3105::Tn10 | Lab collection |
| 40   | 13.25              | CAG12116| MG1655 zbi-3200::Tn10kan | Tn10kan conversion of CAG12021 |
| 41   | 14.50              | RK4342 | pro-3 entA403 supE44 his-218 rpsL109 xyl-5 or xyl-7 | D. Clark (1) |
| 42   | 14.50              | CAG12149| MG1655 zbi-601::Tn10 | P1(RK4342) x MG1655 |
| 43   | 14.50              | CAG18421| MG1655 zbi-3104::Tn10kan | Tn10kan conversion of CAG12149 |
| 44   | 15.00              | SK2257 | thyA6 rpsL120 devC1 zbi-280::Tn10 | B. Bachmann |
| 45   | 15.00              | CAG12077| MG1655 zbi-280::Tn10 | P1(SK2257) x MG1655 |
| 46   | 15.00              | CAG18513| MG1655 zbi-3105::Tn10kan | Tn10kan conversion of CAG12077 |
| 47   | 16.25              | P2217  | lacZ(Am) trpAm) rpsL thi supE44 zbi-3057::Tn10 | P. Reeves, formerly zbi-::Tn10 |
| 48   | 16.25              | CAG18433| MG1655 zbi-3057::Tn10 | P1(P2217) x MG1655 |
| 49   | 16.25              | CAG18514| MG1655 zbi-3106::Tn10 | Tn10kan conversion of CAG18433 |
| 50   | 16.75              | CAG18087| galK rpsL nadA57::Tn10 | Lab collection |
| 51   | 16.75              | CAG12147| MG1655 nadA57::Tn10 | P1(CAG18087) x MG1655 |
| 52   | 16.75              | CAG18341| MG1655 nadA3052::Tn10kan | Tn10kan conversion of CAG12147 |
| 53   | 17.75              | CAG18392| galK2 galT1 cdxE(bisB28) bioC1 bioA24 zbi-29::Tn10 | Lab collection |
| 54   | 17.75              | CAG18493| MG1655 zbi-29::Tn10 | P1(CAG18392) x MG1655 |
| 55   | 17.75              | CAG18531| MG1655 zbi-3108::Tn10kan | Tn10kan conversion of CAG18493 |
| 56   | 18.75              | S43314| pro galK2 rpsL yviH50 conAo41 zbi-3058::Tn10 | K. Hammer, formerly zbi::Tn10 |
| 57   | 18.75              | CAG12034| MG1655 zbi-3058::Tn10 | P1(S43314) x MG1655 |
| 58   | 18.75              | CAG12112| MG1655 zbi-3109::Tn10kan | Tn10kan conversion of CAG12034 |
| 59   | 20.00              | RW1230 | hisG4 thi-1 lacY1 galK2 supE44 mal-1 ΔargF-proA62 xyl-5 or xyl-7, zbi-1230::Tn10 | R. Weisberg |
| 60   | 20.00              | CAG18478| MG1655 zbi-1230::Tn10 | P1(RW1230) x MG1655 |
| 61   | 20.00              | CAG18528| MG1655 zbi-3108::Tn10kan | Tn10kan conversion of CAG18478 |
| 62   | 21.00              | DC304  | zbi-3059::Tn10 | D. Clark, formerly zbi::Tn10 |
| 63   | 21.00              | CAG12094| MG1655 zbi-3059::Tn10 | P1(DC304) x MG1655 |
| 64   | 21.00              | CAG12130| MG1655 zbi-3111::Tn10kan | Tn10kan conversion of CAG12094 |
| 65   | 22.25              | DC305  | pyrD134 his89 galK2 malA1 xyl-7 mal-2 rpsL118 zve-282::Tn10 | D. Clark |
| 66   | 22.25              | CAG18466| MG1655 zve-282::Tn10 | P1(DC305) x MG1655 |
| 67   | 22.25              | CAG18613| MG1655 zve-3112::Tn10kan | Tn10kan conversion of CAG18466 |
| 68   | 22.75              | JY34   | trp locZ rpsL thi supP5::Tn5 | J. Wood (24) |
| 69   | 22.75              | CAG18703| MG1655 supP5::Tn5 | PMJL34 (24) x MG1655 |
| 70   | 24.25              | TL212  | fbbB5301 ppxF25 relA rpsL150 malT100(Con) devC1 araD139 ΔargF-lacY169 ΔmalE144 zve-726::Tn10 | T. Larson (39) |

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### TABLE 1—Continued

| Line | Insertion position | Strain | Genotype | Source or construction |
|------|---------------------|--------|----------|------------------------|
| 71   | 24.25               | CAG12078 | MG1655 :zce-226::Tn10 | P1(TL212) × MG1655 |
| 72   | 24.25               | CAG12124 | MG1655 :zce-311::Tn10kan kan | Tn10kan conversion of CAG12078 |
| 73   | 25.25               | RS3242 | trpE61 :tna-5 dadaR1 trpaA2 zcf-117::Tn10 | B. Bachmann |
| 74   | 25.25               | CAG18463 | MG1655 :zcf-117::Tn10 | P1(RS3242) × MG1655 |
| 75   | 25.25               | CAG18516 | MG1655 :zcf-311::Tn10kan | Tn10kan conversion of CAG18463 |
| 76   | 25.75               | RS3040 | F' :fadR11::Tn10 | B. Bachmann |
| 77   | 25.75               | CAG18497 | MG1655 :fadR11::Tn10kan | P1(RS3040) × MG1655 |
| 78   | 25.75               | CAG18544 | MG1655 :fadR11::Tn10 | Tn10kan conversion of CAG18497 |
| 79   | 26.75               | ORN125 | zcg-3060::Tn10 | P. Orndorff (55), formerly zcg::Tn10 |
| 80   | 26.75               | CAG12016 | MG1655 :zcg-3060::Tn10 | P1(ORN125) × MG1655 |
| 81   | 26.75               | CAG12106 | MG1655 :zcg-311::Tn10kan | Tn10kan conversion of CAG12016 |
| 82   | 27.25               | JW380 | zch-506::Tn10 | B. Bachmann |
| 83   | 27.25               | CAG12169 | MG1655 :zch-506::Tn10 | P1(JW380) × MG1655 |
| 84   | 27.25               | CAG18551 | MG1655 :zch-311::Tn10kan | Tn10kan conversion of CAG12169 |
| 85   | 28.50               | PK1085 | zci-233::Tn10 | T. Hill (28) |
| 86   | 28.50               | CAG12028 | MG1655 :zci-233::Tn10 | P1(PK1085) × MG1655 |
| 87   | 28.50               | CAG12111 | MG1655 :zci-311::Tn10kan | Tn10kan conversion of CAG12028 |
| 88   | 29.50               | EC2111 | araD139 2argE-lacU169 rpsL thi met-1 trp-3 fmr-501 trp-3 zje-301::Tn10 | D. Clark, formerly zci::Tn10 |
| 89   | 29.50               | CAG12081 | MG1655 :zje-301::Tn10 fmr-501? | P1(EC2111) × MG1655 |
| 90   | 31.00               | FM1200 | trg-2::Tn10 | D. Clark (described in reference 10) |
| 91   | 31.00               | CAG12026 | MG1655 :trg-2::Tn10 | P1(FM1200) × MG1655 |
| 92   | 31.00               | CAG12108 | MG1655 :trg-312::Tn10kan | Tn10kan conversion of CAG12026 |
| 93   | 32.00               | PLK1269 | trpA9605 his-85 thyA714 ilv-632 dec-70 trpR55 pro-48 arg-59 tss-84 rac zde-235::Tn10 zdd-230::Tn9 | P. Kuempel (9) |
| 94   | 32.00               | CAG18461 | MG1655 :zde-235::Tn10 | P1(PLK1269) × MG1655 |
| 95   | 32.00               | CAG18576 | MG1655 :zde-311::Tn10kan | Tn10kan conversion of CAG18461 |
| 96   | 32.75               | PK1220 | zdd-230::Tn9 | P. Kuempel (9) |
| 97   | 32.75               | CAG12027 | MG1655 :zdd-230::Tn9 | P1(PK1220) × MG1655 |
| 98   | 33.50               | PLK1253 | trpA9605 his-85 thyA714 ilv-632 dec-70 trpR55 pro-48 arg-59 tss-84 rac zde-234::Tn10 zdd-230::Tn9 | P. Kuempel (9) |
| 99   | 33.50               | CAG18459 | MG1655 :zde-234::Tn10 | P1(PLK1253) × MG1655 |
| 100  | 34.50               | CAG18637 | MG1655 :zdf-3062::Tn5 | This work |
| 101  | 35.75               | UT152 | ara napA argE(Am) rpoB(RpF) thi tyrS565 zdg-603::Tn10 | B. Bachmann |
| 102  | 35.75               | CAG18462 | MG1655 :zdf-603::Tn10 | P1(UT152) × MG1655 |
| 103  | 35.75               | CAG18567 | MG1655 :zdf-3121::Tn10kan | Tn10kan conversion of CAG18462 |
| 104  | 36.00               | CAG18629 | MG1655 :zdf-3198::Tn10kan | This work |
| 105  | 37.50               | DF949 | tonA22 ompF627 gnd-1 relA1 pir-10 spo115 zdh-925::Tn10 | D. Fraenkel (17) |
| 106  | 37.50               | CAG12151 | MG1655 :zdh-925::Tn10 | P1(DF949) × MG1655 |
| 107  | 37.50               | CAG18568 | MG1655 :zdh-3122::Tn10kan | Tn10kan conversion of CAG12151 |
| 108  | 38.25               | BJW72 | thr-1 ara-14 leuB6 lacY1 tss-33 supE44 galK2 xth-3 his44 rbfD1 rpsL51 kgK51 metL xyl-5 argE3 thi-1 dtgnt-proA62 rac zdi-276::Tn10 | B. Bachmann |
| 109  | 38.25               | CAG18464 | MG1655 :zdi-276::Tn10 | P1(BJW72) × MG1655 |
| 110  | 38.25               | CAG18518 | MG1655 :zdi-3122::Tn10kan | Tn10kan conversion of CAG18464 |
| 111  | 39.50               | DC369 | fadR16 btaD12 mel-1 supF58 zdi-225::Tn10 | D. Clark |
| 112  | 39.50               | CAG18465 | MG1655 :zdi-225::Tn10 | P1(DC369) × MG1655 |
| 113  | 39.50               | CAG18578 | MG1655 :zdi-3124::Tn10kan | Tn10kan conversion of CAG18465 |

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TABLE 1—Continued

| Line | Insertion position | Strain | Genotype | Source or construction |
|------|--------------------|--------|----------|------------------------|
| 114  | 40.25              | DC374  | fadR awcC fadF zeu-3098::Tn10 | D. Clark, formerly zeu::Tn10 |
| 115  | 40.25              | CAG12074 | MG1655 zeu-3098::Tn10 | Pl(DC374) × MG1655 |
| 116  | 40.25              | CAG12122 | MG1655 zeu-3125::Tn10kan | Tn10kan conversion of CAG12074 |
| 117  | 40.75              | N3041  | InTerD-rrmE1 edc-51::Tn10 | B. Bachmann |
| 118  | 40.25              | CAG18486 | MG1655 edc-51::Tn10 | Pl(N3041) × MG1655 |
| 119  | 40.75              | CAG18561 | MG1655 edc-3126::Tn10kan | Tn10kan conversion of CAG18486 |
| 120  | 42.25              | N3024  | InTerD-rrmE1 urrC79::Tn10 | B. Bachmann |
| 121  | 42.25              | CAG12156 | MG1655 urrC79::Tn10 | Pl(N3024) × MG1655 |
| 122  | 43.00              | CAG18453 | MG1655 zed-3099::Tn10 | Lab collection |
| 123  | 43.00              | CAG18563 | MG1655 zed-3128::Tn10kan | Tn10kan conversion of CAG18451 |
| 124  | 44.25              | DC411  | thi-1 thr-1 leu-6 metG58 hisC5 proA2 metB his 2GalK2 rpsL urrC72::Tn10 | D. Clark, formerly zee::Tn10 |
| 125  | 44.25              | CAG12099 | MG1655 zee-3129::Tn10 | Pl(DC411) × MG1655 |
| 126  | 44.25              | CAG12176 | MG1655 zee-3189::Tn10kan | Tn10kan conversion of CAG12099 |
| 127  | 45.75              | LA5606 | mgl-500::Tn10 | W. Boos (12) |
| 128  | 45.75              | CAG12179 | MG1655 mgl-500::Tn10 | Pl(LA5606) × MG1655 |
| 129  | 46.50              | LA5651  | thl-1 leuB lacYI phoA14 rpsL14 malA1 metB his 2GalK2 rpsL urrC72::Tn10 | W. Boos (11) |
| 130  | 46.50              | CAG12998 | MG1655 zee-222::Tn10 | Pl(LA5651) × MG1655 |
| 131  | 46.50              | CAG12100 | MG1655 zee-3138::Tn10kan | Tn10kan conversion of CAG12998 |
| 132  | 47.75              | CS1230  | opmC61 gyaA1 zee-298::Tn10 | B. Bachmann, formerly zee-298::Tn10 |
| 133  | 47.75              | CAG12177 | MG1655 zee-298::Tn10 | Pl(CS1230) × MG1655 |
| 134  | 47.75              | CAG18577 | MG1655 zee-3142::Tn10kan | Tn10kan conversion of CAG12177 |
| 135  | 48.50              | DL2    | zee-722::Tn10 | D. Clark |
| 136  | 48.50              | CAG12178 | MG1655 zee-722::Tn10 | Pl(DL2) × MG1655 |
| 137  | 48.50              | CAG12183 | MG1655 zee-3145::Tn10kan | Tn10kan conversion of CAG12178 |
| 138  | 49.50              | DC334  | arcr4 dsdA7 argB1 thi-1 malA1 lacYI or lacZ4 xyl-7 metB::supE44::Tn10 | D. Clark, formerly zee-223::Tn10 |
| 139  | 49.50              | CAG18484 | MG1655 zee-222::Tn10 | Pl(DC334) × MG1655 |
| 140  | 49.50              | CAG18552 | MG1655 zee-3144::Tn10kan | Tn10kan conversion of CAG18484 |
| 141  | 50.50              | RS3338 | fadR901 fadL771::Tn10 | B. Bachmann (described in reference 52) |
| 142  | 50.50              | CAG18483 | MG1655 fadL771::Tn10 | Pl(RS3338) × MG1655 |
| 143  | 51.00              | RS3116  | arcr4 fadL701 dsdA1 ilD188 thi-1 malA1 xyl-7 metB::supE44::Tn10 | B. Bachmann (formerly zaf-1::Tn10) |
| 144  | 51.00              | CAG18467 | MG1655 zaf-1::Tn10 | Pl(RS3116) × MG1655 |
| 145  | 51.00              | CAG18522 | MG1655 zaf-3135::Tn10kan | Tn10kan conversion of CAG18467 |
| 146  | 51.75              | S01024  | relA1 metC99 spoT1 thi-1 supC510::Tn10 | B. Bachmann |
| 147  | 51.75              | CAG18468 | MG1655 supC510::Tn10 | Pl(HisD1024) × MG1655 |
| 148  | 51.75              | CAG18565 | MG1655 supC3146::Tn10kan | Tn10kan conversion of CAG18468 |
| 149  | 52.75              | CAG18632 | MG1655 zaf-3135::Tn10kan | This work |
| 150  | 53.25              | NK6056  | HinfIPO1 relA1 spoT1 thi-1 Δmgt-lacI5 purC80::Tn10 | B. Bachmann |
| 150a | 53.25              | CAG18470 | MG1655 purC80::Tn10 | Pl(NK6056) × MG1655 |
| 150b | 53.25              | CAG18524 | MG1655 purC3147::Tn10kan | Tn10kan conversion of CAG18470 |
| 152  | 53.50              | CAG18631 | MG1655 zaf-3135::Tn10kan | This work |
| 153  | 54.00              | N3007  | InTerD-rrmE1 gau-26::Tn10 | B. Bachmann |
| 154  | 54.00              | CAG18469 | MG1655 gau-26::Tn10 | Pl(N3007) × MG1655 |
| Line | Insertion position | Strain             | Genotype                                      | Source or construction                     |
|------|--------------------|--------------------|-----------------------------------------------|--------------------------------------------|
| 155  | 54.75              | BW280              | HiP(P045) nadB7 ung-1 relA1 spoT1 thi-1      | B. Weiss (18), formerly                   |
|      |                    |                    | zff-208::Tn10                                 | zff-208::Tn10 × MG1655                     |
| 156  | 54.75              | CAG18481           | MG1655 zff-208::Tn10                          | Pl(WB280) × MG1655                        |
| 157  | 54.75              | CAG18570           | MG1655 zff-3139::Tn10kan                      | Tn10kan conversion of CAG18481            |
| 158  | 55.75              | NK6042             | HiP(P045) relA1 spoT1 thi-1 Δgpt-lac5::nadB51::Tn10 | B. Bachmann                              |
| 159  | 55.75              | CAG18480           | MG1655 nadB51::Tn10                          | Pl(NK6042) × MG1655                      |
| 160  | 55.75              | CAG18412           | MG1655 nadB51::Tn10kan                       | Tn10kan conversion of CAG18480            |
| 161  | 56.75              | NK6024             | HiP(P045) relA1 spoT1 thi-1 Δgpt-lac5::pheA18::Tn10 | B. Bachmann                              |
| 162  | 56.75              | CAG12158           | MG1655 pheA18::Tn10                          | Pl(NK6024) × MG1655                      |
| 163  | 56.75              | CAG18608           | MG1655 pheA18::Tn10kan                       | Tn10kan conversion of CAG12158            |
| 164  | 57.50              | CAG18642           | MG1655 zfb-3131::Tn10                         | This work                                 |
| 165  | 58.25              | CAG18562           | MG1655 zfb-3143::Tn10kan                     | This work                                 |
| 166  | 59.25              | N3002              | IN(rrnD-rrnEI) cysC95::Tn10                   | R. W. Lloyd                               |
| 167  | 59.25              | CAG12173           | MG1655 cysC95::Tn10                          | Pl(N3002) × MG1655                       |
| 168  | 59.25              | CAG12182           | MG1655 cysC315::Tn10kan                      | Tn10kan conversion of CAG12173            |
| 169  | 60.25              | JK1015             | fuc-3072::Tn10                               | D. Clark, formerly                       |
| 170  | 60.25              | CAG12079           | MG1655 fuc-3072::Tn10                         | Pl(JK1015) × MG1655                      |
| 171  | 60.25              | CAG12115           | MG1655 fuc-3154::Tn10                         | Tn10kan conversion of CAG12079            |
| 172  | 60.75              | DFB264             | MG1655 recD1901::Tn10                        | D. Biek (8)                               |
| 173  | 62.00              | DC366              | lysA22 argA21 cysC43 rpsL104 zgc-3074::Tn10    | D. Clark (15), formerly                  |
| 174  | 62.00              | CAG18709           | MG1655 zgc-3074::Tn10                         | P1(DC367) × MG1655                       |
| 175  | 62.00              | CAG18212           | MG1655 zgc-3154::Tn10kan                     | Tn10kan conversion of CAG12082            |
| 176  | 63.50              | DF264              | HiP(P0241) relA1 spoT1 pgk-2 pit-10 tonA22    | B. Bachmann                               |
| 177  | 63.50              | CAG12168           | MG1655 zgd-210::Tn10                         | Pl(DF264) × MG1655                       |
| 178  | 63.50              | CAG18604           | MG1655 zgd-3156::Tn10kan                     | Tn10kan conversion of CAG12168            |
| 179  | 64.25              | S01023             | relA1 spoT1 thi-1 napG511::Tn10              | B. Bachmann                               |
| 180  | 64.25              | CAG18472           | MG1655 napG511::Tn10                         | Pl(S01023) × MG1655                      |
| 181  | 64.25              | CAG18559           | MG1655 napG517::Tn10kan                      | Tn10kan conversion of CAG18474            |
| 182  | 65.00              | NK6027             | relA1 spoT1 thi-1 Δgpt-lac5 metC162::Tn10     | B. Bachmann                               |
| 183  | 65.00              | CAG18475           | MG1655 metC162::Tn10                         | Pl(NK6027) × MG1655                      |
| 184  | 65.00              | CAG18527           | MG1655 metC3158::Tn10kan                     | Tn10kan conversion of CAG18475            |
| 185  | 66.25              | P2727              | his rpsL tolC210::Tn10                        | P. Reeves (49)                            |
| 186  | 66.25              | CAG12184           | MG1655 tolC210::Tn10                         | Pl(P2727) × MG1655                       |
| 187  | 67.00              | CAG18164           | zhb-3075::Tn10                               | Lab collection                            |
| 188  | 67.00              | CAG12152           | MG1655 zhb-3075::Tn10                        | Pl(CAG18164) × MG1655                     |
| 189  | 67.00              | CAG18574           | MG1655 zhb-3159::Tn10kan                     | Tn10kan conversion of CAG12152            |
| 190  | 68.75              | SK2262             | leuB6 hisG1 argG6 metB1 lacY1 gal-6 xyl-7 met2 rpsL104 tonA22 tsx-1 supE44 zgi-203::Tn10 | B. Bachmann, formerly                   |
| 191  | 68.75              | CAG12072           | MG1655 zgi-203::Tn10                         | Pl(SK2262) × MG1655                      |
| 192  | 68.75              | CAG12115           | MG1655 zgi-3198::Tn10kan                     | Tn10kan conversion of CAG12072            |
| 193  | 70.00              | DV6                | metB1 panD2 relA1 spoT1 gyrA216 X' zhu-6::Tn10 | C. O. Rock (59)                           |
| 194  | 70.00              | CAG12153           | MG1655 zhu-6::Tn10                           | Pl(DV6) × MG1655                         |
| 195  | 70.00              | CAG18605           | MG1655 zhu-316::Tn10kan                      | Tn10kan conversion of CAG12153            |
| 196  | 71.75              | JW375              | superE44 zhb-3082::Tn10                      | D. Clark, formerly                       |
| 197  | 71.75              | CAG12071           | MG1655 zhb-3082::Tn10                        | Pl(JW375) × MG1655                       |
| 198  | 71.75              | CAG12120           | MG1655 zhb-3169::Tn10kan                     | Tn10kan conversion of CAG12071            |

Continued on following page
| Line | Insertion position | Strain                  | Genotype                                                                 | Source or construction |
|------|------------------|-------------------------|--------------------------------------------------------------------------|------------------------|
| 199  | 72.00            | DV9                     | metB1 panB2 relA1 spoT1 gyrA216 k+ zhe-9::Tn10                            | C. O. Rock (59)        |
| 200  | 72.00            | CAG12159                | MG1655 zhe-9::Tn10                                                      | Pl(DV9) × MG1655       |
| 201  | 72.00            | CAG18606                | MG1655 zhe-317::Tn10                                                    | Tnl0kan conversion of CAG12159 |
| 202  | 72.75            | JK372                   | zhe-3083::Tn10                                                          | D. Clark, formerly zhe-9::Tn10 |
| 203  | 72.75            | CAG12075                | MG1655 zhe-3083::Tn10                                                   | Pl(JK372) × MG1655     |
| 204  | 72.75            | CAG12133                | MG1655 zhe-317::Tn10                                                    | Tnl0kan conversion of CAG12075 |
| 205  | 74.00            | CAG18612                | gal-3 crsG zhe-3084::Tn10                                               | Lab collection         |
| 206  | 74.00            | CAG18456                | MG1655 crsG zhe-3084::Tn10                                              | Pl(CAG18612) × MG1655  |
| 207  | 74.00            | CAG18556                | MG1655 crsG zhe-317::Tn10                                               | Tnl0kan conversion of CAG18456 |
| 208  | 74.50            | CAG8007                 | trp(Am) lac(Am) pho(Am) supC(Ts) spoH165(Am) zhe-3085::Tn10             | Lab collection         |
| 209  | 74.50            | CAG18452                | MG1655 zhe-3085::Tn10                                                   | Pl(CAG8007) × MG1655   |
| 211  | 75.50            | CAG2228                 | relA1 spoT1 thi-1 pveI zib-207::Tn10                                   | T. Yura (57)           |
| 212  | 75.50            | CAG18450                | MG1655 zib-207::Tn10                                                    | Pl(CAG2228) × MG1655   |
| 213  | 75.50            | CAG18573                | MG1655 zib-317::Tn10                                                    | Tnl0kan conversion of CAG18450 |
| 214  | 76.50            | CAG18638                | MG1655 zhe-3086::Tn10                                                   | This work              |
| 215  | 77.75            | CAG18639                | MG1655 zhe-3087::Tn10                                                   | This work              |
| 216  | 78.50            | CAG18640                | MG1655 zib-3076::Tn10                                                   | This work              |
| 217  | 80.00            | CAG12175                | MG1655 zib-3077::Tn10                                                   | This work              |
| 218  | 80.75            | BW322                   | relA1 spoT1 thi-1 pveI zib-207::Tn10                                   | B. Weiss (18), formerly zib-901::Tn10 |
| 219  | 80.75            | CAG12163                | MG1655 zib-207::Tn10                                                    | Pl(BW322) × MG1655     |
| 220  | 80.75            | CAG18569                | MG1655 zib-316::Tn10                                                    | Tnl0kan conversion of CAG12163 |
| 221  | 81.75            | RK4901                  | rbc argH metB his pveL cysE lac mtl rpsL zib-901::Tn10                   | R. Kadner (33), formerly zib-901::Tn10 |
| 222  | 81.75            | CAG18492                | MG1655 zib-901::Tn10                                                    | Pl(RK4901) × MG1655    |
| 223  | 81.75            | CAG18572                | MG1655 zib-316::Tn10                                                    | Tnl0kan conversion of CAG18492 |
| 224  | 83.00            | JW355                   | asusB2 relA1 thyA95 spoT1 thi-1 deo-33 zib-501::Tn10                     | B. Bachmann, formerly zib-501::Tn10 |
| 225  | 83.00            | CAG18499                | MG1655 zib-501::Tn10                                                    | Pl(JW355) × MG1655     |
| 226  | 83.00            | CAG18558                | MG1655 zib-316::Tn10                                                    | Tnl0kan conversion of CAG18499 |
| 227  | 83.75            | SK2210                  | tss-3 supF42 hisG4 rpsL281 xyl-7 mtl-1 argH1 zib-290::Tn10                | B. Bachmann            |
| 228  | 83.75            | CAG18501                | MG1655 zib-290::Tn10                                                   | Pl(SK2210) × MG1655    |
| 229  | 83.75            | CAG18592                | MG1655 zib-316::Tn10                                                    | Tnl0kan conversion of CAG18501 |
| 230  | 84.50            | MBGO                    | araC(Am) araD metB lacZ(Am) rpsL zib-500::Tn10                           | J. Beckwith            |
| 231  | 84.50            | CAG18431                | MG1655 zib-500::Tn10                                                   | Pl(MBGO) × MG1655      |
| 232  | 84.50            | CAG18599                | MG1655 zib-316::Tn10                                                   | Tnl0kan conversion of CAG18431 |
| 233  | 85.50            | RK4349                  | rbc argH metB proB ema rpsL his Δlac metE3079::Tn10                     | R. Kadner              |
| 234  | 85.50            | CAG18491                | MG1655 metE3079::Tn10                                                  | Pl(RK4349) × MG1655    |
| 235  | 86.25            | RS3087                  | fadAB101::Tn10                                                         | R. Simons              |
| 236  | 86.25            | CAG18496                | MG1655 fadAB101::Tn10                                                   | Pl(RS3087) × MG1655    |
| 237  | 86.25            | CAG18557                | MG1655 fadAB165::Tn10                                                   | Tnl0kan conversion of CAG18496 |
| 238  | 87.00            | SK1861                  | rpsL196 trp49825 zib-35::Tn10                                          | S. Kushner             |
| 239  | 87.00            | CAG18495                | MG1655 zib-35::Tn10                                                     | Pl(SK1861) × MG1655    |
| 240  | 87.00            | CAG18601                | MG1655 zib-316::Tn10                                                   | Tnl0kan conversion of CAG18495 |
| 241  | 87.50            | CAG18636                | MG1655 zib-3088::Tn10                                                  | This work              |
| 242  | 88.50            | JW383                    | thr-1 leuB6 eda-50 hisG4 metF159 thi-1 ara-14 lac Y1 xyl-5 mtl-1 rpsL136 tonA31 tsx-78 Δgal-attX)99 zib-501::Tn10 | B. Bachmann, formerly zib-501::Tn10 |
| 243  | 88.50            | CAG18477                | MG1655 zib-501::Tn10                                                  | Pl(JW383) × MG1655     |
| 244  | 88.50            | CAG18560                | MG1655 zib-316::Tn10                                                  | Tnl0kan conversion of CAG18477 |

Continued on following page
| Line | Insertion position | Strain | Genotype | Source or construction |
|------|--------------------|--------|----------|------------------------|
| 245  | 89.50              | CAG8396 | lysA29  | Lab collection          |
| 246  | 89.50              | MG12185 | lacZ(Am) relAI rpsE argE66::Tn10 |
| 247  | 90.25              | NKS139  | I1nrrD-nrrE1 thr-39::Tn10 |
| 248  | 90.25              | CAG18500 | MG1655 thr-39::Tn10 |
| 249  | 90.25              | CAG18618 | MG1655 thr-3178::Tn10kan |
| 250  | 90.75              | RS162   | thr-l lonB6 tonA21 lacY1 supE44 thyA6 rpsL67 thi-l 
|                  |         |         | donB252 dicC1 
|                  |         |         | zji-504::Tn10 |
| 251  | 90.75              | CAG18498 | MG1655 zji-504::Tn10 |
| 252  | 90.75              | CAG18615 | MG1655 zji-3179::Tn10kan |
| 253  | 91.50              | TSI6    | [araD139] f1B5301 ptsF25 relAI epsL150 
|                  |         |         | dcbC1 
|                  |         |         | DeltaF-lacU169 malF3089::Tn10 |
| 254  | 91.50              | CAG12164 | MG1655 malF3089::Tn10 |
| 255  | 91.50              | CAG18609 | MG1655 malF1809::Tn10kan |
| 256  | 92.50              | CAG18630 | MG1655 zji-3181::Tn10kan |
| 257  | 93.75              | AKK231  | Hfr(P2A) relAI spoT1 ilv-299 metB1 ampC1 
|                  |         |         | zji-2231::Tn10 |
| 258  | 93.75              | CAG18488 | MG1655 zji-2231::Tn10 |
| 259  | 93.75              | CAG18671 | MG1655 zji-3182::Tn10kan |
| 260  | 94.50              | AKK241  | Hfr(P2A) relAI spoT1 ilv-299 metB1 ampC1 
|                  |         |         | zji-2241::Tn10 |
| 261  | 94.50              | CAG18487 | MG1655 zji-2241::Tn10 |
| 262  | 94.50              | CAG18555 | MG1655 zji-3183::Tn10kan |
| 263  | 95.75              | GR401   | [araD139] f1B5301 ptsF25 relAI epsL150 
|                  |         |         | dcbC1 
|                  |         |         | rbsR the araE1 DeltaF-lacU169 Deltahis-gbd |
|                  |         |         | cysA30::Tn10 |
| 264  | 95.75              | CAG12073 | MG1655 cysA30::Tn10 |
| 265  | 95.75              | CAG12114 | MG1655 cysA185::Tn10kan |
| 266  | 96.75              | DF1062  | [araD139] galmE5 galkH6 relAI epsL150 spoT1 hsdR2 
|                  |         |         | Deltaarab-17679 (lat17Y)74 zji-920::Tn10 |
| 267  | 96.75              | CAG12019 | MG1655 zji-920::Tn10 |
| 268  | 96.75              | CAG12110 | MG1655 zji-3180::Tn10kan |
| 269  | 98.25              | SK597   | thr-l relAI zji-6::Tn10 |
| 270  | 98.25              | CAG18429 | MG1655 zji-6::Tn10 |
| 271  | 98.25              | CAG18610 | MG1655 zji-3187::Tn10kan |
| 272  | 99.50              | SK472   | relAI thr-l spoT1 serB22 zji-202::Tn10 |
| 273  | 99.50              | CAG18430 | MG1655 zji-202::Tn10 |
| 274  | 99.50              | CAG18619 | MG1655 zji-3188::Tn10kan |

Other strains
1. MG1655 Wild type
2. SC12 lact(Am) trp(Am) pho(Am) mal(Am) supC(Ts) malT
3. CAG12184 SC12 zbi-23::Tn10
4. CAG1284 SC12 cysE zii-4901
5. CAG12185 SC12 cysE zii-207::Tn10
6. CAG12186 SC12 cysE zii-3160::Tn10kan
7. CAG12187 SC12 zgl-3174::Tn10kan
8. CAG12179 MG1656 purE79::Tn10
9. CAG8408 MG1655 btaB3191::Tn10kan
10. CAG3002 MG1655 btaB3192::Tn10
11. CAG1564 rpsL1 lacZ1U18 trpB38::Tn10
12. CAG18579 MG1655 trpB3193::Tn10kan
13. CAG18458 MG1655 trpB38::Tn10
their Tn10 parent, indicating that they contained a Tn10kan at the same chromosomal location as in the parent. The remainder were located close to but separable from the original Tn10. These candidates, which probably arose from one of the types of chromosomal rearrangement known to be mediated by Tn10 (121, 34), were not further analyzed. When the original Tn10 was located in an easily scorable gene, we also determined whether the Tn10kan showed the predicted 100% linkage to that gene by P1 transduction. For example, CAG18425 (Table 1, mapping strains, line 3) should contain a Tn10kan in the thr gene. P1 vir grown on CAG18425 was used to transduce MG1655 strains to KanR, and transductants were scored for a Thr− phenotype. Only candidates which gave 100% KanR Thr− transductants were considered to have a Tn10kan within the thr gene. All of the 13 Tn10kan insertions tested in this way contained a Tn10kan insertion 100% linked to the original Tn10.

Filling in the transduction gaps. In determining the P1 cotransduction frequencies between Tn10 and Tn10kan transposons, we identified several transduction gaps, i.e., regions where two adjacent drug resistance insertions were not linked by P1 transduction. To close these gaps, we obtained additional insertions by a variety of techniques. In general, random insertion pools were made in strain MG1655 as described above. In most cases, P1 vir was then grown on each pool and used to select insertions which closed a transduction gap as outlined below. Each newly isolated insertion was mapped relative to insertions flanking the gap to determine whether the new insertion aided in closing the gap.

(i) Selection of insertions by cotransduction with nearby auxotrophic markers. In several instances, transduction gaps occurred near easily selectable auxotrophic markers. In these cases, P1 vir grown on random Tn10kan pools was used to transduce auxotrophic strains simultaneously to prototrophy and antibiotic resistance, thus targeting the Tn10kan to the region of the gap. The map position of each insertion was determined with respect to the auxotrophic marker used and the Tn10 transposons which defined the transduction gap. New insertions which closed the transduction gap were placed in the collection.

Strains CAG18447, CAG18470, and CAG12164 (Table 1, mapping strains, lines 17, 151a, and 254, respectively), containing proAB, purC, and malF mutations, respectively, were simultaneously transduced to KanR and to either Pro−, Pur−, or Mal− by using P1 vir grown on Tn10kan pools from MG1655. Independent KanR Pro+, KanR Pur+, and KanR Mal− transductants were purified. Strain CAG12158 was simultaneously transduced to Tet− Phe+ by using P1 vir grown on Tn10 pools from MG1655. New insertions were then mapped with respect to the insertions which defined the transduction gap. Strains CAG18580, CAG18633, CAG18632, CAG18631, and CAG18630 (Table 1, mapping strains, lines 15, 19, 152, 149, 164, and 256, respectively) were constructed in this manner. For their cotransduction frequencies with flanking Tn10 transposons, see Table 2 (crosses 8 to 11, 72 to 75, 80, 81, 126 and 127). For linkage to nearby markers, see Table 3 (crosses 6, 7, 37, 38, 88, and 89).

(ii) Selection of insertions by cotransduction with a nearby Tn10. In some instances, an easily selectable auxotrophic marker was not located near one of the identified transduction gaps. In these cases, we targeted Tn10kan transposons to the region of the transduction gap by ampicillin enrichment for KanR transductants that remove the resident Tn10, as first shown by Foster (19). This strategy takes advantage of the fact that tetracycline, a bacteriostatic antibiotic, will protect Tet+ cells from being killed by penicillin.

Strains containing a Tn10 transposon located at one end of the transduction gap were transduced to KanR by using P1 vir grown on a random Tn10kan pool and enriched for transductants which became Tet+. KanR transductants were pooled, diluted 1:100, and then grown overnight. We enriched for KanR Tet− transductants with two rounds of ampicillin treatment as described by Jin and Gross (32), except that KanR survivors were selected on LB-kanamycin plates. To identify KanR Tet− transductants, we replica plated colonies onto LB-kanamycin-tetracycline and LB-kanamycin plates. Between 10 and 70% of the survivors exhibited a KanR Tet− phenotype. To determine the location of the new Tn10kan transposons with respect to nearby Tn10, we then mapped each candidate with respect to nearby Tn10 transposons by P1 transduction. Strains CAG18629, CAG18562, and CAG18636 (Table 1, mapping strains, lines 104, 165, and 241, respectively) were obtained in this manner. For their cotransduction frequencies with flanking Tn10 transposons, see Table 2 (crosses 50, 51, 82, 83, 119, and 120).

(iii) Isolation of insertions by simultaneous cotransduction of two antibiotic resistance markers with phage T4. The two approaches described above were most suitable for gaps that could be filled with a single insertion. A third approach was used to isolate several new insertions to link the one large transduction gap located between 76 and 80 min. In this case we made random pools on strains carrying insertions that defined this transduction gap. New insertions located near the transduction gap were isolated by demanding simultaneous transduction of both antibiotic resistance markers with phage T4G7, which can transduce approximately 3.5 min of the E. coli chromosome. These new insertions would be located within about 3.5 min of the targeted insertion. Each new insertion was then mapped by P1 transduction to determine its map position. Insertions that closed the transduction gap were kept and placed in the mapping collection.

Random Tn10kan pools were made on the Tn10-containing strains CAG8023 and CAG12185 (Table 1, additional strains, lines 3 and 5), and Tn10 pools were made on the Tn10kan-containing strains CAG12186 and CAG12187 (Table 1, additional strains, lines 6 and 7). Each of these strains carries an insertion at one end of the transduction gap. Strains on which the pools were made contained either the malT marker located 1 min to the left of the gap or the cysE marker located 1 min to the right of the gap, or both markers, which enabled us to screen against new insertions located outside the transduction gap. Phage T4G7T7 was grown on these random insertion pools and used to transduce MG1655 simultaneously to Tet+ and KanR, and the transductants were scored for their Mal and Cys phenotypes. Transductants that retained the Mal+ and Cys− phenotypes of the recipient will have the insertion positioned to the right of mal and to the left of cys. Most of these insertions will be located within the transduction gap. P1 vir stocks grown on purified candidates were used to transduce MG1655 to Tet− or KanR, and transductants were scored for the other antibiotic resistance marker. This transduction had two purposes. The first was to separate the new antibiotic resistance markers from the initial antibiotic resistance marker. The new Tn10 and Tn10kan transposons identified were then mapped against each other to establish linkage by P1 transduction. The second was to identify transductants containing the initial antibiotic resistance marker and the newly selected antibiotic resistance marker. These are candidates for new insertions linked to the outermost insertions.
which defined the gap. These Tn10 and Tn10kan transposons were then separated from each other by P1 transduction and mapped with respect to the outside markers, and the other new antibiotic resistance markers were identified. Strains CAG18638, CAG18639, CAG18640, and CAG12175 (Table 1, mapping strains, lines 214 to 217, respectively) were constructed in this manner. For the transduction frequencies for the insertions closing the gap, see Table 2 (crosses 105 to 110).

A similar approach was used to isolate insertions which closed the transduction gap between 33.5 and 35.5 min. In this case, random Tn5 pools were made on strains CAG18459 and CAG18462 (Table 1, mapping strains, lines 99 and 102, respectively), which defined this gap. P1 vir was then grown on each pool, and MG1655 was simultaneously transduced to Tet' and Kan' and analyzed as above. Strain CAG18637 (Table 1, mapping strains, line 100) was isolated by this technique. For the linkage of the insertions to nearby markers, see Table 2 (crosses 48 and 49).

Coordination of insertions to ordered \( \lambda \) clones. To coordinate the insertion map to the ordered clone bank of Kohara et al. (37), we identified \( \lambda \) clones capable of complementing insertions and point mutations in selectable genes. The \( \lambda \)EMBL4 and \( \lambda \)2001 cloning vectors used for the ordered Kohara clone bank are \( \lambda \) cl1: thus, this set of clones is unable to form stable lysogens. To perform complementation analysis, we used \( \lambda \) wild-type (\( \lambda \) cl1) helper phage to supply \( \lambda \) repressor in \( \lambda \)trans. Dicycogen strains containing wild-type \( \lambda \) and a particular \( \lambda \) clone from the region of interest were constructed by coinfecting stationary-phase cells with \( \lambda \) wild type (multiplicity of infection, 5) and the \( \lambda \) clone (multiplicity of infection, 1) being examined. Infected cells were incubated for 2 h at 37°C and then plated on selective plates and NZY plates as a control for dicycogen formation. This allowed the identification of \( \lambda \) clones able to complement or rescue specific auxotrophic mutations.

RESULTS AND DISCUSSION

The mapping kit we present consists of two sets of strains. The first set contains seven Hfr strains and permits the initial mapping of a mutation to a relatively small portion of the chromosome. The second set contains 182 P1 mapping strains with antibiotic resistance elements at approximately 1-min intervals. We have constructed isogenic strains containing one of two different antibiotic resistance markers at most locations on the chromosome. These mapping strains are used as donors for P1 transductions and allow the localization of unknown mutations to about 1 min or less on the chromosome. The overall mapping strategy relies on selecting for transfer of the antibiotic resistance marker from the donor to the recipient and then scoring the transductants for the presence or loss of the mutant phenotype. Both mapping sets are described below.

Hfr Mapping Set

Construction and characterization of Hfr strains. Two subsets of Hfr strains, differing only in the antibiotic resistance marker they carry, have been constructed for initial mapping of mutations and are described in Fig. 1 and Table 1. Subset 1 contains a Tn10 transposon and subset 2 contains a Tn10kan transposon inserted about 20 min from the origin of transfer of each Hfr.

We have determined the mating efficiency of each Hfr strain in the mapping kit by using the mating conditions described in Materials and Methods. Data for the Tn10 derivatives are presented in Table 4. Note that the different Hfr strains exhibit different efficiencies of mating. HfrH, for example, mates with high efficiency, whereas Hfr strains KL228 and KL14 have a relatively low efficiency of mating. The mating efficiency is not affected by the type of insertion, either Tn10 or Tn10kan, used (data not shown).

We have mapped the origin of transfer of each Hfr with respect to nearby insertions. Each Hfr was mated with strains containing independent antibiotic resistance elements near the reported origin of transfer. Exconjugants were scored for loss of the antibiotic resistance element in the recipient. Antibiotic resistance elements that are removed by the incoming Hfr are transferred early by the Hfr; those that remain are transferred later. Using this strategy, we have been able to place the origin of each Hfr between two adjacent antibiotic resistance markers. The earliest insertion transferred by each Hfr is shown in Fig. 1.

Hfr mapping strategy. Hfr strains transferring overlapping regions of the chromosome were used to localize the position of mutations to a small region of the chromosome. Each Hfr strain carries a selectable antibiotic resistance element approximately 20 min from its respective origin of transfer. Matings were performed as described in Materials and Methods, and the strains were plated onto selective plates. Wild-type alleles of mutations located between the origin of transfer and the selected antibiotic resistance marker were incorporated into exconjugants with a high probability (within the range of 15 to 100%). Scoring as few as 10 to 30 exconjugants is sufficient to establish whether a particular Hfr strain transfers the region in which the mutation is located. By comparing which Hfr strains give wild-type

Fig. 1. Hfr mapping set. 1. HfrH; 2. KL227; 3. KL208; 4. KL196; 5. KL16; 6. KL228; 7. KL14. Arrowheads indicate the origin and direction of transfer for each Hfr; bars indicate the positions of insertions used as selective markers. See Table 1 for genotypes. The position of each origin has been mapped between two insertions, and the first insertion transferred is shown for each Hfr.
recombinants, the position of the mutation can be determined to about a 5- to 15-min interval. This mapping strategy has been used by Wanner (60).

This method involves a single timed mating and only one type of selective plate, and it does not require the use of multiply marked donor or recipient strains, thereby allowing more flexibility in the choice of strain background for mutant selections. By using this approach, a large number of mutations can be easily mapped to a small region of the chromosome in a short time. Once the unknown mutation has been placed in a 5- to 15-min interval on the chromosome, its position can be localized further by P1 transduction, using the mapping strains discussed below. This allows for mapping to within a 1-min interval on the E. coli chromosome. It should be noted that the Hfr strains presented here are a subset of the various Hfr strains available (42, 43, 60), and this method can be used with other Hfr strains. In addition, other Hfr mapping techniques, such as determination of the time of entry (47, 53), can be used to narrow down the map position of mutation.

**P1 Mapping Set**

Our goal was to assemble a collection of isogenic strains, each containing a single antibiotic resistance marker, such that the set of strains would contain antibiotic resistance markers around the entire chromosome with adjacent insertions linked by P1 transduction. These strains are then used in conjunction with the Hfr strains described above to locate the map positions of mutations to within a 1-min region. This is similar to an approach described by Kukral et al. (38), who constructed 279 S. typhimurium strains carrying randomly spaced Tn10Δ163Δ17 elements. Below, we present a collection of strains that contain at least one of two different antibiotic resistance elements at known positions and link the entire E. coli chromosome by P1 transduction, coordinate the positions of these insertions to the standard E. coli map of Bachmann (2), and explain the use of these strains in the mapping of mutations.

**Construction of the P1 mapping set.** To construct the P1 mapping strains, we started with a collection of strains containing Tn10 insertions located around the chromosome. In addition, a few strains contained either a Tn9 or a Tn5 transposon. The origin and type of insertion present in each strain are indicated in Table 1. Each insertion was then transduced into MG1655 by using phage P1 as discussed in Materials and Methods.

To determine the linkage between nearby Tn10 transposons, it was necessary for adjacent Tn10 transposons to encode different antibiotic resistances. To accomplish this, we constructed each Tn10 transposon to a Tn10kan transposon by homologous recombination between the IS10 elements of the resident Tn10 on the chromosome and the IS10 elements of the incoming Tn10kan, carried on a defective λ phage (see Materials and Methods for the procedure and criteria for conversion). About 90% of the Tn10 transposons were amenable to conversion to Tn10kan; despite several attempts, the remainder were resistant. Resistance to conversion is most likely to result from some property of the insertion itself, for example, lack of homology between the resident Tn10 and the Tn10kan on either side of the tetracycline resistance marker as in the case of the mini-Tn10 element Tn/0163Δ17 (61). Less likely possibilities are that the insertion is in a region of the chromosome in which homologous recombination is low or that it is in a gene affecting the conversion process.

To determine the linkage between adjacent insertions, we mapped the position of each Tn10 by P1 transduction relative to each nearby Tn10kan (or Tn9 or Tn5). In general, P1 vir stocks grown on Tn10 strains were used to transduce Tn10kan (or Tn9 or Tn5) strains to Tet', and transductants were scored for Kan' (or Cam' in the case of Tn9). For the cotransduction frequencies obtained, see Table 2. Because the packaging of the DNA by phage P1 is not completely random, transduction frequencies between two markers can vary depending upon whether the donor or recipient carries that marker. In some cases, transductions were performed in both directions, using strains containing a particular insertion as both donor and recipient. In such cases, both sets of data are presented in Table 2. Variability depending upon the direction of transduction can be observed in Table 2 (crosses 93, 94, 136, and 137).

In several places, existing Tn10 transposons were unlinked by P1 transduction to the nearest converted Tn10kan. This led to the identification of several transduction gaps. To close these transduction gaps, we isolated additional Tn10, Tn10kan, and Tn5 insertions. Several strategies, described in detail in Materials and Methods, were used to target insertions to the regions containing these transduction gaps. All newly identified insertions were subsequently mapped with respect to existing antibiotic resistance insertions, which defined the transduction gap. Insertions that were linked to existing antibiotic resistance elements by P1 transduction were added to the collection. Complete cotransduction data for all of the insertions are presented in Table 2.

**Location of the antibiotic resistance elements on the standard E. coli map.** To coordinate the linkage data for the antibiotic resistance insertions with the standard E. coli map, we mapped a number of insertions with respect to nearby genes. Table 3 contains our data for the linkage of these insertions to additional markers, as well as data taken from the literature when appropriate. The sources of data are indicated in Table 3. As a result of these mapping studies, we have changed the alphanumeric map position designation for several insertions to more accurately reflect their map positions. In each case the allele number of the original insertion has been kept as described by Chumley et al. (14) and is indicated in Table 1.

We constructed the accumulated linkage data presented in Tables 2 and 3 to map distance (in minutes) by using the empirical equation $F = (1 - D/L)$ (63). In this equation, $F$ is the cotransduction frequency, $D$ is the distance in minutes, and $L$ is the length of chromosomal DNA packaged by P1. $L$ is normally considered to be 2 min; however, we have adjusted it to 1.8 min because our mapping procedure requires each transducing particle to carry an antibiotic insertion of about 0.2 min in length in addition to chromosomal DNA.

Once the cotransduction frequencies were converted into minutes, the position of each insertion was aligned to the standard E. coli genetic map. We first assigned map locations to insertions in known genes and to insertions with high linkage (>0.8 cotransduction frequencies) to known genes. These insertions are designated by an asterisk in Fig. 2. These map locations served as reference points for aligning the remainder of the insertions to the standard E. coli genetic map. The remainder of the insertions were intercalated between the insertions already aligned on the standard map on the basis of the calculated map distances between each insertion. The insertion map generated is shown in Fig. 2. In cases in which two P1 transductions were performed with the same insertion strains as the donor in one cross and the
TABLE 2. Linkage between insertion elements

| Cross no. | Position of insertion (min)* | Recipient | Insertion allele no. | Donor | Cotransduction by PI Transduction |
|-----------|-----------------------------|-----------|----------------------|-------|----------------------------------|
|           | Recipient | Donor | thr-3091::Tnl0kan | car-96::Tnl0kan | car-96::Tnl0kan | car-96::Tnl0kan |
| 1         | 0.00      | 0.75  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 2         | 0.75      | 0.00  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 3         | 0.75      | 2.00  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 4         | 3.50      | 2.00  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 5         | 3.50      | 4.75  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 6         | 4.75      | 3.50  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 7         | 4.75      | 4.75  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 8         | 4.75      | 6.25  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 9         | 6.25      | 4.75  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 10        | 6.25      | 6.75  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 11        | 7.75      | 6.75  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 12        | 8.00      | 7.75  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 13        | 8.00      | 9.00  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 14        | 9.00      | 8.00  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 15        | 9.00      | 9.50  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 16        | 9.50      | 9.00  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 17        | 9.50      | 10.50 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 18        | 10.50     | 9.50  | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 19        | 10.50     | 11.50 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 20        | 11.50     | 12.25 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 21        | 13.25     | 12.25 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 22        | 13.25     | 14.50 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 23        | 15.00     | 14.50 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 24        | 16.25     | 15.00 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 25        | 16.25     | 16.75 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 26        | 16.75     | 17.75 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 27        | 17.75     | 16.75 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 28        | 18.75     | 17.75 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 29        | 18.75     | 17.75 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 30        | 20.00     | 20.00 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 31        | 20.00     | 21.00 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 32        | 21.00     | 22.25 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 33        | 22.75     | 22.25 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 34        | 22.75     | 24.25 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 35        | 24.25     | 25.25 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 36        | 25.25     | 25.75 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 37        | 25.75     | 25.75 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |
| 38        | 25.75     | 26.75 | 0.00                 | 0.00   | 0.00                             | 0.00   | 0.00 |

continued on following page
| Cross no. | Position of insertion (mm)* | Insertion allele no. | Cotransduction by P1 Transduction† | Frequency | Avg value* |
|---|---|---|---|---|---|
| Recipient | Donor | Recipient | Donor | |
| 39 | 26.75 | 27.25 | zeb-3066::Tn10kan | zeb-506::Tn10 | 0.40 | |
| 40 | 27.25 | 26.75 | zeb-3117::Tn10kan | zeb-3066::Tn10 | 0.51 | 0.45 |
| 41 | 28.50 | 27.50 | zeb-3118::Tn10kan | zeb-506::Tn10 | 0.04 | |
| 42 | 28.50 | 29.50 | zeb-3118::Tn10kan | zeb-3061::Tn10 | 0.11 | |
| 43 | 29.50 | 31.00 | zeb-3061::Tn10 | trg-3120::Tn10kan | 0.02 | |
| 44 | 32.00 | 31.00 | zeb-3117::Tn10kan | trg-2::Tn10 | 0.03 | |
| 45 | 32.00 | 32.75 | zeb-225::Tn10 | zeb-230::Tn9 | 0.18 | |
| 46 | 32.75 | 32.00 | zeb-220::Tn9 | zeb-235::Tn10 | 0.24 | 0.21 |
| 47 | 32.75 | 33.50 | zeb-230::Tn9 | zeb-234::Tn10 | 0.18 | |
| 48 | 33.50 | 34.50 | zeb-3062::Tn5 | zeb-234::Tn10 | 0.04 | |
| 49 | 34.50 | 35.75 | zeb-3062::Tn5 | zeb-235::Tn10 | 0.01 | |
| 50 | 35.75 | 36.00 | zeb-603::Tn10 | zeb-3198::Tn10kan | 0.32 | |
| 51 | 36.00 | 37.50 | zeb-3198::Tn10kan | zeb-925::Tn10 | 0.05 | |
| 52 | 38.25 | 37.50 | zeb-3123::Tn10kan | zeb-925::Tn10 | 0.14 | |
| 53 | 38.25 | 39.50 | zeb-3123::Tn10kan | zeb-223::Tn10 | 0.08 | |
| 54 | 39.50 | 40.25 | zeb-3124::Tn10kan | zeb-3068::Tn10 | 0.10 | |
| 55 | 40.25 | 39.50 | zeb-3068::Tn10 | zeb-3124::Tn10kan | 0.19 | 0.15 |
| 56 | 40.25 | 41.00 | zeb-3068::Tn10 | ede-3126::Tn10kan | 0.10 | |
| 57 | 41.00 | 42.25 | ede-3126::Tn10kan | uvrC279::Tn10 | 0.07 | |
| 58 | 42.25 | 41.00 | uvrC279::Tn10 | ede-3126::Tn10kan | 0.06 | 0.06 |
| 59 | 42.25 | 43.00 | uvrC279::Tn10 | zeb-3128::Tn10kan | 0.25 | |
| 60 | 43.00 | 42.25 | zeb-3128::Tn10kan | uvrC279::Tn10 | 0.16 | 0.18 |
| 61 | 44.25 | 43.00 | zeb-3129::Tn10 | zeb-3128::Tn10kan | 0.03 | |
| 62 | 44.25 | 45.75 | zeb-3189::Tn10kan | mgl-500::Tn10 | 0.005 | |
| 63 | 45.75 | 46.50 | mgl-500::Tn10 | zeb-3130::Tn10kan | 0.06 | |
| 64 | 46.50 | 47.75 | zeb-3130::Tn10kan | zeb-298::Tn10 | 0.03 | |
| 65 | 47.75 | 46.50 | zeb-3142::Tn10kan | zeb-223::Tn10 | 0.01 | 0.02 |
| 66 | 47.75 | 48.50 | zeb-3142::Tn10kan | zeb-723::Tn10 | 0.30 | |
| 67 | 48.50 | 49.50 | zeb-723::Tn10 | zeb-3144::Tn10kan | 0.05 | |
| 68 | 50.50 | 49.50 | fasI771::Tn10 | zeb-3144::Tn10kan | 0.02 | |
| 69 | 50.50 | 51.00 | fasI771::Tn10 | zfb-3135::Tn10kan | 0.32 | |
| 70 | 51.00 | 51.75 | zfb-1::Tn10 | nupC3146::Tn10kan | 0.22 | |
| 71 | 51.75 | 51.00 | nupC3146::Tn10kan | zfb-1::Tn10 | 0.12 | 0.18 |
| 72 | 52.75 | 51.75 | zfb-3071::Tn10kan | nupC510::Tn10 | 0.19 | |
| 73 | 52.75 | 53.25 | zfb-3071::Tn10kan | purC81::Tn10 | 0.42 | |
| 74 | 53.25 | 53.50 | purC81::Tn10 | zfe-3138::Tn10kan | 0.45 | |
| 75 | 53.50 | 54.00 | zfe-3138::Tn10kan | gna-26::Tn10 | 0.35 | |

*Continued on following page*
TABLE 2—Continued

| Cross no. | Position of insertion (min) | Insertion allele no. | Cotransduction by PI Transduction† | Frequency | Avg value
|-----------|-----------------------------|----------------------|-----------------------------------|-----------|-----------
| 76        | 54.00                       | gna-26::Tn10         | zff-3139::Tn10kan                 | 0.25      |           |
| 77        | 55.75                       | nadB3140::Tn10kan    | zff-209::Tn10                     | 0.17      |           |
| 78        | 55.75                       | nadB3140::Tn10kan    | pheA1B::Tn10                      | 0.04      |           |
| 79        | 56.75                       | pheA3141::Tn10kan    | nadB51::Tn10                      | 0.08      | 0.06     |
| 80        | 56.75                       | pheA3141::Tn10kan    | zff-3131::Tn10                    | 0.32      |           |
| 81        | 57.50                       | zff-3131::Tn10       | pheA3141::Tn10kan                 | 0.35      | 0.33     |
| 82        | 57.50                       | zff-3131::Tn10       | zff-3143::Tn10kan                 | 0.30      |           |
| 83        | 58.25                       | zff-3143::Tn10kan    | cysC95::Tn10                      | 0.03      |           |
| 84        | 59.50                       | cysC95::Tn10         | fuc-3154::Tn10kan                 | 0.05      |           |
| 85        | 60.25                       | fuc-3154::Tn10kan    | recD901::Tn10                     | 0.48      |           |
| 86        | 60.75                       | recD901::Tn10        | zge-3155::Tn10kan                 | 0.05      |           |
| 87        | 63.50                       | zge-3156::Tn10kan    | zge-3074::Tn10                    | 0.02      |           |
| 88        | 63.75                       | zge-3156::Tn10kan    | nupG51::Tn10                      | 0.37      |           |
| 89        | 64.25                       | nupG3157::Tn10kan    | zge-3156::Tn10                    | 0.28      | 0.32     |
| 90        | 64.25                       | nupG3157::Tn10kan    | metC162::Tn10                     | 0.11      |           |
| 91        | 65.00                       | metC3158::Tn10kan    | nupG51::Tn10                      | 0.08      | 0.06     |
| 92        | 66.25                       | tolC210::Tn10        | metC3158::Tn10kan                 | 0.24      |           |
| 93        | 66.25                       | tolC210::Tn10        | zgb-3159::Tn10kan                 | 0.24      |           |
| 94        | 67.00                       | tolC210::Tn10        | zgb-3159::Tn10                    | 0.05      | 0.15     |
| 95        | 67.00                       | zgb-3159::Tn10       | zgb-203::Tn10                     | 0.005     |           |
| 96        | 70.00                       | zgb-3168::Tn10kan    | zgb-203::Tn10                     | 0.07      |           |
| 97        | 70.00                       | zgb-3168::Tn10kan    | zgb-3082::Tn10                    | 0.02      |           |
| 98        | 71.75                       | zgb-3169::Tn10kan    | zgb-3082::Tn10                    | 0.06      | 0.04     |
| 99        | 72.00                       | zgb-3170::Tn10kan    | zgb-3082::Tn10                    | 0.31      |           |
| 100       | 72.00                       | zgb-3170::Tn10kan    | zgb-3083::Tn10                    | 0.18      |           |
| 101       | 72.75                       | zgd-3083::Tn10       | zhe-3172::Tn10kan                 | 0.05      |           |
| 102       | 74.00                       | zhe-3172::Tn10kan    | zhe-3085::Tn10                    | 0.32      |           |
| 103       | 74.50                       | zhe-3173::Tn10kan    | zhe-3085::Tn10                    | 0.26      |           |
| 104       | 75.50                       | zhe-3174::Tn10kan    | zhe-3085::Tn10                    | 0.29      | 0.27     |
| 105       | 75.50                       | zhe-3174::Tn10kan    | zhe-3086::Tn10                    | 0.22      |           |
| 106       | 76.50                       | zhe-3174::Tn10kan    | zhe-3086::Tn10                    | 0.26      | 0.24     |
| 107       | 76.50                       | zhe-3086::Tn10       | zhe-3087::Tn10kan                 | 0.02      |           |
| 108       | 77.75                       | zhe-3087::Tn10kan    | zhe-3076::Tn10                    | 0.17      |           |
| 109       | 78.50                       | zhe-3076::Tn10       | zhe-3077::Tn10kan                 | 0.02      |           |
| 110       | 80.00                       | zhe-3076::Tn10       | zhe-207::Tn10                     | 0.20      |           |
| 111       | 81.75                       | zhe-3076::Tn10       | zhe-207::Tn10                     | 0.11      |           |
| 112       | 83.00                       | zhe-3076::Tn10       | zhe-4901::Tn10                    | 0.04      |           |

Continued on following page
TABLE 2—Continued

| Cross no. | Position of insertion (min)<sup>a</sup> | Recipient | Donor | Insertion allele no. | Recipient | Donor | Cotransduction by P1 Transduction<sup>b</sup> | Frequency | Avg value<sup>c</sup> |
|-----------|---------------------------------------|-----------|-------|----------------------|-----------|-------|------------------------------------------|-----------|-----------------|
| 113       | 83.75                                 | zic-3163::Tn10kan | zid-501::Tn10 | 0.14 |
| 114       | 84.50                                 | zic-3164::Tn10kan | zie-296::Tn10 | 0.25 |
| 115       | 84.50                                 | metE3079::Tn10 | 0.07 |
| 116       | 85.50                                 | metE3079::Tn10 | 0.11 | 0.09 |
| 117       | 85.50                                 | fadA3165::Tn10 | 0.50 |
| 118       | 87.00                                 | fadAB101::Tn10 | 0.33 |
| 119       | 87.00                                 | zih-35::Tn10 | 0.35 |
| 120       | 87.50                                 | zii-3167::Tn10kan | zii-3088::Tn10kan | 0.03 |
| 121       | 88.50                                 | zii-3167::Tn10kan | argE396::Tn10 | 0.7 |
| 122       | 89.50                                 | argE396::Tn10 | 0.4 | 0.55 |
| 123       | 90.25                                 | thi-3178::Tn10kan | argE396::Tn10 | 0.14 |
| 124       | 90.75                                 | thi-39::Tn10 | 0.17 |
| 125       | 91.50                                 | malF3180::Tn10kan | zjb-504::Tn10 | 0.28 |
| 126       | 91.50                                 | malF2::Tn10 | 0.05 |
| 127       | 92.50                                 | zic-3181::Tn10 | zic-3081::Tn10kan | 0.05 |
| 128       | 93.50                                 | zic-3182::Tn10kan | zic-2241::Tn10 | 0.11 |
| 129       | 94.50                                 | zic-3183::Tn10kan | zic-2241::Tn10 | 0.18 | 0.15 |
| 130       | 94.50                                 | cycA30::Tn10 | 0.02 |
| 131       | 95.75                                 | cycA30::Tn10 | 0.02 |
| 132       | 96.75                                 | zic-3186::Tn10kan | zih-920::Tn10 | 0.51 |
| 133       | 96.75                                 | cycA30::Tn10 | 0.40 | 0.45 |
| 134       | 96.75                                 | zic-3186::Tn10kan | zic-6::Tn10 | 0.005 |
| 135       | 98.25                                 | zic-3187::Tn10kan | zic-6::Tn10 | 0.005 | 0.005 |
| 136       | 98.25                                 | zic-3188::Tn10kan | zic-6::Tn10 | 0.28 |
| 137       | 99.50                                 | zic-3187::Tn10kan | zic-202::Tn10 | 0.07 | 0.17 |
| 138       | 99.25                                 | zic-3188::Tn10kan | thr-H4::Tn10 | 0.21 |
| 139       | 00.00                                 | thr-H4::Tn10 | 0.24 | 0.22 |

<sup>a</sup> Positions are based on assigning thr-14::Tn10 a position of 0 min. All other positions were calculated from the equation by Wu (83), modified as described in the text.

<sup>b</sup> Linkage determinations were based on scoring at least 100 transductants. In cases in which cotransduction frequencies were <0.05, at least 200 transductants were scored.

<sup>c</sup> When available, the average of transduction data in both directions was used to determine the map position listed in Fig. 2.

reciprocal in the second, the map position was determined by averaging the two frequencies obtained (Table 2).

The map positions we derived were in excellent agreement with the standard genetic map of E. coli, indicating the strength of these mapping techniques. It is important to remember that the standard genetic map is a compilation of many data, including results from Hfr and P1 transduction experiments from many laboratories (2). The insertion map we present in Fig. 2 is based on the P1 cotransduction frequencies between a series of insertions that are converted into minutes and then fitted onto the standard map. In general, the map in Fig. 2 should be used as a guide to determine which insertions one may wish to use for mapping purposes (see below). In some instances, cotransduction frequencies may vary as a result of differences in strain backgrounds. Once mutations have been mapped between insertions, more detailed mapping should include the mapping of mutations with respect to known genes in any given region. This can be done by referring to the standard genetic map (2).

**P1 mapping strategy.** The 182 mapping strains described above were designed to be used in conjunction with the Hfr mapping set presented. Once a mutation has been mapped with either of the Hfr subsets to a 5- to 15-min region, P1 transductions are performed to locate the mutation to an interval of approximately 1 min. The mapping strains co-
TABLE 3. Linkage of insertions to known nearby markers

| Cross no. | Position of insertion | Insertion | Marker(s) | Cotransduction frequency by P1 transduction | Reference (if not this work) |
|-----------|-----------------------|-----------|-----------|---------------------------------------------|-----------------------------|
| 1         | 00.00                 | thr-34::Tn10 | ser       | 0.76                                        |                             |
| 2         | 00.75                 | car-96::Tn10 | leu       | 0.16                                        |                             |
| 3         | 00.75                 | car-96::Tn10 | araBAD    | 0.62                                        |                             |
| 4         | 02.00                 | zac-3051::Tn10 | araBAD | 0.40                                        |                             |
| 5         | 02.00                 | zac-3051::Tn10 | leu       | 0.80                                        |                             |
| 6         | 04.75                 | zac-3095::Tn10::kan | proAB | 0.07                                        |                             |
| 7         | 06.75                 | zac-3198::Tn10::kan | proAB | 0.12                                        |                             |
| 8         | 08.00                 | lac-42::Tn10 | proC      | 0.08                                        |                             |
| 9         | 08.00                 | zac-3053::Tn10 | proC      | 0.60                                        |                             |
| 10        | 16.75                 | nadA57::Tn10 | bio       | 0.12                                        |                             |
| 11        | 16.75                 | nadA57::Tn10 | gal       | 0.80                                        |                             |
| 12        | 17.75                 | zhi-29::Tn10 | gal       | 0.07                                        |                             |
| 13        | 21.00                 | zeb-3059::Tn10 | pyrD      | 0.70                                        |                             |
| 14        | 22.25                 | zec-282::Tn10 | pyrD      | 0.10                                        |                             |
| 15        | 22.25                 | zec-282::Tn10 | fabA      | 0.09                                        | D. Clark                    |
| 16        | 22.25                 | zec-282::Tn10 | purA      | 0.40                                        | D. Clark                    |
| 17        | 25.25                 | zecf-117::Tn10 | purB     | 0.90                                        |                             |
| 18        | 27.25                 | zecf-506::Tn10 | trpA      | 0.43                                        | B. Bachmann                 |
| 19        | 27.25                 | zecf-506::Tn10 | tyrT      | 0.83                                        |                             |
| 20        | 28.50                 | zecf-233::Tn10 | trpA      | 0.25                                        |                             |
| 21        | 29.50                 | zecf-3061::Tn10 | fmr      | 0.95                                        |                             |
| 22        | 37.50                 | zecf-925::Tn10 | pki       | 0.55                                        |                             |
| 23        | 37.50                 | zecf-925::Tn10 | pps       | 0.65                                        |                             |
| 24        | 39.50                 | zecf-225::Tn10 | ptsM      | 0.25                                        | D. Clark                    |
| 25        | 39.50                 | zecf-225::Tn10 | fabI      | 0.42                                        | D. Clark                    |
| 26        | 39.50                 | zecf-225::Tn10 | gap       | 0.58                                        | D. Clark                    |
| 27        | 43.00                 | zecf-3069::Tn10 | his       | 0.05                                        |                             |
| 28        | 44.25                 | zecf-3129::Tn10 | his       | 0.68                                        |                             |
| 29        | 47.75                 | zecf-298::Tn10 | gyrA      | 0.30                                        |                             |
| 30        | 47.75                 | zecf-298::Tn10 | ompC      | 0.77                                        | B. Bachmann                 |
| 31        | 48.50                 | zecf-723::Tn10 | gyrA      | 0.86                                        |                             |
| 32        | 49.50                 | zecf-223::Tn10 | purF      | 0.18                                        |                             |
| 33        | 51.00                 | zecf-1::Tn10 | purF      | 0.11                                        |                             |
| 34        | 51.00                 | zecf-1::Tn10 | araC      | 0.20                                        |                             |
| 35        | 51.00                 | zecf-1::Tn10 | fadL      | 0.35                                        |                             |
| 36        | 51.00                 | zecf-1::Tn10 | bsdA      | 0.80                                        | B. Bachmann                 |
| 37        | 52.75                 | zecf-3071::Tn10::kan | purC | 0.45                                        |                             |
| 38        | 53.75                 | zecf-3138::Tn10::kan | purC | 0.52                                        |                             |
| 39        | 55.00                 | zecf-208::Tn10 | ung       | 0.04                                        |                             |
| 40        | 55.00                 | zecf-208::Tn10 | nadB      | 0.08                                        |                             |
| 41        | 55.00                 | zecf-208::Tn10 | gviA      | 0.90                                        |                             |

Continued on following page.
| Cross no. | Position of insertion\(^t\) | Insertion     | Marker(s) | Cotransduction frequency by PI transduction | Reference (if not this work) |
|----------|-----------------------------|---------------|-----------|---------------------------------------------|-----------------------------|
| 42 60.25 | fuc-3972::Tn10              | relA          | 0.45      |                                             |                             |
| 43 60.25 | fuc-3972::Tn10              | arg           | 0.59      |                                             |                             |
| 44 60.25 | fuc-3972::Tn10              | cynC          | 0.38      |                                             |                             |
| 45 61.75 | zgc-3074::Tn10              | thy           | 0.40      |                                             |                             |
| 46 65.00 | metC162::Tn10               | rpoD          | 0.09      |                                             |                             |
| 47 66.25 | tolC210::Tn10               | metC          | 0.45      |                                             |                             |
| 48 66.25 | tolC210::Tn10               | rpoD          | 0.60      |                                             |                             |
| 49 67.00 | zgh-3075::Tn10              | rpoD          | 0.90      |                                             |                             |
| 50 68.75 | zgi-203::Tn10               | argG          | 0.80      |                                             |                             |
| 51 70.00 | zhu-6::Tn10                 | argG          | 0.27      |                                             |                             |
| 52 71.75 | zih-3082::Tn10              | aroE          | 0.81      |                                             |                             |
| 53 71.75 | zih-3082::Tn10              | rpsL          | 0.60      |                                             |                             |
| 54 72.00 | zhe-9::Tn10                 | rpsL          | 0.30      |                                             |                             |
| 55 72.00 | zhe-9::Tn10                 | aroE          | 0.50      |                                             |                             |
| 56 72.75 | zbd-3083::Tn10              | aroE          | 0.12      |                                             |                             |
| 57 74.00 | zhe-3085::Tn10              | rpsL          | 0.29      |                                             |                             |
| 58 74.00 | zhe-3085::Tn10              | madT          | 0.25      |                                             |                             |
| 59 74.50 | zhe-3084::Tn10              | rpsL          | 0.50      |                                             |                             |
| 60 74.50 | zhe-3084::Tn10              | madT          | 0.60      |                                             |                             |
| 61 75.50 | zhf-50::Tn10                | rpoH (rpsH)   | 0.60      |                                             |                             |
| 62 75.50 | zhf-50::Tn10                | madT          | 0.70      |                                             |                             |
| 63 76.50 | zku-3886::Tn10              | rpoH          | 0.50      |                                             |                             |
| 64 80.75 | zib-207::Tn10               | cysE          | 0.33      |                                             |                             |
| 66 81.75 | zic-4901::Tn10              | gltC (gltS)   | 0.52      |                                             |                             |
| 67 81.75 | zic-4901::Tn10              | pvrE          | 0.70      |                                             |                             |
| 68 83.00 | zid-501::Tn10               | asa           | 0.50      |                                             | B. Bachmann                 |
| 69 83.00 | zid-501::Tn10               | bgi           | 0.72      |                                             | B. Bachmann                 |
| 70 83.00 | zid-501::Tn10               | dnaA          | 0.90      |                                             |                             |
| 71 83.75 | zic-296::Tn10               | ilv           | 0.66      |                                             |                             |
| 72 83.75 | zic-296::Tn10               | cya           | 0.06      |                                             |                             |
| 73 84.50 | itv-500::Tn10               | cya           | 0.48      |                                             |                             |
| 74 85.50 | metE3079::Tn10              | cya           | 0.42      |                                             |                             |
| 75 86.25 | fadAB101::Tn10              | metE          | 0.15      |                                             |                             |
| 76 87.00 | zib-15::Tn10                | polA          | 0.64      |                                             | S. Kushner                  |
| 77 88.50 | zib-501::Tn10               | argE          | 0.58      |                                             |                             |
| 78 88.50 | zib-501::Tn10               | metF          | 0.90      |                                             |                             |
| 79 88.50 | zib-501::Tn10               | rpoB          | 0.42      |                                             |                             |
| 80 89.50 | argE86::Tn10                | thi           | 0.14      |                                             |                             |
| 81 89.50 | argE86::Tn10                | rpoB          | 0.53      |                                             |                             |
| 82 89.50 | argE86::Tn10                | metA          | 0.05      |                                             |                             |
| 83 90.25 | thi-39::Tn10                | rpoB          | 0.90      |                                             |                             |
| 84 90.25 | thi-39::Tn10                | metA          | 0.05      |                                             |                             |
| 85 90.25 | thi-39::Tn10                | argH          | 0.34      |                                             |                             |

Continued on following page
containing an antibiotic resistance marker in the appropriate region serve as donor strains for P1 transductions, and the mutant strain serves as the recipient. Transductants are selected on the basis of the antibiotic resistance marker of the donor used and then scored for the presence or absence of the mutant phenotype. The cotransduction frequencies between the insertion and the mutation can then be used to place the mutation between two linked antibiotic resistance insertions, and the relative map position can be determined. Additional markers from the standard genetic map can then be used to perform three-factor crosses to align the mutation to other genes in the region, when additional markers are available in the region of interest.

A unique practical advantage of this system is that the P1 mapping set contains two different antibiotic resistance markers at most positions on the chromosome and allows for the mapping of mutations in genetic backgrounds that already contain one antibiotic resistance marker (either Tet' or Kan'). For example, the recipient strain may contain an antibiotic resistance marker linked to a transcriptional or translational fusion necessary to score the mutant phenotype. Alternatively, the original mutation may have been selected by insertion mutagenesis, bringing with it either a Tn10 or a Tn5. As long as the recipient contains only one of the antibiotic resistance markers described here (Tet' or Kan'), the P1 mapping strains containing an alternative antibiotic resistance gene can be used as described above to locate the mutation.

An additional benefit of this mapping set is the ability to generate linked antibiotic insertions (with one of two antibiotic resistances) on either side of the mutation of interest. Once obtained, these insertions can be used to facilitate subsequent genetic manipulations involving the mutation. For example, they can be used to construct strains containing markers closely linked on either side of the original mutation. Conversely, the insertions can be used to remove markers on either side of the desired mutation. This is often important when selection for a particular phenotype generates multiple, closely linked mutations.

The mapping strategy described above is most easily applicable for locating mutations with strong, selectable phenotypes. Mutations that have weak or unstable phenotypes or phenotypes that must be analyzed by biochemical assay are not amenable to this approach. In these cases, a strategy of chromosome replacement can be used. This strategy takes advantage of the mapping strains containing alternating antibiotic resistance elements. We can replace DNA segments between two insertions by selecting for one antibiotic resistance marker and scoring for the loss of the adjacent marker. Using this technique, we can walk down the chromosome in the region in which the mutation has been located, systematically replacing one segment after another. For each replacement, only one or two transductants need be assayed to determine whether the mutation has been removed. This is in contrast to the usual method, in which 100 or so transductants must be scored to determine accurate linkage to an insertion.

Once the mutation has been located between two antibiotic resistance markers, a similar strategy can be used to move it from one strain background to another. In this case, phage P1 would be grown on the strain containing the mutation and a linked antibiotic resistance marker. The recipient strain would have the linked antibiotic resistance marker on the other side of the mutation. Transductants would be selected for the antibiotic resistance marker from the donor and screened for transductants that had lost the flanking antibiotic resistance marker on the other side. Virtually all of these transductants should have the mutant phenotype.

| Cross no. | Position of insertion | Insertion | Marker(s) | Cotransduction frequency by P1 transduction | Reference (if not this work) |
|-----------|----------------------|-----------|-----------|---------------------------------------------|-----------------------------|
| 86        | 90.75                | zjb-504::Tn10 | metA      | 0.16                                        |                             |
| 87        | 91.50                | malF::Tn10  | metA      | 0.09                                        |                             |
| 88        | 92.50                | zje-318I::Tn10kan | malF | 0.10                                        |                             |
| 89        | 92.50                | zje-318I::Tn10kan | melAB | 0.16                                        |                             |
| 90        | 93.75                | zjd-2231::Tn10 | ampC      | 0.66                                        | B. Bachmann                 |
| 91        | 93.75                | zjd-2231::Tn10 | melB      | 0.80                                        |                             |
| 92        | 94.50                | zje-2241::Tn10 | ampC      | 0.51                                        | B. Bachmann                 |
| 93        | 94.50                | zje-2241::Tn10 | melB      | 0.23                                        |                             |
| 94        | 99.50                | zji-202::Tn10 | thr       | 0.01                                        |                             |
| 95        | 99.50                | zji-202::Tn10 | serB      | 0.06                                        |                             |

* As in Table 2.

### Table 3—Continued

**Table 4. Hfr mating efficiencies**

| Hfr strain | Total no. of exconjugants | Mating efficiency |
|------------|---------------------------|-------------------|
| HfrH       | $2.1 \times 10^4$         | $2.1 \times 10^3$ |
| KL14       | $8.2 \times 10^2$         | $8.2 \times 10^5$ |
| KL16       | $2.2 \times 10^3$         | $2.2 \times 10^4$ |
| KL96       | $1.3 \times 10^3$         | $1.3 \times 10^4$ |
| KL208      | $4.2 \times 10^4$         | $4.2 \times 10^4$ |
| KL227      | $8.9 \times 10^3$         | $8.9 \times 10^4$ |
| KL228      | $3.6 \times 10^2$         | $3.6 \times 10^5$ |

* Mating efficiency is defined as the number of exconjugants divided by the number of donors used. Matings were done as described in Materials and Methods.
FIG. 2. Insertion map. Asterisks indicate insertions used to align the insertion map to the standard E. coli genetic map of Bachmann (2). Each insertion was placed onto the standard genetic map as described in the text. Numbers between insertions represent the P1 cotransduction frequency indicated as percent cotransduction (Table 2). Minute designations are to the left of the bar.
TABLE 5. Correlation between the Kohara physical map and the insertion map showing identification of complementing λ clones.

| Location   | Position (min) | Comple-     | Location of point mutation | Position (min) | Comple-     |
|------------|----------------|-------------|----------------------------|----------------|-------------|
|            |                | menting λ clone no. |                          |                | menting λ clone no. |
| thr        | 0.00           | 9E4         | dnaK                       | 0.00           | 6H3         |
| proAB      | 0.25           | 8G4         | ara                        | 0.50           | 8H1         |
| proC       | 0.90           | 6A12        | leu                        | 0.75           | 6F3         |
| purE       | 12.25          | 6E7         | his                        | 44.00          | 2H10        |
| argA       | 20.25          | 6H3         | argG                       | 69.00          | 18H7        |
| trp        | 27.75          | AF1         |                            |                |             |
| purC       | 53.25          | 4C11        |                            |                |             |
| cysC       | 59.25          | 12G1        |                            |                |             |
| ltv        | 84.50          | 2E6         |                            |                |             |
| metE       | 85.50          | 7G1         |                            |                |             |
| argE       | 89.50          | 4G11        |                            |                |             |

* Clone number as in Kohara et al. (37).

Correlation between Our Insertion Map and the Physical Map of E. coli

Once mutations have been mapped and novel loci have been identified, it is usually important to clone the novel gene. To facilitate the cloning of such genes, we have correlated our insertion map to the ordered clone bank of Kohara et al. (37) by determining clones that complement or rescue auxotrophic mutations. As described in Materials and Methods, coinfection with λ λ' was necessary to form stable lysogens required for complementation analysis. The λ clones able to complement or rescue strains carrying insertions or point mutations in genes with selectable phenotypes were identified and are listed in Table 5. The λ clones which identify the position of additional markers determined by Kohara et al. (37) are listed in Table 6. These clones should be used as reference points to determine which clones may carry the wild-type allele of the mutation of interest. Once a novel locus has been mapped, a λ clone carrying the wild-type allele can be identified by screening clones in the vicinity of the mapped locus. This method will allow for the quick screening of a small number of λ clones, from 5 to 10, to identify clones carrying the wild-type gene of interest.

This particular strategy required for cloning depends upon the nature of the mutation. If the mutant allele is recessive, λ clones carrying the wild-type allele can be obtained by complementation. When the wild-type allele is selectable, λ clones which span the location of the mutation are used to make lysogens and the wild-type phenotype is selected. If the wild-type allele cannot be selected but can be scored, it may be useful to first confirm the formation of dilsogenic clones, by demonstrating the release of both turbid (helper) and clear (clone) plaques, before screening dilsogenic for the wild-type phenotype. If the mutant allele is dominant, λ clones carrying the wild-type allele must be obtained by recombination. In this case, confirmed dilsogenic containing λ clones spanning the location of the mutation, are plated under selective conditions, Wild-type recombinants should appear at significantly higher frequency than revertants on the selective plates. The survivor recombination approach can also be used in instances when λ clones of interest do not express the wild-type gene on the cloned fragment. In such cases, wild-type recombinants can be obtained as described above.

TABLE 6. Correlation between the Kohara physical map and the insertion map indicating additional markers

| Gene  | Position (min) | λ clone no. | λ clone no. | λ clone no. | λ clone no. |
|-------|----------------|-------------|-------------|-------------|-------------|
| lacZ  | 08.00          | 10A6        | 28.25       | 18H6        |             |
| ntrR  | 29.50          | 3G3         | 37.50       | 12H         |             |
| mgl   | 45.75          | 7F1         | 54.00       | 8E3         |             |
| pyrG  | 59.75          | 8B9         | 67.00       | 19F2        |             |
| cml   | 79.75          | 6F2         | 80.75       | 17G2        |             |
| cya   | 85.00          | 12G1        | 90.00       | 18B7        |             |
| melAB | 93.50          | 8H1         | 99.50       | 12A4        |             |

* Clone numbers are from Kohara et al. (37). Only one clone number is given for each position. Additional overlapping clone numbers are not given.

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