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Rapid Mapping in *Salmonella typhimurium* with Mu*d*-P22 Prophages

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A new method for mapping mutations in the *Salmonella typhimurium* chromosome is described and applied to the localization of novel regulatory mutations affecting expression of the *nirB* (nitrite reductase) gene. The mapping technique is also illustrated by the mapping of mutations in genes affecting carbohydrate catabolism and biosynthetic pathways. The new mapping method involves use of the hybrid phage Mu*d*P and Mu*d*Q (together referred to as Mu*d*-P22), originally constructed by Youderian et al. (Genetics 118:581–592, 1988). This report describes a set of Mu*d*-P22 lysogens, each member of the set containing a different Mu*d*-P22 insertion. The insertions are scattered along the entire *Salmonella* genome. These lysogens, when induced by mitomycin C, generate transducing lysates that are enriched (45- to 1,400-fold over the background, generalized transducing particle population) for transducing particles containing bacterial DNA that flanks one side of the insertion. We demonstrate that within the set of lysogens there can be found at least one Mu*d*-P22 insertion that enriches for any particular region of the *Salmonella* chromosome and that, therefore, all regions of the chromosome are discretely enriched and represented by the collection as a whole. We describe a technique that allows the rapid and facile determination of which lysate contains enriched sequences for the repair of a mutant locus, thereby allowing the determination of the map position of the locus. This technique is applicable to those mutations for which the wild-type allele is selectable. We also describe a procedure whereby any Tn*10* insertion can be mapped by selecting for the loss of Tet^r.

The localization of genes on the genetic and physical map is one of the most basic tasks of genetics. Currently, the most widely used technique for genetic mapping in *Salmonella typhimurium* involves the isolation of a Tn*10* or Tn*10*dTet insertion that is either transductionally linked to the mutation of interest or is itself the mutation of interest. This unmapped Tn*10* is used to direct Hfr formation (8); the Hfr thus created is used in matings with known mutations representing different regions of the chromosome. By using this technique, a Tn*10* insertion (and consequently the linked mutation) can be localized to a 10-min region of the chromosome. This positioning can be refined to within a 1-min region by testing transductional linkage to standard *Salmonella* markers or by using the Kukral et al. (19) collection of Tn*10*dTet insertions. Alternatively, the Tn*10*dTet collection can be used to directly map the mutation.

Both mapping procedures suffer from the multiple steps required, the number of strains required (the Tn*10*dTet collection consists of 279 strains), the time involved in the mapping, and, in the case of Hfr mapping, the accuracy of the method. Mapping with the standard set of Tn*10*dTet insertions can be difficult because not all the insertions are mapped and a 5-min gap in the distribution of these insertions is thought to be present at min 90. These limitations can be eliminated by use of the P22/Mu hybrid phage Mu*d*-P22 (35). This paper describes a collection of *S. typhimurium* Mu*d*P and Mu*d*Q lysogens that can be used to generate transducing lysates; these lysates can be used as transductional donors and allow the mapping of mutations to within several minutes on the *S. typhimurium* chromosome. The procedure can be carried out on a single petri plate, and a map position can be determined in 24 to 48 h.

Transduction refers to the transfer of genetic information (DNA) from one bacterium to another via a phage particle. Generalized transduction refers to the ability of the transducing mechanism to transfer information from all parts of the donor genome. P22 is one of several phage that mediate generalized transduction in salmonellae (21). The mechanism whereby P22 mediates generalized transduction is understood at a general level, although some of the specific molecular details require further elucidation (for a review of P22 molecular genetics, see references 25 and 31).

P22 packages its genome by processive packaging of 43.4-kb (±0.75 kb [3]) fragments derived from a double-stranded, linear, concatemeric DNA molecule. Gene 3 is believed to code for a nuclease which recognizes a specific nucleotide sequence within the gene 3 coding sequence called the *pac* site (4, 20, 26). Once the *pac* site is recognized, a cut is made near the *pac* site (3, 17) and unidirectional packaging of the concatemer into phage heads begins. The direction of packaging is from gene 3, towards the late genes and the immunity I region. Continuous filling of heads from one concatemer may continue for 3 to 8 headfuls, depending on the genotype of the virus (1).

Lysates made from wild-type P22 are found to contain small amounts of host rather than viral DNA (approximately 3% of the plaque-forming particles [10]). This subpopulation of particles contains most if not all regions of the host chromosome, although in widely varying concentrations (29). It is believed, although this has not been absolutely proven, that host DNA is substituted for viral DNA owing to the presence of pseudo "*pac*" sites present on the host chromosome (7, 18, 26, 32). Since its discovery (21), P22 generalized transduction has played a pivotal and indispensable role in the genetics of salmonellae (28).

Weaver and Levine (33) demonstrated that the induction

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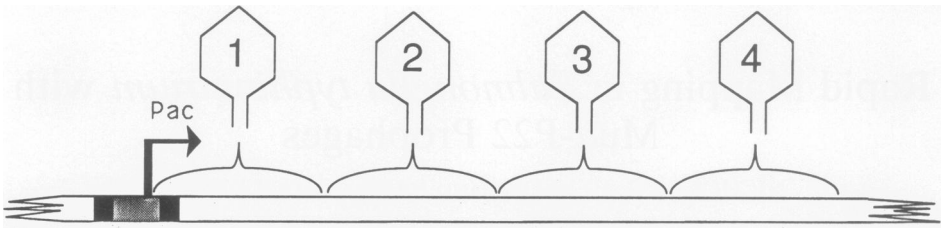

FIG. 1. Representation of a packaging series. Mu*d*-P22 is inserted in foreign DNA (white bar) which, after replication and amplification, is packaged beginning at the *pac* site. The first headful contains both Mu*d*-P22 sequences and foreign sequences. Subsequent headfuls contain only foreign DNA.

of an excision-defective (also known as "locked-in") P22 prophage residing at the standard integration site between *proAB* and *proC* results in the amplification of the entire *pro* region and packaging of the *proC* side of the prophage. Under these conditions, the specificity and unidirectional nature of packaging is identical to standard P22 packaging but extends into the neighboring bacterial DNA (Fig. 1). These induced lysates were not only generalized transducing lysates but were also specialized transducing lysates for the *proC* region, as they contained many more *proC*-derived particles than particles derived from other chromosomal regions. These early studies were limited to P22 resident at the normal bacterial attachment site. Youderian et al. (35) realized that if an excision-deficient P22 prophage was inserted at another site in the *Salmonella* genome, it would be possible to amplify that region of the chromosome and package those sequences under the domain of the prophage's unidirectional *pac* site.

Random insertion of P22 was achieved by construction of the phage Mu*d*-P22 (35). Two hybrid phage were constructed, Mu*d*P and Mu*d*Q (Fig. 2). The majority of the hybrid's genome is derived from P22, except for the region between *sieA* and *xis* (approximately 12 kb). Included in this deleted region are the ImmI genes, the tail gene (gene 9), the phage attachment site, and the *int* and *xis* genes, which are required for integration and excision at the bacterial attachment site. The practical difference between Mu*d*P and Mu*d*Q is that all the P22 material is inverted with respect to the Mu ends (Fig. 2). Therefore, when Mu*d*P and Mu*d*Q lysogens are made from the same Mu insertion, the Mu*d*P and Mu*d*Q will package opposite sides of the insertion.

The Mu-derived sequences are limited to the ends of a Mu prophage, *attL* and *attR*, which are required in *cis* for Mu transposition. The Mu *A* and *B* genes, which are required in *trans* for transposition, are not supplied on Mu*d*-P22 and therefore must be supplied exogenously, usually on a plasmid, in order for Mu*d*-P22 to transpose. A gene specifying resistance to chloramphenicol is included in Mu*d*-P22 so that transposition events in the host chromosome can be selected as chloramphenicol-resistant Mu*d*-P22 lysogens.

Figure 3 illustrates the events leading to the production of a Mu*d*-P22 lysate. Figure 3A depicts a Mu*d*-P22 element in the host chromosome. The addition of mitomycin C effects induction of the prophage. Induction causes bidirectional replication, beginning at the phage origin of replication and extending into the neighboring host sequences (Fig. 3B). Simultaneously, specific replication from the bacterial origin of replication is halted (10). As multiple copies of the phage and adjacent host DNA are made, packaging of these copies is initiated at the prophage *pac* site and continues into the bacterial genome (Fig. 1). The result of this packaging is the production of three general classes of particles. One class of particles contains the first headful of packaged DNA. This first headful contains the junction between P22 sequences (approximately 14 kb) and bacterial sequences (28 kb). The second class of particles contains purely bacterial DNA that was contiguous with the bacterial sequences contained in the first headful. These two classes compose the majority of the transducing population. A third (and minor) class of particles is composed of diverse regions of the chromosome. This third class is derived from P22's mechanism of generalized transduction, which is still operational.

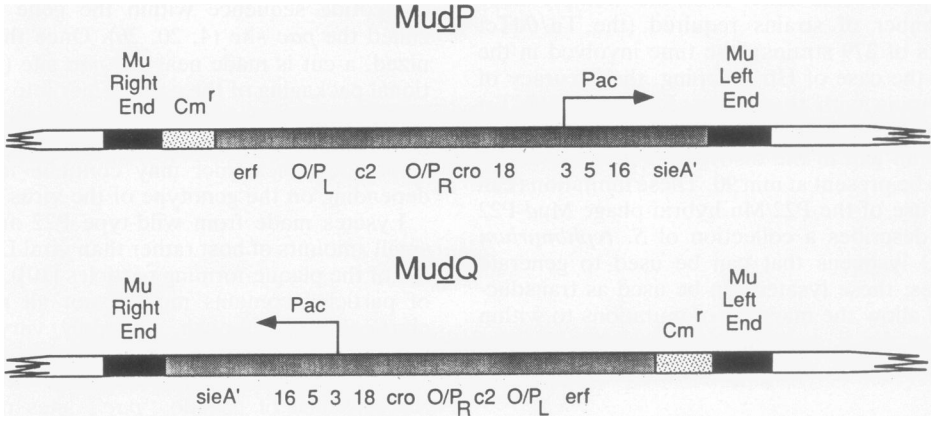

FIG. 2. The structure of Mu*d*P and Mu*d*Q elements when inserted into foreign DNA. Black bars represent Mu sequences, dark dotted regions represent P22 DNA, light dotted regions represent the Cm^r determinant, and white regions represent foreign (chromosomal or episomal) DNA. The numbers and letters below P22 indicate P22 genes. O/P$_L$ and O/P$_R$ represent the left and right operator/promoter control region, respectively.

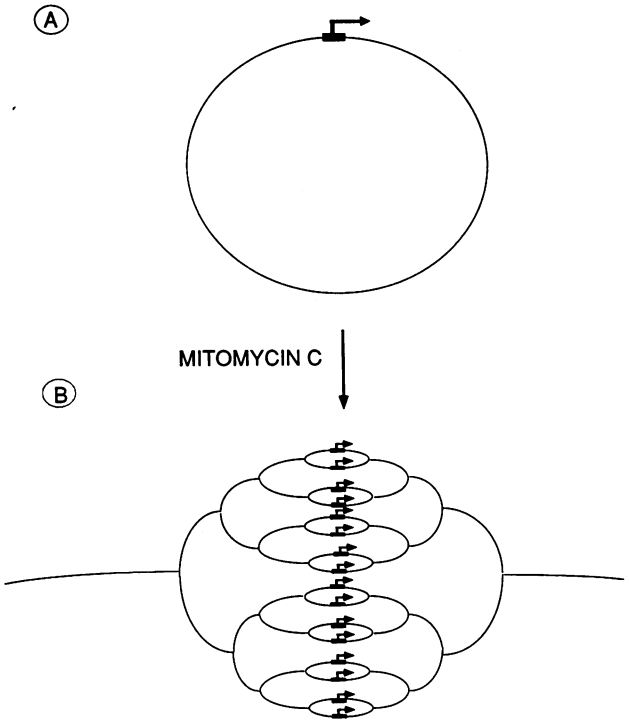

FIG. 3. (A) Mud-P22 in the *Salmonella* chromosome. (B) The addition of mitomycin C results in the replication of the Mud-P22 and flanking chromosomal DNA.

Youderian et al. (35) isolated Mud-P22 insertions in the first 9 min of the *Salmonella* chromosome and into F factors. The lysates derived from these insertions were enriched for transducing particles containing DNA to one side of the insertion, as predicted by the orientation of the *pac* site. This enrichment could be detected for a region approximately 5 min in length. We have extended the collection of Mud-P22 lysogens of Youderian et al. to include insertions along the entire chromosome. In this paper, we present a set of Mud-P22 lysogens and describe how lysates derived from this set permit rapid mapping of many types of mutations. We demonstrate the method by mapping mutations in a sugar utilization gene and in the arginine biosynthetic pathway. We also use the technique to map novel regulatory mutations for the *nirB* (nitrite reductase) gene.

MATERIALS AND METHODS

Bacteria, phage, and genetic methods. Unless specifically stated, all bacterial strains (Tables 1 and 2) are derivatives of MS1868, described by Youderian et al. (35). Bacterial methods and general phage techniques not described below are described by Youderian et al. (35) or by Davis et al. (9). All standard P22 transductions were done with phage MS2104, which contains the high-transducing allele HT12/4 (29) and was the kind gift of Mimi Susskind. MudA, MudJ, and MudF are transposition-defective derivatives of Casadabon's Mud1 (here referred to as MudA [15]) or Mud1/1734 (referred to here as MudJ [5]). MudF is a kanamycin-resistant derivative of MudD (6) containing an intact *lac* operon and was made by Ramesh Sonti in the Roth laboratory (30a). The selection for tetracycline sensitivity is that of Bochner et al. (2) as modified by Maloy and Nunn (23). The set of Mud-P22 mapping strains listed in Table 2 can be obtained from Ken Sanderson, curator of the Salmonella Genetic Stock Center, Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

TABLE 1. *Salmonella* strains

Strain	Genotype	Source
TT12915	*leuA414*(Am) r⁻; *fels2* F'114ts (*lac-ZZF20*::Tn*10*; *ZZF3551*::MudP)	P. Youderian
TT12916	*leuA414*(Am) r⁻ *fels2* F'114ts (*lac-ZZF20*::Tn*10 ZZF3553*::MudQ)	P. Youderian
TT16716	*his-644 pro-621* F'128(*pro*⁺ *lacZ477*::Tn*10*dTet)	J. Roth
MS1868	*leuA414*(Am) r⁻; *fels2*	P. Youderian
NB116	*fels2 leuA414*(Am) r⁻ *thr458*::MudA	This work
NB140	*fels2 leuA414*(Am) r⁻ *pyrF2690*::MudA	This work
TT15814	*nirB2*::MudJ	This work

Media. E medium (minimal medium) and Luria-Bertani (LB) broth (rich medium) are described by Davis et al. (9). E medium was supplemented with glucose or the appropriate carbohydrate at 0.2%. Amino acid supplements, when needed, were added at the final concentrations suggested by Davis et al. (9). LB plates were made by the addition of agar to LB broth to a final concentration of 1.5% agar. Green plates are described by Youderian et al. (35). Bochner plates (2, 23), used for the selection of tetracycline-sensitive transductants in Mud-P22 mapping experiments, were made as follows. Two solutions, A and B, were made up separately, autoclaved, and then mixed to give a final volume of 1 liter. Solution A was 15 g of agar, 5 g of tryptone, 5 g of yeast extract, and 50 mg of chlortetracycline-HCl in a final volume of 500 ml of water. Solution B was 10 g of NaCl, 10 g of $NaH_2PO_4 \cdot H_2O$ in a final volume of 500 ml of water. After mixing, 5 ml of 20 mM $ZnCl_2$ (aqueous) and 12 mg of fusaric acid dissolved in 1 ml of *N*,*N*-dimethyl-formamide were added. These plates were used within 48 h of being poured. Final concentrations of antibiotics in solid or liquid medium were as follows: tetracycline hydrochloride, 20 μg/ml in LB broth; kanamycin sulfate, 50 μg/ml in LB broth; ampicillin (sodium salt), 50 μg/ml in LB broth; chloramphenicol, 40 μg/ml in LB broth or green plates. Phage medium is described by Youderian et al. (35). All chemicals, amino acids, and antibiotics were purchased from Sigma Chemical Company. Agar was purchased from Becton Dickinson Co.; yeast extract and tryptone were purchased from Difco.

Source of MudA, -J, and -F insertions. Most of the insertions used to create the collection were already in the Roth laboratory collection or were obtained from other laboratories. Exceptions were the MudJ insertions used to make strains TT16706, TT16707, TT16708, TT16709, TT15638, and TT15633. The methodology used to make these and all other Mud insertions is described by Hughes and Roth (16). The MudJ insertion used to make strains TT16706 and TT16707 was linked to *metC*, and the MudJ insertion used to make strains TT16706 and TT16707 was linked to *metC*, and the MudJ insertion used to make strains TT16708 and TT16709 was linked to *serA*. Strains TT15638 and TT15633 were made from a MudJ linked to *argI*.

Preparation of MudP or MudQ transducing lysates. The procedure used to prepare lysates that are enriched for localized regions of chromosomal DNA is a modification of the procedure of Youderian et al. (35). A fresh 5-ml overnight culture of the desired Mud-P22 lysogen (grown in LB

TABLE 2. Mu*d*-P22 mapping strains

Minute[a]	Strain	Genotype of Mu*d* insertion[b]	Packaging direction[c]
0.00	TT15223	*thr-469*::Mu*d*P	A
0.00	TT15224	*thr-469*::Mu*d*Q	B
3.5	TT15226	*nadC218*::Mu*d*P	A
3.5	TT15227	*nadC218*::Mu*d*Q	B
7	TT15229	*proA692*::Mu*d*Q	A
7	TT15231	*proA692*::Mu*d*P	B
8.5	TT15230	*proC963*::Mu*d*P	A
12	TT15232	*purE2154*::Mu*d*Q	A
12	TT15235	*purE2154*::Mu*d*P	B
14	TT15237	*cobD498*::Mu*d*P	A
14	TT15236	*cobD498*::Mu*d*Q	B
17	TT15238	*nadA219*::Mu*d*P	A
17	TT15629	*nadA219*::Mu*d*Q	B
21.5	TT15239	*putA1019*::Mu*d*Q	A
21.5	TT15240	*putA1019*::Mu*d*P	B
28.5	TT15244	*aroD561*::Mu*d*P	A
28.5	TT15243	*aroD561*::Mu*d*Q	B
33	TT15245	*pyrF2690*::Mu*d*Q	A
30	TT15246	*pyrF2690*::Mu*d*P	B
36	TT15630	*tre-152*::Mu*d*P	A
36	TT15625	*tre-152*::Mu*d*Q	B
40.5	TT15249	*zea-3666*::Mu*d*Q	A
40.5	TT15250	*zea-3666*::Mu*d*P	B
50	TT15254	*cysA1586*::Mu*d*P	A
50	TT15258	*cysA1586*::Mu*d*Q	B
52	TT15632	*guaAB5641*::Mu*d*Q	A
52	TT15255	*guaAB5641*::Mu*d*P	B
54	TT15256	*purG2149*::Mu*d*P	A
54	TT15257	*purG2149*::Mu*d*Q	B
57	TT15261	*proU1884*::Mu*d*P	A
57	TT15628	*proU1884*::Mu*d*Q	B
60	TT15263	*cysHIJ1547*::Mu*d*P	B
62.7	TT16706	*zgc-1715*::Mu*d*P	A
62.7	TT16707	*zgc-1715*::Mu*d*Q	B
65	TT16708	*zgf-1716*::Mu*d*P	A
65	TT16709	*zgf-1716*::Mu*d*Q	B
68.5	TT17190	*zgi-3717*::Mu*d*P	A
68.5	TT17191	*zgi-3717*::Mu*d*Q	B
72	TT15264	*cysG1573*::Mu*d*P	B
73.6	TT17165	*envZ1005*::Mu*d*Q	A
73.6	TT15265	*envZ1005*::Mu*d*P	B
79.7	TT15267	*pyrE2419*::Mu*d*P	A
79.7	TT15266	*pyrE2419*::Mu*d*Q	B
83	TT15269	*ilvA2648*::Mu*d*Q	A
83	TT15268	*ilvA2648*::Mu*d*P	B
84	TT15270	*metE2131*::Mu*d*Q	A
84	TT15271	*metE2131*::Mu*d*P	B
86.7	TT15273	*pnuE41*::Mu*d*Q	A
86.7	TT15272	*pnuE41*::Mu*d*P	B
93	TT15276	*melAB396*::Mu*d*P	A
93	TT15275	*melAB396*::Mu*d*Q	B
96.1	TT15277	*purA1881*::Mu*d*P	A
97	TT15638	*zjh-3725*::Mu*d*P	A
97	TT15633	*zjh-3725*::Mu*d*Q	B

[a] All map positions are from Sanderson and Roth (28).
[b] All strains contain the background genotype *fels2 leuA414*(am) r^-.
[c] A and B, clockwise and counterclockwise packaging, respectively.

broth plus 40 μg of chloramphenicol per ml [LB Cm_{40}]) was added to 25 ml of LB broth. Mitomycin C was added to a final concentration of 2.0 μg/ml, and the mixture was shaken overnight at 37°C. After overnight shaking, 3 ml of $CHCl_3$ was added and the flask was vigorously shaken for 3 min. The LB broth was decanted from the $CHCl_3$, and the cell debris was removed by centrifugation at 8,000 rpm in a Sorval RC-5 (SS-34 rotor) for 10 min. The supernatant was then incubated with excess tail protein (35) for 2 h at 37°C. These tailed phage heads were pelleted by centrifugation at 17,000 rpm for 1 h in a Sorval RC-5 (SS-34 rotor). The pelleted phage particles were resuspended by shaking with 5 ml of phosphate-buffered saline. Lysates were stored with 0.2 ml of $CHCl_3$ in small screw-cap tubes at 4°C. Eventually, the $CHCl_3$ in the tube evaporated, and it was not replenished.

Preparation of Mu*d*P and Mu*d*Q donor lysates for replacing existing Mu*d* derivatives. In order to prepare lysates that served as donors of Mu*d*P and Mu*d*Q, strains TT12915 (donor of Mu*d*P) and TT12916 (donor of Mu*d*Q) were induced as described above, except that these strains were propagated and lysates were made at 30°C rather than 37°C. Note also that the practice of moving Mu*d*P and -Q phage by transduction with high-transducing derivatives of P22 is unnecessary and is absolutely not recommended. Autotransduction (by the addition of mitomycin C) of Mu*d*-P22 prophage should be the method of choice for moving these phage because it does not risk recombination with a carrier phage and avoids the usual problems associated with "live" phage transductions.

Replacement of Mu*d*A, Mu*d*J, and Mu*d*F insertions with Mu*d*P or Mu*d*Q. The recipient in this cross (i.e., the Mu*d*A, -F, or -J that is to be replaced with a Mu*d*P or -Q) was grown to a density of 4×10^8 cells/ml. To 0.2 ml of these freshly grown cells was added 0.2 ml of the Mu*d*P or -Q donor lysate diluted to 10^5 tail-dependent phage per ml. The mixture was incubated at room temperature for 20 min to allow adsorption. The adsorbed mixture of donor and recipient (usually 0.2 ml) was spread on an LB plate and incubated at 37°C for 3 to 15 h. The LB plate was replica printed to a green plate containing chloramphenicol (40 μg/ml), and this green plate was incubated at 37°C overnight. Green colonies were then patched to LB Cm_{40} and LB $Cm_{40}Amp_{50}$ (for Mu*d*A replacements) or LB $Cm_{40}Kn_{50}$ (for Mu*d*J or -F replacements). Candidates for further analysis were Cm^r and sensitive to the antibiotic that the recipient was resistant to. Typical replacement efficiencies for Mu*d*P were 50 to 0.1%, depending on the location of the element to be replaced. Replacement efficiencies for Mu*d*Q were much more variable, ranging from 50% to no replacement found.

Quantitative transduction procedure. The following procedure was used to determine the precise number of CFU in a Mu*d*-P22 lysate for a particular marker. Each lysate in Table 3 was assayed for a nearby (and therefore enriched) marker and a faraway marker. The recipients in the crosses are all simple derivatives of MS1868 and are therefore leucine auxotrophs as well as auxotrophs for an additional marker specified by a single Mu*d*A, -J, or -P insertion (listed in Table 3, column 4). These experiments measure the repair of auxotrophy specified by these Mu*d* insertions. Recipients were grown to 4×10^8 cells/ml in LB Amp_{50} (Mu*d*A insertions) or LB Kn_{50} (Mu*d*J insertions) and pelleted. The cells were resuspended in an equal volume of phosphate-buffered saline, and 0.4 ml of cells was added to 0.1 ml of a dilution of the lysate to be titered. After adsorption at room temperature for 20 min, 0.2 ml of this cell-phage particle mixture, or an appropriate dilution in saline, was spread on E-glucose-leucine (E-glu-leu) plates. The standard concentration of the lysate for which transduction was to be measured was set at 5×10^4 CFU/ml for a distant marker. The distant markers used to generate the enrichment data in Table 3 (column 8) were *thr-458*::Mu*d*A (min 52 to 87), *cysG1573*::Mu*d*P (min 0 to 50), and *pyrF2690*::Mu*d*A (min 93 to 99). Lysates were assigned one of these reference markers

TABLE 3. Repair of near and distant chromosomal markers by Mu*d*-P22 transducing lysates

Strain	Minute	Genotype of insertion	Genotype of repaired marker	Location of repaired marker	Repair of near marker (CFU)[a]	Repair of distant marker (CFU)[a]	Transductional enrichment[b]
TT15223	0.0	*thr-469*::MudP	*nadC*	3.5	2.7×10^9	1.3×10^7	210
TT15226	3.5	*nadC218*::MudP	*proC*	8.5	4.5×10^9	9.8×10^6	427
TT15230	8.5	*proC963*::MudP	*purE*	12	1.7×10^9	9.7×10^6	175
TT15232	12	*purE2154*::MudQ	*nadA*	17	8.9×10^6	1×10^5	89
TT15238	17	*nadA219*::MudP	*putA*	21.5	1.2×10^9	5.1×10^6	234
TT15239	21.5	*putA1019*::MudQ	*aroD*	28.5	1.5×10^9	1.2×10^6	1,213
TT15246	33	*pyrF2690*::MudP	*aroD*	28.5	3.1×10^9	7.3×10^6	423
TT15630	36.0	*tre-152*::MudP	*pyrF*	33	2.0×10^9	1.0×10^7	200
TT15250	40.5	*zea-3666*::MudP	*pyrF*	33	4.5×10^9	2.1×10^7	216
TT15249	40.5	*zea-3666*::MudQ	*hisH*	42	1.1×10^9	7.0×10^7	155
TT15258	50	*cysA1585*::MudP	*hisH*	42	1.1×10^8	1.3×10^6	85
TT15255	52	*guaAB5641*::MudP	*aroC*	47.6	1.5×10^7	1.5×10^5	100
TT15632	52	*guaAB5641*::MudQ	*purG*	54	9.7×10^7	6.4×10^5	151
TT15628	57	*proU1884*::MudQ	*purG*	54	1.0×10^8	3.7×10^5	270
TT15261	57	*proU1884*::MudP	*srl*	58.8	1.2×10^9	8.2×10^5	1,463
TT15263	60	*cysHIJ1574*::MudP	*srl*	58.8	1.1×10^8	2.4×10^5	458
TT16707	62.7	*zgc-3715*::MudQ	*cysJIH*	60	2.6×10^7	5.5×10^5	47
TT16709	65	*zgf-3716*::MudQ	*serA*	62.7	1.1×10^9	1.3×10^6	846
TT17191	68.5	*zgi-3717*::MudQ	*metC*	65	9.7×10^7	1.8×10^6	54
TT17190	68.5	*zgi-3717*::MudP	*cysG*	72.8	7.2×10^8	2.6×10^6	277
TT15265	73.6	*envZ1005*::MudP	*cysG*	72.8	9.7×10^7	4.9×10^5	198
TT17165	73.6	*envZ1005*::MudQ	*pyrE*	79.7	3.4×10^8	2.1×10^6	162
TT15267	79.7	*pyrE2419*::MudP	*ilvA*	83	4.6×10^7	1.8×10^5	256
TT15269	83	*ilvA2648*::MudP	*metE*	84	9.3×10^8	1.6×10^6	581
TT15272	86.7	*pnuE41*::MudP	*metE*	84	1.1×10^8	4.1×10^5	268
TT15273	86.7	*pnuE41*::MudQ	*melAB*	93	1.9×10^8	4.6×10^5	411
TT15276	93	*melAB396*::MudP	*purA*	96.1	1.6×10^8	1.8×10^5	889
TT15633	97	*zjh-3725*::MudQ	*purA*	96.1	2.2×10^8	4.3×10^5	512
TT15638	97	*zjh-3725*::MudP	*thr*	0.00	1.2×10^8	2.2×10^5	545

[a] The method used to calculate numbers in these columns is described in Materials and Methods.
[b] Transductional enrichment is determined by dividing the number in column 6 by that in column 7.

so that the relative enrichment of a marker near the insertion could be determined. The nearby marker used to determine the degree of enrichment for a particular interval is listed in Table 3, column 4, for each Mu*d*-P22 insertion. For each transduction, three independent experiments were performed for the distant and near markers. The results of the triplicate transductions were averaged to give the average titer of a lysate. Enrichment is defined as the average number of CFU for the nearby marker divided by the average number of CFU for the distant marker. Individual experiments rarely varied by more than 50% from each other. When replicate experiments varied by more than a factor of two, additional transductions were performed and the results were averaged. Occasionally, the pelleting and resuspension of the recipient in phosphate-buffered saline was omitted and the adsorption was done in LB growth medium. This variation in the procedure had no significant effect on the transduction results. In these transductions, it is not necessary to make the recipients lysogenic for Sie$^-$ P22 because the titer of live tail-dependent phage in these lysates is far below the titer of transducing particles. Thus, killing by phage does not interfere with the recovery of transductants.

Rapid mapping. The following procedure was used for the repair of most (non-Tn*10* insertion) single auxotrophic mutations. Lysates made from Mu*d*-P22 lysogens (Table 2 or 3) were diluted 100-fold in phosphate-buffered saline. Three microliters of each dilution was spotted on an E-glu-leu plate upon which had been previously spread 0.2 ml of the freshly grown (4×10^8 cells/ml) culture of the mystery mutant. Incubation at 37°C for 48 h typically resulted in a single confluent patch of transductants corresponding to one Mu*d*-P22 lysate. Identification of the lysate responsible for this patch of transductants localized the mutation to a 1- to 5-min region of the chromosome.

Rapid mapping of Tn*10* insertions. The procedure for mapping Tn*10* insertions differed from the above procedure for auxotrophic mutations because higher concentrations of the lysates were required. The recipient carrying the Tn*10* to be mapped was grown to 4×10^8 cells/ml in LB broth containing tetracycline. The cells were washed once in phosphate-buffered saline and diluted 10-fold, and 0.1 ml was plated on fresh (no more than 48 h old) Bochner plates (see Media). The Mu*d*-P22 lysates to be tested were diluted 15-fold, and 5-μl spots were applied to the prespread Bochner plate. The plate was then incubated at 42°C for 24 to 48 h. Typically, the Mu*d*-P22 insertion close to the site of Tn*10* insertion gave a confluent spot of Tet^s transductants, while the remaining spots appeared similar to the background of cells that did not receive any phage. We have found that Tn*10* insertions can give variable results, mostly because of heavy background. This problem can be minimized by using Tn*10*dTet to make the original insertion mutation.

RESULTS

Constructing the mapping collection. The collection of Mu*d*-P22 lysogens in Table 2 was created by converting a collection of Mu*d*A, Mu*d*J, and Mu*d*F insertions to Mu*d*P and/or Mu*d*Q derivatives.

The process of converting a Mu derivative into a Mu*d*-P22 requires recombination between shared homologies of the

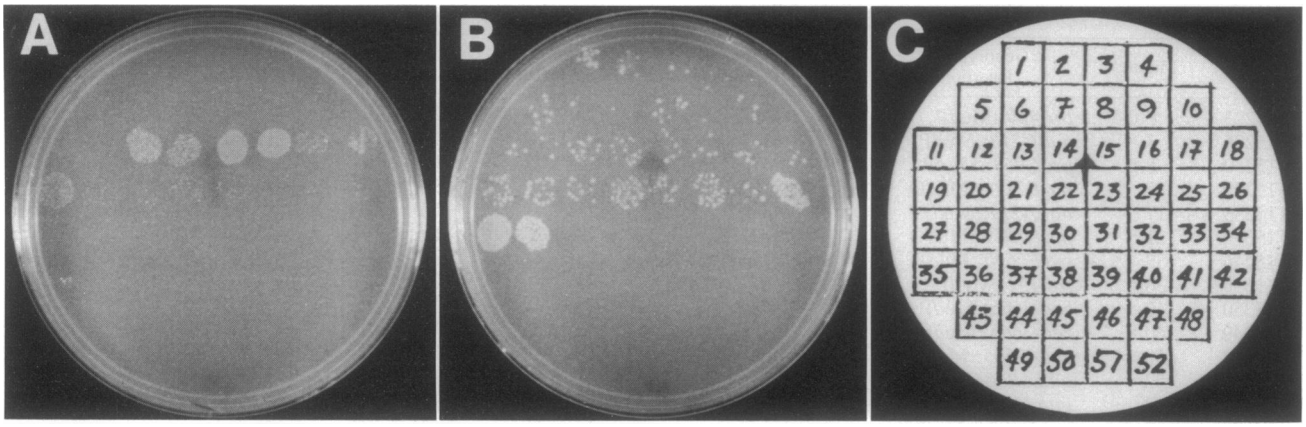

FIG. 4. Mapping of mutations. (A) The lysates from Table 3 were diluted 100-fold, and 3-μl volumes were spotted as described in Materials and Methods. (B) Mapping an arginine auxotrophy. Spotting is the same as in panel A. (C) The grid (boxes 1 to 29) portrayed here is used to spot the lysates from Table 3 in the same order in which they are listed there.

Mu's. *Salmonella* donors of Mu*d*-P22 contain a Mu*d*P or -Q inserted in an F′ derived from *Escherichia coli*. Because there is no homology between the F′ and the *Salmonella* chromosome, the only homology shared between a DNA-transducing fragment containing a Mu*d*-P22 element inserted in the F′ and a Mu residing in the recipient chromosome is between the left and right ends of Mu. Therefore, replacement of a Mu*d* element by Mu*d*-P22 must occur by homologous recombination between both Mu arms, resulting in a substitution of Mu*d*-P22 material for Mu material.

The set of Mu*d*-P22 lysates allows localization of mutant loci to specific regions of the *Salmonella* chromosome. In order for our mapping protocol to be effective, it is necessary to ensure that every region of the chromosome is enriched for and therefore represented by at least one member of the collection. We therefore need to demonstrate (i) that each strain lysogenic for a particular Mu*d*-P22 insertion amplifies that region of the chromosome adjacent to the Mu*d*-P22 and under the domain of the *pac* site and (ii) that while each lysate only enriches for a discrete region, adjacent enriched regions overlap so that no "holes" exist.

Rather than presenting data for the entire set of lysogens (Table 2), we have chosen a subset of lysogens (Table 3) and present evidence that each member of this subset is itself enriched for by a neighboring insertion. We use one of two genetic methods to prove mutual enrichment for each strain listed in Table 3. For instance, specific enrichment of the interval 0.0 to 12.0 min is demonstrated by showing that the region of insertion of each Mu*d*-P22 in this interval is enriched for by the Mu*d*-P22 insertion preceding it. To illustrate this, let us start at the insertion *thr-465*::Mu*d*P at min 0.00. Table 3 (line 1) demonstrates that enrichment of the *nadC* locus (min 3.5) is 210 times greater with a distant marker. This enrichment is demonstrated by the increased repair of a *nadC* insertion with respect to repair of a distant marker by the same lysate (Table 3, line 1, column 6 versus column 7). We then show that a *nadC218*::Mu*d*P insertion enriches for the *proC* region (Table 3, line 2, column 6 versus column 7). Line 3 of Table 2 shows that the *proC963*::Mu*d*P insertion enriches for the *purE* locus (min 12) and that a Mu*d*Q insertion in *purE* enriches for regions farther down the chromosome.

Some insertions in Table 3 are separated by more than 10 min. In these cases, we do not show that an insertion on one side of this region can enrich for a locus 10 min away. Rather, we show that the interval is enriched for by picking a locus in the center of the interval and demonstrating that the Mu*d*-P22 insertions on either side of the interval both enrich for a point within the interval. Consider the region 21.5 to 33 min. At min 21.5 is *putA1019*::Mu*d*Q, which packages the *aroD* gene (min 28.5; Table 3, line 7) 1,213 times as much as the distant marker. On the other side of the interval, at min 33, is the *pyrF2690*::Mu*d*P insertion, which packages the same *aroD* locus with an enrichment factor of 423 (Table 3, line 8). Thus, the 21.5- to 33-min region is represented in the collection. Inspection of Table 3 shows that some point on the chromosome is enriched for by at least one insertion in the collection. Therefore, we claim that the minimal set of strains in Table 3 is enough to ensure that any given point on the *Salmonella* chromosome can be found enriched for by at least one lysate derived from our lysogen collection.

Mapping of mutations. To illustrate the mapping technique, we describe the application of the method to three different types of mutations. The first mutation is an insertion of Tn*10*dCm resulting in the inability to utilize sorbitol as a carbon source. The experiment was performed as described in the legend to Fig. 4 and in Materials and Methods. The mutant cells were spread on a minimal sorbitol-leucine plate onto which were spotted dilutions of the lysates from Table 3. In Fig. 4A, spots of lysate on grid numbers 13 to 19 have given rise to many Srl$^+$ transductants. Grid numbers 15 and 16 are confluent with Srl$^+$ transductants. These confluent spots correspond to lysates made from *proU1884*::Mu*d*P (min 57) and *cysHIJ1574*::Mu*d*P (min 60) insertions. Both these lysates package the 57- to 60-min interval (Table 3). Further analysis demonstrated that *srl*-Tn*10*dCm is linked to the *recA* gene at min 58.5 (data not shown). The presence of Srl$^+$ transductants on the other grids is due to the low level of generalized transducing particles (containing Srl$^+$ DNA) present in all the lysates.

A second example of the mapping technique involves mapping a Tn*10* insertion, resulting in the inability to synthesize the amino acid arginine. In Fig. 4B, it can be seen that spots of lysates on grid numbers 27 to 29 have high numbers of Arg$^+$ transductants. Only grid 28 (corresponding to insertion *zjh-38725*::Mu*d*Q at min 97) shows confluent growth. This suggests that the Tn*10* insertion lies between min 93 and 97 and is close to min 97. In fact, the insertions *zjh-3725*::Mu*d*P and -Q are linked to the Tn*10*, and the Tn*10*

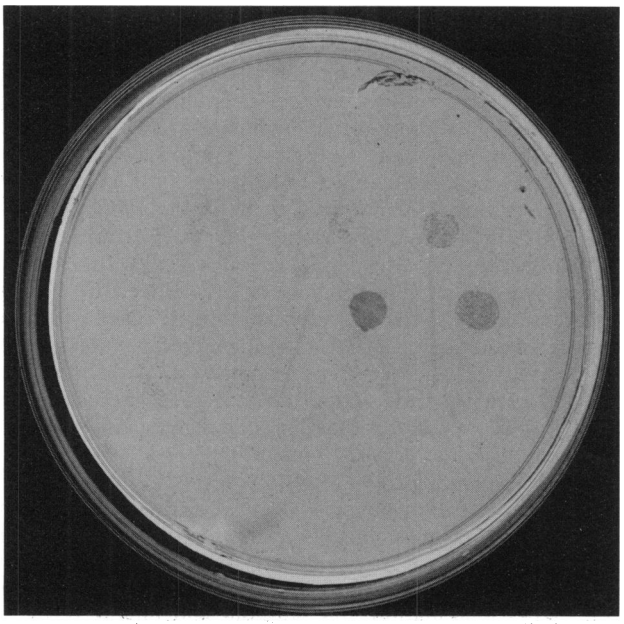

FIG. 5. Mapping a Tn*10*dTet linked to a *nirB* regulatory mutation. A subset of the lysates from Table 2 was spotted as described in Materials and Methods.

probably lies in the *argI* gene at min 97. The fact that insertion *zjh-3725*::Mu*d*P, which packages away from the Tn*10*, gave rise to so many Arg$^+$ transductants can be accounted for by the increased gene dosage (caused by the amplification of the region [Fig. 3]), which would allow for more generalized transducing particles containing this region to be packaged than would usually be the case. Quantitation of enrichment by both lysates, for repair of the Arg$^-$ phenotype, unambiguously establishes that the Tn*10* is before min 97 (data not shown).

Mapping of novel regulatory mutations for the *nirB* locus and mapping insertions of Tn*10*dTet. A procedure similar to that described in the preceding section can be applied to the mapping of mutations conferring tetracycline resistance, such as the insertion of the transposable element Tn*10* or its transposition defective derivative, Tn*10*dTet. This procedure differs from the above procedure because the Tn*10* insertion is mapped by virtue of the ability to select for loss of the drug resistance element (i.e., by selecting for Tet^s transductants; see Materials and Methods) and not for repair of a biosynthetic gene.

In order to isolate mutations with effects on the expression of the *nirB* gene, a strain containing a *nirB*::Mu*d*J insertion was isolated. The Mu*d*J element contains a promotorless *lacZ* gene; the *nirB2*::Mu*d*J insertion makes a transcriptional fusion at the *nirB* locus with *nirB* regulatory elements. Colonies of this strain are white (Lac$^-$) on MacConkey lactose plates. When this strain was mutagenized with diethyl sulfate, colonies appeared that were red (Lac$^-$) on MacConkey lactose plates, indicating that they were constitutive for the production of β-galactosidase (the product of the *lacZ* gene). In order to map these mutations, Tn*10*dTet insertions were isolated that were transductionally linked to these mutations.

These Tn*10*dTet insertions were mapped with the Mu*d*-P22 collection as described in Materials and Methods. Figure 5 shows the result of such a mapping experiment. A subset of the collection was spotted onto Bochner plates on which was spread the strain containing the Tn*10*dTet linked to the regulatory mutation. The petri plate in Fig. 5 shows three spots containing various numbers of Tet^s transductants. These spots correspond to insertions *purE2154*::Mu*d*Q (min 12), *nadA219*::Mu*d*P (min 17), and *putA1019*::Mu*d*P (min 21.5). The lysate that gave the most transductants was derived from the *nadA219*::Mu*d*P insertion at min 17. This Mu*d*P element packages clockwise; therefore, the Tn*10*dTet must be clockwise of min 17 and no farther than min 21.5 (the position of the counterclockwise packaging *putA* insertion). On the basis of this information, the linkage of the Tn*10*dTet was tested with various operons in the region. The insertion was found to be linked to *bio* (20%) and *chlA* (90%; min 18). Thus, the original *nirB* regulatory mutation is at min 18 on the *Salmonella* map. This mutation and other *nirB* regulatory mutations will be discussed in more detail elsewhere (12).

DISCUSSION

We have described a collection of *S. typhimurium* strains each containing a "locked in" Mu*d*-P22 prophage (35). We have extended the analysis of these prophages of Youderian et al. to the entire *Salmonella* chromosome. We have shown that each lysogen in the set can be induced and that the resulting Mu*d*-P22 lysate is enriched for transducing particles containing sequences on one side of the Mu*d*-P22 prophage. The magnitude of this enrichment, as determined by transduction, can be 47 to 1,400 times the concentrations of individual particles derived from distant regions of the chromosome, which are generated by P22's system of generalized transduction. This discrete enrichment of chromosomal regions is the basis of the mapping strategy. We have shown that simple auxotrophic lesions and Tn*10* insertions can be quickly mapped with a resolution of 1 to 5 min.

In the course of constructing the collection, several general rules became apparent with respect to replacing various Mu derivatives. The success in forming a replacement is variable and almost always less than the probability of retrieving a strain that exhibits both the drug resistance of the Mu*d*-P22 (Cm^r) and that of the Mu derivative to be replaced. The frequency with which a replacement is formed seems to vary with the location of the recipient Mu*d* element in the chromosome and does not depend on the type of Mu derivative that is being replaced. In most instances, and for unknown reasons, it was easier to retrieve a P conversion for a given Mu*d* than a Q conversion. Very rarely, it was impossible to retrieve a Q convertant, and in other exceptional cases when a rare Q convertant was found, it was a very poor donor of amplified sequences.

The mapping set is redundant. The mapping set is composed of more strains than is required to assure that a given sequence is enriched for by at least one lysogen in the set (cf. Tables 2 and 3). These extra strains have been included in the mapping set for several reasons. The redundancy is added insurance that "gaps" in our collection do not exist. The additional strains add to the resolution of the mapping technique by narrowing the distance between Mu*d*-P22 elements. In most cases, the resolution of the set is to within 3 min, but in certain regions a mutation can be resolved to 1 min. Redundancy in the collection also gives added assurance that a particular map assignment is correct, and the surplus of strains also helps to eliminate possible "end" effects which are discussed below.

Limitations and variability of the genetic method. Our data

indicate quite a large variation in the transductional repair of markers along the chromosome. Table 3 shows enrichment values of 47- to 1,463-fold compared with those obtained with distant markers. We believe there are at least two distinct mechanisms operating here.

One possible contribution to the wide variation in enrichment values is that different distant markers have been used to compute enrichment for different regions of the chromosome. It is known that wild-type P22 transduces different regions of the chromosome with different frequencies (29); thus, differential transduction of the distant markers could play a role in the variation. The inherent variation of lysates could also play a role. It can be seen from Table 3 that different lysates repair the same distant marker with efficiencies that vary by as much as a factor of 10.

The efficiency of repair for enriched markers also varies over 2 orders of magnitude. This variability could originate from the nature of Mu*d*-P22 itself. While Mu*d*-P22 contains the wild-type packaging apparatus of P22, the nature of the chromosomal substrate is significantly different. Wild-type P22 packages a chromosome that has ceased replication, and P22 probably initiates this packaging from various diverse pseudo "*pac*" sites. Thus, transducing particles capable of repairing a particular region are biochemically heterogeneous over the 43.4-kb piece of DNA. A given region that is packaged might be found in the center of one transducing DNA but at the end in another, because packaging of that region might be initiated from many *pac* sites (7, 24, 27). In contrast, the majority of the transducing particles in Mu*d*-P22 lysates are derived from a region of the chromosome that is undergoing repeated replication, and the initiation of packaging for these particles is predominantly from the single P22 *pac* site within the Mu*d*-P22 (Fig. 2) and less from diverse sites. Thus, transducing DNAs from the amplified region of a Mu*d*-P22 lysogen are likely to be substantially homogeneous.

This homogeneity is important because Ebel-Tsipis et al. (11) have demonstrated that successful P22 transduction is associated with the degradation of two-thirds of the incoming DNA fragment, with the incorporation of the remaining one-third as double-stranded replacement of the chromosome. While they did not explicitly show that the ends of the fragment were degraded, this is very likely the case. If an amplified lysate is asked to repair a region that lies close to an end, it would be expected to repair that region less efficiently because of the likelihood of the end sequences being degraded before repair could be effected. One would expect that a region lying in the middle of a transducing fragment would have a greater probability of surviving and repairing a lesion on the chromosome than would a sequence near the end of a transducing fragment. Thus, we suggest that one significant contribution to the variability in our enrichment data could be the positioning of donor repair sequences with respect to the ends of packaged, amplified DNA. Sequences close to the ends would appear to have limited enrichment. This could also represent a significant limitation to the genetic mapping technique. If a mutation to be mapped happens to lie close to the end of a packaged region, it could very likely be degraded during transduction and therefore not be detected by our system. This is another reason that we have chosen to make the mapping collection sufficiently redundant to represent all regions of the chromosome at least twice. These genetic limitations can be overcome by the use of physical mapping, which will be discussed below.

Additional genetic uses of the Mu*d*-P22 collection. While we have stressed the use of this collection for genetic mapping of auxotrophic markers and Tn*10* insertions, we emphasize that any mutation can be mapped if inheritance of its wild-type allele can be selected. It is also possible to map Mu*d* insertions by converting them to Mu*d*-P22 elements and testing the lysate against a battery of known auxotrophs.

We have also developed a technique for mapping Mu derivatives that contain a *lacZ* gene (such as Mu*d*A, Mu*d*J, and Mu*d*F). Strain TT16716 contains an F′ from *E. coli* in which the *lacZ* gene has an insertion of Tn*10*dTet. Any Mu derivative containing *lacZ* can be transduced to Tet^r (by using strain TT16716 as a donor) and, in cases of operon fusions, simultaneously effect a change of phenotype from Lac^+ to Lac^-. The Tet^r transductants obtained should have the Tn*10*dTet inserted within the *lacZ* gene of the recipient. The Tn*10*dTet (and thus the Mu*d*) can then be mapped by the protocol described for Tn*10* insertions.

Physical mapping using Mu*d*-P22. Mu*d*-P22 lysates can be extremely useful for physical studies for precisely the same reason that they are valuable genetic tools: they amplify and package DNA from a specific region of the bacterial chromosome. In addition, physical techniques are not susceptible to the degradation and possible end effects discussed above.

Physical techniques can be used to map any Mu*d* insertion, regardless of phenotype, by converting the insertion to a Mu*d*-P22 and then making an induced lysate. DNA purified from the lysate can be used as a probe against lysate DNA from the collection that has been cut with a variety of appropriate restriction enzymes, allowing the electrophoretic separation of the amplified Mu*d*-P22 sequences from the amplified bacterial sequences. While the Mu*d*-P22 sequences from the unmapped insertion will hybridize to the Mu*d*-P22 sequences in all the known DNAs, the separated chromosomal sequences will hybridize to and give the strongest signal with that lysate that has amplified the same region. Thus, if one can isolate a Mu*d* insertion linked to an unmapped mutation, that mutation can be physically mapped.

Several laboratories have used our collection of Mu*d*-P22 strains to physically map cloned DNA and to clone amplified DNA. Eisenstadt and coworkers (30) probed Mu*d*-P22 DNA with oligonucleotide probes specific for the *umuDC* locus, *icd* locus, and *phoP* and *dadB* genes. Their data allowed them to map the *umuDC* genes (between min 35.9 and 40.5) and to map one end of the inversion breakpoint that differentiates *Salmonella* gene order from *E. coli* gene order.

Hughes (14) has cloned and sequenced the *nadC* gene by use of *nadC*::Mu*d*-P22 insertions and the unique restriction sites present in the Mu ends.

Heffron and Bowe (13) have isolated insertions that make salmonellae avirulent to mice. These insertions and flanking chromosomal DNA were cloned, and the clones were used as probes against a membrane containing amplified DNA from the lysate collection. This procedure allowed the physical mapping of loci that, when disrupted, made salmonellae avirulent.

Liu and Sanderson (22) and Wong and McClelland (34) describe *Xba*I and *Bln*I restriction maps of the *Salmonella* genome. The restriction fragments generated by digesting genomic DNA with these enzymes were ordered in part by using Mu*d*-P22-derived bacterial DNAs as probes.

In conclusion, we have presented a collection of Mu*d*-P22 lysates that allow the rapid mapping of most mutations in salmonellae by either genetic or physical means. We believe

that this collection of Mu*d*-P22 mapping strains will facilitate physical and genetic mapping in salmonellae.

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