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High-Resolution Restriction Map for a 240-Kilobase Region Spanning 91 to 96 Minutes on the *Salmonella typhimurium* LT2 Chromosome

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A hierarchical approach allows the completion of contiguous sets of overlapping clones for small regions of a genome, one at a time rather than tackling the whole genome at once. On the basis of the *Bln*I restriction map for *Salmonella typhimurium* LT2, we dissected the chromosome into 21 different fragments by using a Tn*5* transposon carrying a *Bln*I site. Dissected chromosomal fragments were purified by pulsed-field gel electrophoresis and used as probes for sorting a lambda DASHII genomic library of 2,304 primary clones. A total of 129 clones identified as spanning the region from 91 min to 98 min were partly ordered on the basis of the intensity of hybridization with mitomycin-induced Mud-P22 phage DNAs from insertions with *pac* sites in opposite orientations at 93 min used as probes. Decreased signal intensity with the Mud-P22 probes corresponded to the increased distance of the clone from the site of Mud-P22 insertion and allowed the clones to be placed in two groups from 91 min to 93 min and from 93 min to 98 min and into four intensity categories within the two groups. A member of each category was used to generate a riboprobe from the T3 promoter flanking the insert. This probe identified overlapping clones among the 129 clones. This subchromosomal library was then screened again with riboprobes from nonoverlapping clones. After four cycles of this strategy, a minimal contiguous sequence of 19 partly overlapping clones was selected for restriction mapping. A detailed map of 378 sites for eight restriction enzymes is presented for a region of about 240 kb. Working clockwise, the following genes were placed on this physical map on the basis of their restriction maps: *malFEK*, *lamB*, *malM*, *lexA*, *qor*, *dnaB*, *alr*, *uvrA*, *proP*, *pmrB*, *pmrA*, *melA*, *melB*, *phoN*, *amiB*, *mutL*, and *miaA*.

In 1987, a restriction map of the *Escherichia coli* genome was produced by using partial restriction digests of 3,400 bacteriophage lambda clones generated with the eight restriction enzymes *Bam*HI, *Hin*dIII, *Eco*RI, *Eco*RV, *Bgl*I, *Kpn*I, *Pst*I, and *Pvu*II (7). The genomic sequence of *E. coli* is going to be completed soon (2). Among other enterobacteria, only *Salmonella typhimurium* has been analyzed to a considerable extent at the molecular and genetic level, and the level of genetic detail of the chromosome map is second only to that of *E. coli* among prokaryotes. *E. coli* and *S. typhimurium* have been extensively compared and contrasted at the genetic level (e.g., see reference 9). While these organisms have about 70% DNA sequence conservation and share approximately the same linkage map, with one major inversion, there are many regions scattered throughout the genome that are unique to one species or the other that probably encode some of the genes involved in their different lifestyles (9). Nevertheless, a detailed restriction map and complete genomic library of *S. typhimurium* have not yet been produced.

We intend to provide a resource that is comparable to that available for the *E. coli* genome. The end products will be extremely useful not only to researchers working with *S. typhimurium* but also to researchers interested in *E. coli* who wish to compare the equivalent genomic regions from these two related but divergent organisms. The detailed restriction map of a fragment of *S. typhimurium* LT2 presented here is an initial step toward this goal. The same set of restriction enzymes used for *E. coli* was employed here. However, we took a different approach which allows the completion of contiguous sequences (contigs) for manageable regions of the genome, one at a time rather than tackling the whole genome at once, and which requires restriction mapping of only a small number of the lambda clones.

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MATERIALS AND METHODS

Phage and bacterial strains. All bacterial strains were LT2 derivatives. Strains were constructed via P22 HT12/4 *int-3* transductions (6) as described previously (16). Tn*10* insertions (13), Tn*5*(pfm) insertions (15), and Mud-P22 strains (1) have been described previously.

Construction of pulsed-field gel electrophoresis strains. A set of random Tn*5*(pfm) insertions were generated and mapped as described previously (14). Known Tn*10* insertions were chosen for the construction of specific "drop-out" fragments. Different combinations of Tn*5*(pfm) insertions and Tn*10* insertions were transduced sequentially into the same strain, MS1017 (3), by selecting the corresponding tetracycline and kanamycin markers. The strains used for pulsed-field electrophoresis are listed in Table 1.

Construction of lambda DASHII genomic library. *S. typhimurium* LT2 DNA was isolated and partially digested with *Sau*3A at four different concentrations. DNA fragments of about 14 to 23 kb were isolated by low-melting-temperature agarose electrophoresis. Low-melting-temperature agarose (NuSieve; FMC, Rockland, Maine) was digested with GELase (Epicentre Technologies, Madison, Wis.) and the DNA fragments were ethanol precipitated. Isolated fragments were ligated to Lambda DASHII cleaved with *Bam*HI. Ligation

TABLE 1. Pulsed-field mapping strains

Strain[a]	Genotype	Restriction fragment	Map location in min (size in kb)
KKW2001	*zag*::Tn*5*(pfm) *pyrB692*::Tn*10*	B	90–98 (310)
		A	98–6 (380)
KKW2002	*zbc*::Tn*5*(pfm) *panBCD*::Tn*10*	C	5–12 (380)
KKW2003	*zag*::Tn*5*(pfm) *purE884*::Tn*10*	D	6–12 (360)
KKW2004	*zbc*::Tn*5*(pfm) *gal*::Tn*10*	E	12–18 (300)
		F	18–21 (240)
KKW2005	*zdc*::Tn*5*(pfm) *pncA148*::Tn*10*	G	21–27 (350)
		H	27–32 (220)
		I	32–38 (250)
KKW2006	*zef*::Tn*5*(pfm) *hisD8578*::Tn*10*	J	38–42 (230)
		K	42–45 (190)
KKW2007	*zef*::Tn*5*(pfm) *zej-1031*::Tn*10*	L	45–49 (180)
		M	49–56 (290)
TT14	*metC1975*::Tn*10*	O	56–60 (200)
		P	60–64 (260)
		Q	64–72 (330)
SA1985	*xyl-183*::Tn*10*	R	72–78 (280)
		S	78–83 (260)
		T	86–89 (150)

[a] All strains have two small fragments, U (95 kb) and V (42 kb), at 83 to 86 and 89 to 90 min, respectively.

mixtures were incubated with an in vitro packaging mixture, GigapackII Gold packaging extract, and propagated on *E. coli* SRB(P2) for Spi$^-$ selection. Phage, packaging mixes, and strains were from Stratagene Cloning Systems, La Jolla, Calif. Phage from the resultant large plaques were transferred to 200 μl of SM medium (0.1 M NaCl, 8. mM $MgSO_4$, 50 mM Tris-HCl [pH 7.5], 0.01% gelatin) in sterilized 96-well microtiter plates. A set of 2,304 independent clones were picked and established as the *Sau*3A library. A further aliquot of the packaging mix has been stored.

Screening of lambda DASHII library for clones covered by the 90- to 98-min pulsed-field fragment. The 2,304 individually picked primary phage plaques in a total of 24 96-well plates were transferred and inoculated onto a lawn of SRB(P2) on NZY medium (5 g of NaCl, 2 g of $MgSO_4$, 5 g of yeast extract, 10 g of NZ amine [ICN Pharmaceuticals]) (15 cm in diameter) by using a sterile transfer block with 96 prongs. The NZY plates were incubated at 40°C overnight. The plaques were transferred to Duralon membranes (Stratagene) by standard procedures (12). Two to three replicas were made from each NZY plate.

DNA from the strain KKW2001 was prepared (15) in InCert agarose (FMC, Rockland, Maine). The DNA was cleaved with 10 U of *Bln*I (Takara Biochemical, Inc., Berkeley, Calif.) in a volume of 100 μl. Digested DNA was resolved by a transverse alternating-field electrophoresis system (Beckman Instruments, Palo Alto, Calif.). A 310-kb *Bln*I pulsed-field fragment which covers 90 to 98 min of the chromosome was purified and labeled with the Prime-It random-primer kit (Stratagene). Membranes were probed by hybridization in Quickhyb solution (Stratagene). Clones giving a positive hybridization signal were used to make membranes for Mud-P22 DNA hybridization analysis.

Preparation of DNA from lambda clones. Liquid lysates were used for the preparation of DNA. Two hundred microliters of 10^8-cell/ml *E. coli* SRB(P2) at log phase, grown in Luria broth–10 mM $MgSO_4$–0.2% maltose, and 20 μl of 10^7-PFU/ml phage were added to 300 μl of adsorption buffer (10 mM $CaCl_2$, 10 mM $MgSO_4$) in 15-ml Falcon conical (no. 1059) tubes, corresponding to a multiplicity of infection of 0.01. The mixture was incubated at 37°C for 15 min, and then 10 ml of Luria broth–10 mM $MgSO_4$–0.2% glucose was added. The lysate was grown overnight at 37°C with shaking. Cell debris was removed from the lysate by spinning at 3,000 rpm in a Sorval RT6000B centrifuge. Lambda phage particles were then pelleted by ultracentrifugation with an SW40 rotor at 30,000 rpm for 30 min. Phage pellets were resuspended in 1× universal buffer. For preparation of DNA for partial restriction digestion, resuspended phage particles were phenol-chloroform extracted and ethanol precipitated. For preparation of DNA for riboprobe synthesis, resuspended phage particles were treated with DNase and RNase before phenol-chloroform extraction.

Preparation of Mud-P22 DNA. Mud-P22 DNA was prepared from mitomycin-induced Mud-P22 lysate as described by Youderian et al. (17).

Partial digestion of lambda clones. Two hundred microliters of DNA solution was prepared from 10 ml of lambda lysates. A 100-μl solution of 4× universal buffer containing 16 U of *Not*I was added to 100-μl DNA samples and digested to completion at 37°C. To each 20 μl of *Not*I-digested DNA in a 96-well microtiter plate, the following amount of enzyme was added in a volume of 7 μl of sterile H_2O (except *Kpn*I [14 μl]): 0.6 U for *Bgl*I, 0.3 U for *Bam*HI, 0.7 U for *Eco*RI, 0.1 U for *Eco*RV, 0.4 U for *Hin*dIII, 0.3 U for *Kpn*I, 0.35 U for *Pst*I, and 0.1 U for *Pvu*II. The reaction mixtures were incubated at 37°C for 30 min and then were inactivated by adding loading buffer on ice.

Electrophoresis, Southern hybridization, and restriction mapping. The partial digests were resolved in 0.8% low endoosmosis agarose. The size of the gel was 20 by 24 cm, and it had a thickness of 0.5 cm. Digests of three clones with the eight enzymes, for a total of 24 samples, were loaded on each gel. *Bgl*I partially digested lambda DASHII DNA was loaded on the same gel as the size markers. After electrophoresis, fragments were transferred to a Duralon membrane (Stratagene) and probed with alkaline phosphatase-conjugated T3 or T7 oligonucleotide probes from the Flash nonradioactive gene mapping kit (Stratagene). The filters were then incubated with the chemiluminescent substrate. Light emanating from the target probe was detected by exposure to Kodak XAR-5 film for 30 to 60 min.

Identification of linking clones. Riboprobes were generated by in vitro synthesis of RNA transcripts from *Not*I-digested lambda DASHII clones which contain T3 and T7 promoters. In vitro transcription was carried out in a 25-μl reaction mixture, which consisted of 40 mM Tris-HCl (pH 8.0), 8 mM $MgCl_2$, 50 mM NaCl, 2 mM spermidine, 30 mM dithiothreitol, 400 μM each ribonucleoside triphosphate NTP (except ribo-UTP [40 μM]), 2 to 3 μg of DNA template, 10 U of polymerase, and 40 μCi of α-^{32}P-labeled UTP. The reaction mixture was incubated at 37°C for 30 min.

Sequencing strategy. Thirty microliters of lambda clone DNA (2 to 3 μg), prepared as described above for partial digestion, was denatured with 8 μl of 2 M NaOH for 10 min at room temperature. Twelve microliters of 3 M Na acetate (pH 5.2), 28 μl of sterile distilled water, and 240 μl of absolute ethanol were then added, and the mixture was chilled at −70°C for 1 h. Precipitated denatured DNA was then sequenced with T7 or T3 primer with a Sequenase kit (U.S. Biochemical Corp., Cleveland, Ohio).

Data analysis. The software Genescape (11) was used to compile the restriction map generated with the nine restriction enzymes *Bam*HI, *Bgl*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Not*I, *Pst*I, and *Pvu*II. The individual restriction maps of the lambda clones and restriction maps derived from GenBank sequences were aligned with the consensus genomic restriction map by using the program MapSearch (11).

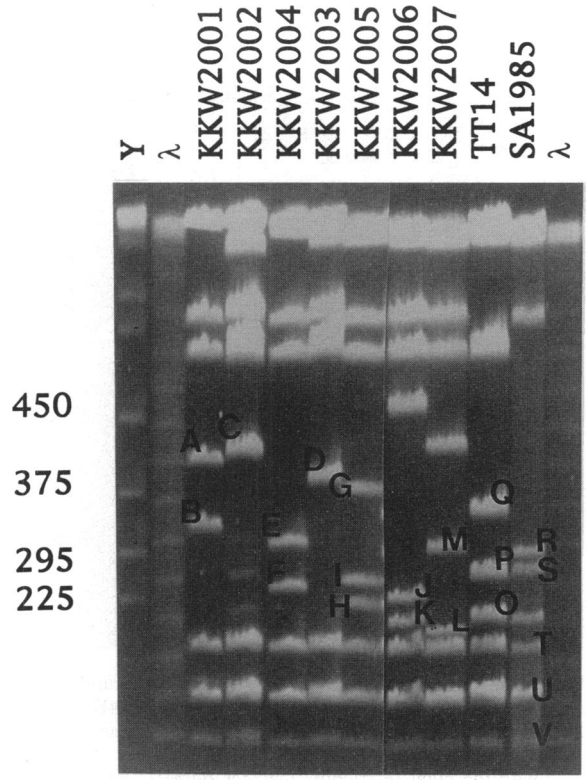

FIG. 1. *Bln*I restriction digestion of pulsed-field mapping strains, resolved by pulsed-field gel electrophoresis. The molecular sizes are given in kilobases on the left. Fragments A to V, described in Table 1, collectively represent the whole genome of *S. typhimurium*.

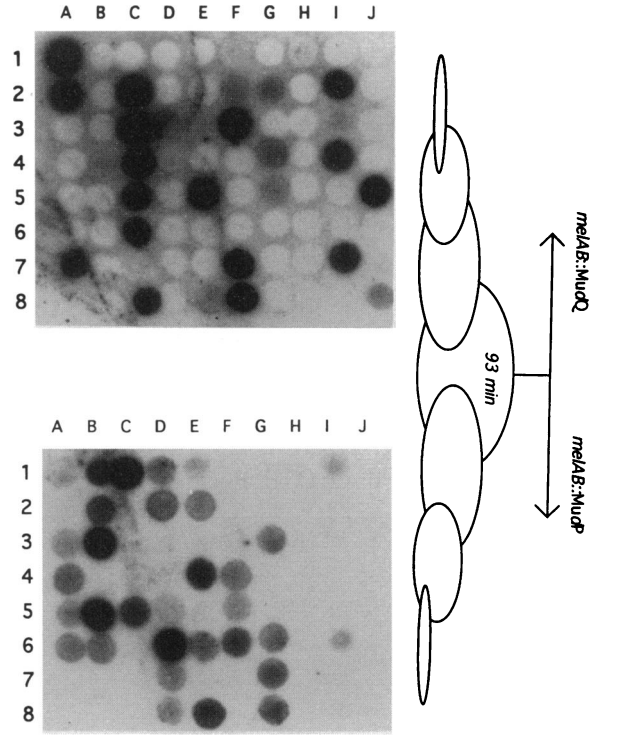

FIG. 2. Sorting of lambda DASHII clone with Mud-P22. DNAs prepared from mitomycin-induced Mud-P22 lysate from strain TT15275 (*melAB*::MudQ) and TT15276 (*melAB*::MudP) were radiolabeled as probes. Sequential headfuls of chromosomal DNA from this pair of strains were packed in opposite orientations starting at the site of insertion. The degree of amplification of chromosomal DNA decreases from the site of insertion.

RESULTS

Mapping strategy. A top-down approach was followed for the mapping strategy. A long-range restriction map with the rare cutting enzymes *Bln*I and *Xba*I has been constructed for *S. typhimurium* LT2 (8, 14). By taking advantage of the *Bln*I restriction map of *S. typhimurium* LT2 and the presence of *Bln*I sites in both Tn*5*(pfm) and Tn*10* transposons, we were able to construct a set of pulsed-field gel electrophoresis strains, each of which produces specific *Bln*I drop-out fragments in the range 190 to 380 kb (Table 1). The drop-out fragments were clearly resolved by pulsed-field gel electrophoresis and purified for subsequent manipulation. This set of restriction fragments (A to V [Fig. 1]) collectively represent the whole genome. Each of the fragments has been used as a probe to identify the corresponding clones from a lambda DASHII genomic library having about 8.5 genome equivalents, which consist of 2,304 individually picked plaques with an average insert size of 17 kb.

As an initial step toward a restriction map for the whole genome, pulsed-field-purified fragment B (90 to 98 min), with a size of about 310 kb, was used as a probe for hybridization to 24 membranes carrying the array of 2,304 primary clones. One hundred twenty-nine positive clones were obtained with this probe. These clones were further subdivided into different groups by using DNA prepared from mitomycin-induced Mud-P22-specific insertions. Induced Mud-P22 phage pack several headfuls of DNA from the site of insertion in one orientation and may cover up to 5-min regions, but the amount of DNA packed from the adjacent region by Mud-P22 phages decreases as the distance from the site of insertion increases. Phage lysates were induced from a pair of strains with Mud-P22 insertions with *pac* sites in opposite orientations at the *melAB* operon located at 93 min. Hybridization results with probes made from DNA prepared from each of the two lysates are shown in Fig. 2. Clones which were positive when probed with DNA from the *melAB*::MudQ insertion strains were negative when a probe from the *melAB*::MudP insertion strain was used, with three exceptions. Clones which were positive with both probes contained DNA flanking the Mud-P22 insertion region in *melAB*.

As a result, these 129 clones spanning 91 to 98 min were subdivided into two groups—91 to 93 min and 93 to 98 min. Clones were further subdivided into different hybridization intensity groups. Clones with the highest intensities were suspected to contain inserts from the chromosomal region close to or at 93 min, the insertion site of the corresponding Mud-P22. Four intensity groups could be distinguished by each of the Mud-P22 DNA probes. This assumption was validated by subsequent analysis.

Contig assembly. The vector lambda DASHII contains T3 and T7 promoter sequences adjacent to the *Bam*HI cloning site. This feature enabled us to generate riboprobes by in vitro transcription. From each of the hybridization intensity groups, we picked one clone to prepare a riboprobe. Clones that did not hybridize to the first probe were chosen to make riboprobes for a second hybridization. A similar rationale was used for the third and subsequent hybridizations so as to link all of the clones. An example of a linking clone found by using the riboprobes generated from clones 8B12 and 12A5 is shown in Fig. 3. A linking clone between 8B12 and 12A5 is indicated by

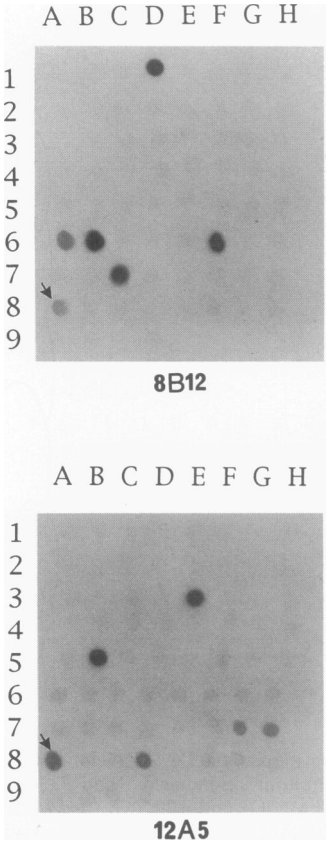

FIG. 3. Identification of linking clone. Riboprobes were generated from clones 8B12 and 12A5. A linking clone between 8B12 and 12A5 is indicated by an arrow.

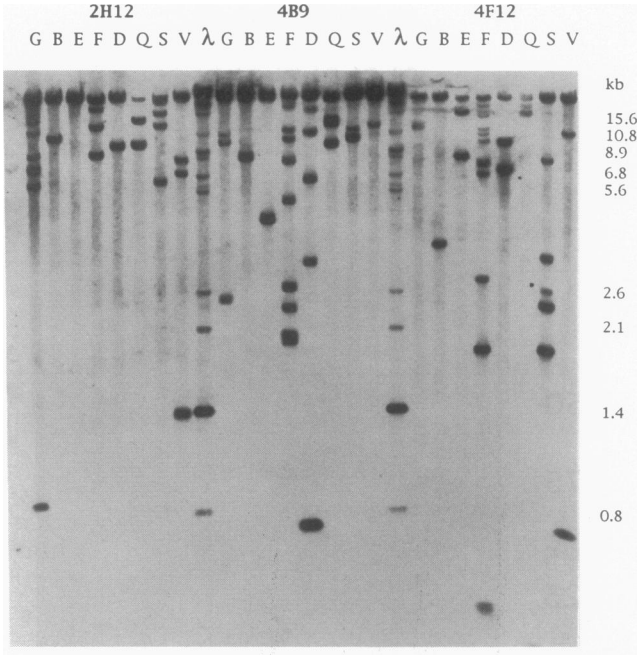

FIG. 4. Partial digestion of lambda DASHII clones and restriction mapping by indirect end labeling. Three clones (2H12, 4B9, and 4F12) were completely digested with *Not*I to release the cloned insert and adjacent T7 and T3 promoter regions. These DNAs were then partially digested with the eight restriction enzymes *Bam*HI (B), *Hin*dIII (D), *Eco*RI (E), *Eco*RV (F), *Bgl*O (G), *Kpn*I (Q), *Pst*I (S), and *Pvu*II (V). The lambda DNA size marker was *Bgl*I partially digested lambda DASHII DNA (predigested completely with *Not*I). Alkaline phosphatase-conjugated T3 oligonucleotide was used for the hybridization and chemiluminescent detection.

an arrow. After four steps with clones in each intensity group, all of the clones were linked, with the exception of some clones from the lowest-intensity group, which may be unlinked clones near the ends of the *Bln*I fragment. Among the linked clones, we were able to choose 19 of the 129 clones as the minimal set for restriction analysis.

Detailed restriction map of the 240-kb fragment spanning 91 to 96 min. A restriction map for each clone in the minimal set was determined by partial digestion with eight restriction enzymes (*Bam*HI, *Bgl*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Kpn*I, *Pst*I, and *Pvu*II) which are the same enzymes used for the *E. coli* map (7). One microgram of DNA from each lambda clone in the putative minimal set was digested completely with *Not*I restriction enzyme to release the insert DNA as well as the T3 and T7 promoter regions adjacent to the *Bam*HI cloning site and then was partially digested with a serial dilution of each of the eight enzymes and separated by electrophoresis on a 0.8% agarose gel. The gel was blotted onto a filter, which was then hybridized with alkaline phosphatase-conjugated T3 oligonucleotide, and incubated with a chemiluminescent substrate (Fig. 4). The T3 probe was stripped off from the membrane, which was reprobed with alkaline phosphatase-conjugated T7 probe. The mobilities of individual bands on the autoradiograms were measured, and their sizes relative to those of the *Bgl*I partially digested lambda DASHII DNA were calculated. This process also mapped any *Not*I sites internal to the clone. Only one *Not*I site occurred, at about 146 kb in the contig presented in Fig. 5.

From the restriction map of each lambda clone, we relied on an overlap of two or more restriction sites between adjacent clones to construct the contig. To improve the accuracy of the map, we have also generated another 23 restriction maps from other clones in addition to the 19 minimal clones. The program Genescape was used to generate a bar graph display of restriction sites for each clone. The graphs from adjacent clones were examined for overlapping sites. The final 240-kb map (Fig. 5) was then generated by linking each pair of adjacent clones by using the Genescape program. The restriction maps for nine GenBank entries containing genes known to genetically map in this region were aligned with the consensus genomic map, and their physical locations and orientations are given.

The ends of a few of the minimal clones from the *S. typhimurium* contig have been directly sequenced as described in Materials and Methods. One end of lambda 13E11 encodes a region adjacent to *hfq* (gene encoding host factor 1) (5) that maps to the expected location.

DISCUSSION

We have used a top-down approach to identify a minimum set of lambda clones that need to be restriction mapped for the generation of a high-resolution restriction map from a specific dissected pulsed-field fragment. Figure 5 summarizes the restriction map data for a 240-kb fragment spanning 91 to 96 min of the *S. typhimurium* genome.

The detailed restriction map for the region spanning 91 to 96 min consists of 376 restriction sites (1.6 sites per kb). These

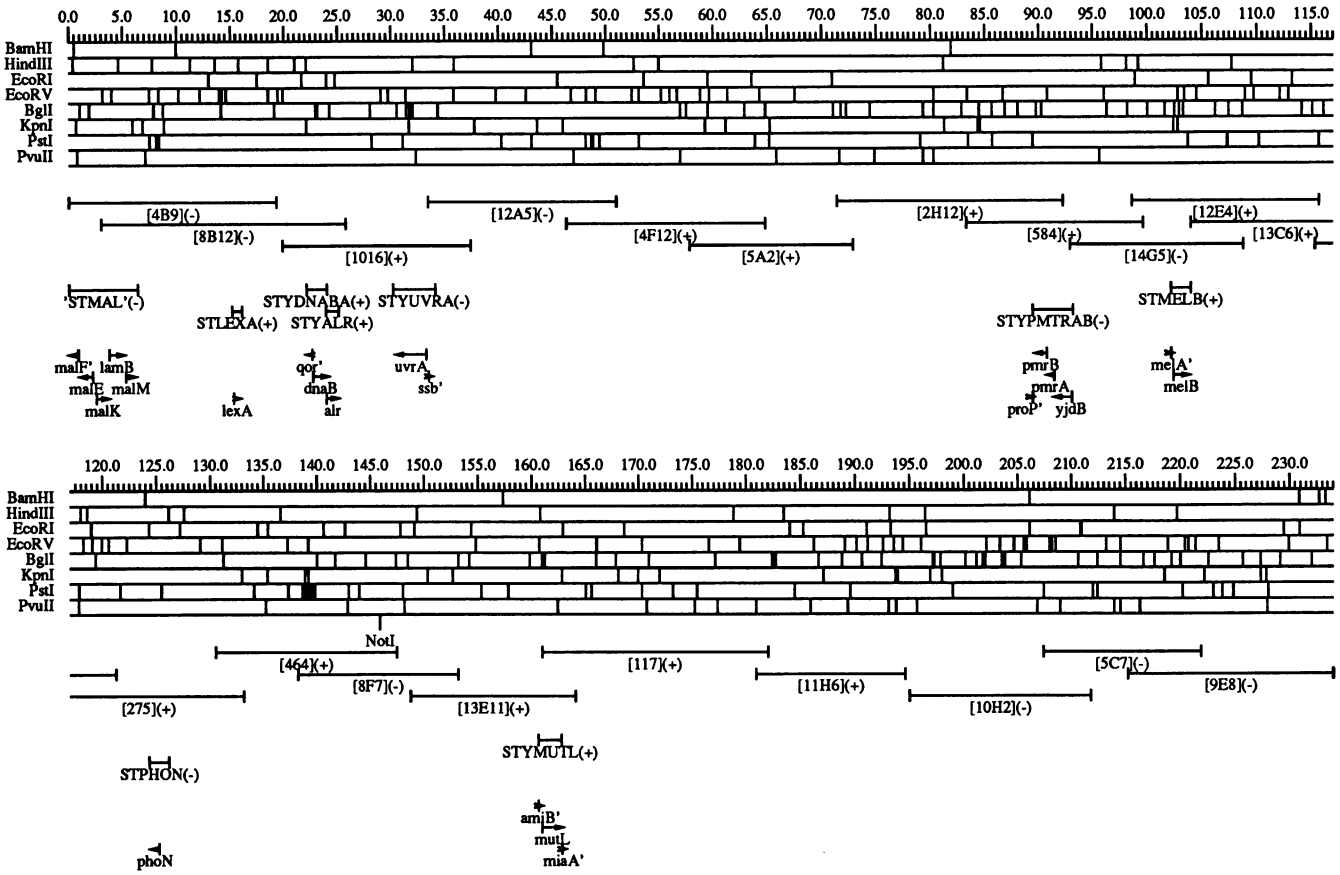

FIG. 5. Restriction map of a 240-kb fragment covering the 91- to 96-min region of the *S. typhimurium* chromosome. The map depicts 376 sites for eight restriction enzymes (and one *Not*I site), the positions of 20 selected lambda DASHII clone inserts, and the positions of GenBank entries and the genes they contain. The orientations of inserts relative to the vector arms and GenBank entries relative to the genomic map are denoted as (+) or (−). In the (+) orientation, the T3 cloning vector arm is to the left of the insert and the T7 arm is to the right. The length and direction of transcription of genes (clockwise or counterclockwise) are indicated by arrows. The *E. coli* counterpart of *pmrB* is *basS*, and that of *pmrA* is *basR*. The open reading frame preceding *pmrA* is named *vidB* after its partially sequenced *E. coli* counterpart. Prime marks (') indicate that STMAL is truncated at the beginning and end (see text) and that gene sequences are partial. GenBank-EMBL accession numbers are as follows: X54292, STMAL; X63002, STLEXA; J03390, STYDNABA; M12847, STYALR; M93014, STYUVRA; L13395, STYPMTRAB; X62101, STMELB; X63599, STPHON; and M29687, STYMUTL. This figure was produced with the PrintMap drawing program (11).

sites were mapped after the cloned insert DNA was released from the vector by *Not*I cleavage, allowing for much greater accuracy than the lambda vector used by Kohara et al. (7), in which sites were mapped relative to the lambda ends 9 and 20 kb from the insertion. Nevertheless, the accuracy of the map varies within each clone. The mobility of each band in the agarose gel is logarithmic and is inversely proportional to the size of the band. Thus, sites mapped to the middle of each clone have a greater error than those at the ends. To increase the reliability of the map, when there were overlapping data, we preferentially used data from near the end of the clones to assemble the final contig map. To ensure this greater accuracy, the final map is the result of mapping a further 23 lambda clones in addition to the 19 minimally overlapping clones.

A transductional analysis by Youderian et al. (17) indicated that during mitomycin induction of Mud-P22 phage, there was a gradual decrease in chromosomal amplification with increasing distance from the site of Mud-P22 integration. We obtained evidence by DNA hybridization to support this interpretation. Use of amplified DNA from induced Mud-P22 as a probe revealed different hybridization groups with different intensities. Restriction digests of clones within each hybridization group confirmed that these clones are closely linked (data not shown). The order of the final minimally overlapping clones also confirmed that the degree of chromosome amplification progressively decreases from the site of Mud-P22 insertion during mitomycin induction. Although not absolutely vital to the success of the strategy, such probes assigned clones to four intensity categories, from which clones were used to start the contig assembly.

The T7 and T3 promoters in the lambda DASHII vector, and thus the ability to produce riboprobes, were not available to Kohara et al. (7). By using T7 and T3 probes, the process of contig assembly was faster and more systematic. The sequential use of riboprobes from the ends of clones rapidly completed the contig, and as a result, only a small number of the clones were needed to complete the restriction map (less than 10% of the number needed to complete a region of the same size if the clones are not ordered with riboprobes).

Some of the success in closing the contig with a relatively small number of clones can be attributed to qualities of the vector and library. Chimeric clones arising from coligation of multiple fragments are unlikely in this library because the maximum insert size that the DASHII vector can hold is about

23 kb. *Sau*3A partially digested fragments were fractionated by low-melting-temperature agarose, and sizes from 14 kb to 23 kb were recovered from the gel for library construction. Second, the library consisted of only sorted primary plaques, minimizing loss of representation. Also, the chance of deletion of genes that are toxic to *E. coli* is minimized in a lytic phage.

One of the major uses of the high-resolution *E. coli* genomic restriction map of Kohara et al. has been the integration of sequenced genes with the genomic map by using restriction map alignments (7, 11). It is now possible to align and orient sequenced *Salmonella* genes in the same fashion and with the same computer programs. The high-resolution *Salmonella* genetic map that is produced this way can be directly and accurately compared with the *E. coli* physically derived genetic map to search for minor rearrangements and gene insertion or deletion events. Nine DNA sequences from GenBank (containing 14 complete and 7 partial gene sequences) were expected to map to the 91- to 96-min region. At least one of the genes in each of these GenBank entries has been genetically mapped to this region of the *Salmonella* chromosome. All nine sequences were successfully aligned to this segment of the high-resolution *S. typhimurium* genomic restriction map (Fig. 5). The STLEXA sequence contained only one restriction site (*Hin*dIII) but was provisionally aligned with a particular *Hin*dIII site on the basis of a comparison with the *E. coli* map in this region. The other eight alignments were all statistically significant best-ranked MapSearch computer alignments, with every sequenced restriction site having a corresponding gel-derived site in the consensus map. The first 336 bases of the STMAL GenBank entry were found to be M13 cloning vector and were removed. The end of the STMAL sequence, including *malG* and part of *malF*, lies to the left of the region mapped in this study and was also excluded. A few of the partially sequenced *Salmonella* genes were identified in the GenBank entries in this study by using the homologous *E. coli* protein sequences (e.g., *amiB*, *qor*, and *proP*). All aligned genes, with the exception of *phoN*, have counterparts in *E. coli*, and the relative distances and orientations are comparable.

In addition, we have started to determine the sequences of the ends of a few of the minimal clones from the *S. typhimurium* contig. Soon, the sequence of the whole genome for *E. coli* will be determined (2). The exact locations of the two ends of the minimal clones from *Salmonella* can then be placed on this map. As an example, one end of lambda 13E11 which encodes a region adjacent to the gene *hfq* (gene encoding host factor 1) (5) matches the homologous region in *E. coli* and maps to the expected location relative to the genetic map of *E. coli*, although *hfq* has not been found in *S. typhimurium*.

With the strategy outlined, it is possible to choose the order in which the map is to be completed. Thus, we have concentrated on the major known differences between the maps of *S. typhimurium* and *E. coli* that may be partly responsible for their different lifestyles and pathogenicities. Some genes have been ascribed to five of the loops that are unique to *Salmonella* spp., and some *Salmonella*-specific clones have been obtained from these loops for functional and evolutionary analysis (4). For example, the region between 91 min and 96 min presented here has a loop at 92 min which is unique to *S. typhimurium* compared with *E. coli*, and an *S. typhimurium*-specific gene, *inlA*, that is responsible for utilization of inositol has been mapped to 92 min (10). With this first contig map, we have accurately mapped the precise extent of the loops at 92 and 94 min, and a contig and restriction map spanning the loops at 1 and 7.5 min is in progress (unpublished data).

The approach outlined here will allow completion of a detailed restriction map for the whole genome, starting with those regions of the map that span the 16 known loops specific to *S. typhimurium*. Once the extent of these loops is precisely delineated, this contig will greatly facilitate sequencing of the loops and junction regions shared with *E. coli*.

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REFERENCES

1. **Benson, N. R., and B. S. Goldman.** 1992. Rapid mapping in *Salmonella typhimurium* with Mud-P22 prophages. J. Bacteriol. **174:**1673–1681.
2. **Daniels, D. L., G. Plunkett III, V. Burland, and F. R. Blattner.** 1992. Analysis of the *Escherichia coli* genome: DNA sequence of the region from 84.5 to 86.5 minutes. Science **257:**771–778.
3. **Grana, D., P. Youderian, and M. M. Susskind.** 1985. Mutations that improve the *ant* promoter of *Salmonella* phage P22. Genetics **110:**1–16.
4. **Groisman, E. A., M. A. Sturmoski, F. R. Soloman, R. Lin, and H. Ochman.** 1993. Molecular, functional, and evolutionary analysis of sequences specific to *Salmonella*. Proc. Natl. Acad. Sci. USA **90:**1033–1037.
5. **Kajitani, M., and A. Ishihama.** 1991. Identification and sequence determination of the host factor gene for bacteriophage Q_β. Nucleic Acids Res. **19:**1063–1066.
6. **Kleckner, N., R. K. Chan, B.-K. Tye, and D. Botstein.** 1975. Mutagenesis by insertion of a drug-resistance element carrying an inverted repetition. J. Mol. Biol. **97:**561–575.
7. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50:**495–508.
8. **Liu, S.-L., and K. E. Sanderson.** 1992. A physical map of the *Salmonella typhimurium* LT2 genome made by using *Xba*I analysis. J. Bacteriol. **174:**1662–1672.
9. **Riley, M., and S. Krawiec.** 1987. Genome organization, p. 967–981. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
10. **Riley, M., and K. E. Sanderson.** 1990. Comparative genetics of *Escherichia coli* and *Salmonella typhimurium*, p. 85–95. *In* K. Drlica and M. Riley (ed.), The bacterial chromosome. American Society for Microbiology, Washington, D.C.
11. **Rudd, K. E., G. Bouffard, and W. Miller.** 1992. Computer analysis of *Escherichia coli* restriction maps. Genome Anal. **4:**1–38.
12. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
13. **Sanderson, K. E., and J. R. Roth.** 1988. The linkage map of *Salmonella typhimurium*, edition VII. Microbiol. Rev. **52:**485–532.
14. **Wong, K. K., and M. McClelland.** 1992. A *Bln*I restriction map of the *Salmonella typhimurium* LT2 genome. J. Bacteriol. **174:**1656–1661.
15. **Wong, K. K., and M. McClelland.** 1992. Dissection of the *Salmonella typhimurium* genome by use of a Tn*5* derivative carrying rare restriction sites. J. Bacteriol. **174:**3807–3811.
16. **Wong, K. K., K. L. Suen, and H. S. Kwan.** 1989. Transcription of *pfl* is regulated by anaerobiosis, catabolite repression, pyruvate, and *oxrA*:*pfl*::MudA operon fusions of *Salmonella typhimurium*. J. Bacteriol. **171:**4900–4905.
17. **Youderian, P., P. Sugino, K. L. Brewer, N. P. Higgins, and T. Elliot.** 1988. Packaging specific segments of the *Salmonella* chromosome with locked-in Mud-P22 prophages. Genetics **118:**581–592.