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Sequence Analysis of Tn*10* Insertion Sites in a Collection of *Escherichia coli* Strains Used for Genetic Mapping and Strain Construction

BRIAN P. NICHOLS,* OBAID SHAFIQ, AND VICTORIA MEINERS

Laboratory for Molecular Biology, Department of Biological Sciences, University of Illinois at Chicago, Chicago, Illinois 60607

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The chromosomal insertion sites of Tn*10*-containing *Escherichia coli* strains were amplified by inverse PCR, and the nucleotide sequences of the junctions were determined. In 95 strains analyzed, 88 unique Tn*10* positions were determined and matched to the *E. coli* chromosome sequence. Two gaps in insertion site positions were noted, one including the terminus of DNA replication and another bounded by recombination hot spots *RhsA* and *RhsB*.

A collection of *Escherichia coli* strains with Tn*10* insertions located at approximately 1-min intervals around the chromosome was reported in 1989 (12) and has been used in many laboratories for strain construction and genetic mapping. The versatility of this collection of strains is based on its regularity of map positions around the chromosome and its combination of Tn*10* and positionally equivalent Tn*10kan* members. To clarify an occasional inconsistency in map position in certain members of the collection in our laboratory, we developed an inverse PCR scheme to allow determination of the precise positions of the Tn*10* insertion sites by DNA sequence analysis. We have determined the nucleotide positions of nearly all of the Tn*10* insertion sites in the collection of strains originally reported by Singer et al. (12) and subsequently catalogued by Berlyn et al. (2).

Strains used in this study were obtained either from the Carol Gross laboratory or from the *E. coli* Genetic Stock Center. DNA preparations were done by a modification of standard methods (10, 13). Cells from 5 ml of an overnight culture grown in Luria broth-tetracycline (10 μg/ml) were harvested by centrifugation and resuspended in 2.5 ml of lysis solution (25 mM Tris-HCl [pH 7.4], 50 mM glucose, 10 mM EDTA, 2-μg/ml lysozyme). Cells were lysed by the addition of 0.25 ml of 10% sodium dodecyl sulfate, and DNA was extracted once with an equal volume of phenol saturated with 0.3 M sodium acetate (NaOAc). The aqueous phase was retained and made 0.3 M in NaOAc by addition of 0.1 volume of 3 M NaOAc, and DNA was precipitated by addition of 2.5 volumes of ethanol. The precipitate was transferred to 0.5 ml of 70% ethanol by using a Pasteur pipette. Following a brief centrifugation (30 s at 13,000 × *g*), the supernatant was removed and the DNA was resuspended in 0.25 ml of 10 mM Tris-HCl (pH 7.4)–0.1 mM EDTA. Chromosomal DNA was digested with *Hpa*II and circularized with DNA ligase preparatory to inverse PCR (8).

Inverse PCR was performed as described by Ochman et al. (8) by using Platinum *Taq* DNA Polymerase (Life Technologies). The PCR primers (Integrated DNA Technologies) were designed from the Tn*10* sequence (5, 7, 9, 11) and are illustrated in Fig. 1. The product of the first PCR using primers 1 and 2 was diluted 1/10, and 1 μl was used as the template for a second round of PCR using primers 3 and 4. The sequences of the primers were as follows: primer 1, ACATGAAGGTC ATCGATAGCAGGA; primer 2, GGCTGTTGAGTTGAGG TTGACGAA; primer 3, AACAGTAATGGGCCAATAACA CCG; primer 4, CGAGTTCGCACATCTTGTTGTCTG.

PCR products were sequenced by using a PCR sequencing kit (Amersham). The sequencing primer was a 19-mer situated at positions −62 to −53 relative to the end of IS*10*R. Twenty- to 40-nucleotide-long sequences at the junction of IS*10*R were determined, and the positions were identified by a BLAST search (1) of the *E. coli* genome sequence (3).

Of 95 strains analyzed, 88 yielded sufficient sequences for confident definition of positions on the *E. coli* chromosome (Table 1 and Fig. 2). Two strains (CAG18463 and CAG12099) yielded short sequences that occurred twice in the genome sequence, once within 0.5 min of the reported map position and once distant. The site near the reported map position was taken as the site of insertion for these strains. The sequence derived from the junction of CAG18491 was very short, but inspection of the sequence near *metE* showed only two identities, one in the *metR-metE* intergenic region and one downstream from the *metE* coding region. We presumed that the intergenic insertion was more likely to yield a *metE* phenotype and placed the insertion site at that position. One additional sequence (from CAG18429 *zjh-6*::Tn*10*) was too short for unambiguous assignment, and no phenotype was available to assist in placement. The remaining five strains yielded sequences identical to others in the collection. These duplicate strains

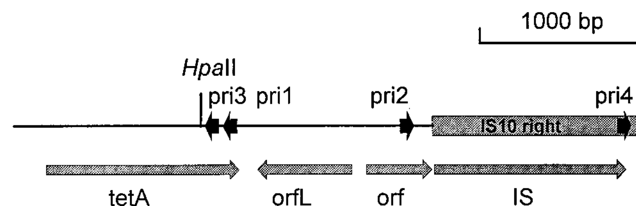

FIG. 1. Map of a portion of Tn*10* including *tetA* and IS*10*R. Open reading frames (orfs) are indicated, as are the positions of the PCR primers (pris) used in this study. The single *Hpa*II site in this region is also shown.

* Corresponding author. Mailing address: Laboratory for Molecular Biology, Department of Biological Sciences, Molecular Biology Research Bldg. m/c 567, University of Illinois at Chicago, 900 S. Ashland Ave., Chicago, IL 60607. Phone: (312) 996-5064. Fax: (312) 413-2691. E-mail: brian.p.nichols@uic.edu.

TABLE 1. Locations of Tn*10* insertion sites

Strain	Genotype[a]	Map position (min)		Nucleotide position	D[d]	Gene	Accession no.	Position (bp)	Flanking sequence
		Reported[b]	Calculated[c]						
CAG18442	*thr-34*::Tn*10*	0.0	0.0	425	<	*thrA*	AE000111	425	TGCCTGGCATTGCTTTCCAG
CAG12093	*car-96*::Tn*10*	0.7	0.7	33,963	<	*carB*	AE000113	13,304	GGCAAAGCCGCCGTTGAGGG
CAG12095	*zab-3051*::Tn*10*	1.9	1.8	84,350	<	*leuO*	AE000118	805	CACTTAACTCCCTTTCCCTT
CAG12025	*zad-220*::Tn*10*	3.2	3.2	150,100	>	*yadC*	AE000123	472	GGTGAAGCGCCATTTTTAAT
CAG18436	*zae-502*::Tn*10*	4.4	5.0	230,099	>	*yafC*	AE000129	10,075	CACTTTATCTGCCATTAACT
CAG18447	*proAB81*::Tn*10*	5.7	5.6	261,286	>	*proA*	AE000132	8,002	TGCTGCGTATGGATAAATAC
CAG18439	*lacI42*::Tn*10*	7.9	7.9	365,945	>	*lacI*	AE000141	9,129	CGCGCAGCCCGG
CAG12080	*zah-281*::Tn*10*	7.5	8.0	371,180	>	b0349	AE000142	3,414	CGCTTTGTGCCGATGGATGC
CAG18091	*zaj-3053*::Tn*10*	9.0	8.5	393,633	>	b0374	AE000144	4,231	TACCTGGTAATATTCTTCAC
CAG12148	*tsx-247*::Tn*10*	9.3	9.3	430,667	<	*tsx*	AE000147	9,036	TACTTTGTGCCGATTACCGA
CAG12017	*zba-3054*::Tn*10*	10.1	9.9	461,486	>	b0441	AE000150	7,331	GGTTAAACAGGCGATTTTCG
CAG12154	*zba-3055*::Tn*10*	10.9	10.8	500,025	>	*gsk*	AE000154	782	CGCTGGGCACCAAGTTTGTC
CAG12171	*purE79*::Tn*10*	11.9	11.9	551,279	<	*purK*	AE000158	5,564	CGCATAACCTGCATCAGGAC
CAG12149	*zbe-601*::Tn*10*	14.3	13.7	637,765	>	b0604	AE000166	1,930	AGCAGAGCCAGTAAAAGTAT
CAG12077	*zbe-280*::Tn*10*	14.7	14.1	655,952	<	*ybeG*	AE000167	4,670	TGCCAGGTGATGGCAGGAAT
CAG18433	*zbf-3057*::Tn*10*	15.9	15.1	698,404	<	*asnB*	AE000171	1,888	GGTTATGTGTTCAATTTTTG
CAG12147	*nadA57*::Tn*10*	16.9	16.8	781,532	>	*nadA*	AE000177	8,094	TGCAAAGCATCCCGCTTCTA
CAG18493	*zbi-29*::Tn*10*	18.0	17.7	819,871	>	ig,[e] b0786-b0787	AE000181	3,833	CACTTAACAAGTACCAGGTA
CAG12034	*zbi-3058*::Tn*10*	18.7	19.3	897,129	<	*potI*	AE000187	9,890	TGTCGCGTATCCGCTGTTTT
CAG18478	*zbj-1230*::Tn*10*	19.8	20.4	946,252	>	ig, *ycaD*-b0899	AE000192	1,345	AGCAGAGTGTGAACTTACTG
CAG12094	*zcb-3059*::Tn*10*	21.1	21.6	1,000,952	<	b0940	AE000196	4,074	AGCTTCGCCCCAGCGCACGC
CAG18466	*zcc-282*::Tn*10*	22.6	22.9	1,064,111	<	*yccE*	AE000202	2,444	TACTCTGCCCCTGAATTTGG
CAG12078	*zce-726*::Tn*10*	24.6	24.9	1,154,216	>	*yceG*	AE000210	6,298	TGCTGCGCATCCGG
CAG18463	*zcf-117*::Tn*10*	25.5	26.1	1,211,084	>	b1160	AE000215	1,599	GACTTAACCGG
CAG18497	*fadR13*::Tn*10*	26.6	26.6	1,234,734	<	*fadR*	AE000217	2,438	AGCCCAGCGCCAGACTGCGC
CAG12016	*zch-3060*::Tn*10*	28.0	27.4	1,269,233	<	ig, *kdsA-cha*	AE000220	156	GACGTAGTATCCACACCAAG
CAG12169	*zci-506*::Tn*10*	28.0	28.1	1,302,475	>	*oppC*	AE000223	1,625	ATCGGGCATTGTTATTCGCC
CAG18455[f]	*trpB83*::Tn*10*	28.3	28.4	1,317,596	<	*trpC*	AE000224	5,761	CGCATTGCCGCCATTTATAA
CAG12028[f]	*zcj-233*::Tn*10*	29.0	29.5	1,368,905	>	b1309	AE000229	1,260	TGCTGAGTATGTCCGCCTGG
CAG12179[f]	*mg1-500*::Tn*10*	48.2	30.2	1,399,003	>	*ydaH*	AE000231	9,081	TGCTATGCGTAAACCAAAAC
CAG12081	*zda-3061*::Tn*10*	30.4	30.4	1,412,228	<	*recT*	AE000232	9,574	TGCGCAGCCTGAGTAAAGCT
CAG18640[f]	*zhj-3076*::Tn*10*	79.7	30.9	1,434,058	>	b1377	AE000234	8,385	TACTGTGCAGTGACTTCAAA
CAG12026	*trg-2*::Tn*10*	32.1	32.2	1,491,930	<	*trg*	AE000239	6,837	CGTTATGCCTCTACTTTGTT
CAG18461	*zdd-235*::Tn*10*	33.3	33.1	1,533,652	<	*yddE*	AE000243	5,163	AGCTGCGCACTATGTACGTG
CAG12151	*zdi-925*::Tn*10*	38.6	38.3	1,776,386	<	ig, b1695-b1696	AE000264	9,608	TGCGCCGCCTGCAGATTATT
CAG18464	*zdj-276*::Tn*10*	39.4	39.5	1,834,488	<	b1754	AE000270	4,289	AGCTGTGTGTCGACATAGCG
CAG18465	*zea-225*::Tn*10*	40.3	40.3	1,869,535	>	b1785	AE000273	8,941	GACATCGTGTGGGTGATAAA
CAG12068	*zeb-3190*::Tn*10*	41.3	41.0	1,903,614	<	ig, b1820-b1821	AE000276	7,274	GGCATAGCGATTGATGTGCA
CAG18486	*eda-51*::Tn*10*	41.6	41.6	1,930,416	<	*eda*	AE000279	1,608	TGCTGGGTATGGACTACGGT
CAG12156	*uvrC279*::Tn*10*	43.0	42.9	1,991,244	>	*uvrC*	AE000284	5,249	CGCCAAGTAGCAGCGGATGA
CAG18451	*zed-3069*::Tn*10*	43.9	43.9	2,038,762	<	b1972	AE000288	8,420	GGCAAAGCACCATAATCCTA
CAG12099	*zef-3129*::Tn*10*	45.4	44.9	2,085,192	>	ig, *yeeF*-b2015	AE000293	1,586	TACGAAGCCCGG
CAG12163[f]	*zib-207*::Tn*10*	81.8	46.9	2,173,967	>	*gatZ*	AE000298	10,901	CGCATCAAGATGAATTTTAC
CAG12021[f]	*zbd-3105*::Tn*10*	13.1	46.9	2,175,213	>	*gatY*	AE000298	12,147	TACCACGTACATTTTCATAT
CAG12098	*zei-722*::Tn*10*	48.3	49.6	2,299,610	<	*napA*	AE000309	11,314	CAACCTGCACCTGCTGACCG
CAG12177	*zej-298*::Tn*10*	49.6	50.0	2,318,294	>	*atoS*	AE000311	3,377	ATCCGCGCATTAAATGCAGA
CAG12178	*zfa-723*::Tn*10*	50.4	50.5	2,341,772	<	*yfaL*	AE000313	4,289	GGCGCAATCTATTCTTCTGG
CAG18484	*zfb-223*::Tn*10*	51.5	51.5	2,387,022	<	b2274	AE000317	5,073	TGCTCCGTATCTTCTGACCA
CAG18467	*zfd-1*::Tn*10*	53.4	53.7	2,491,451	<	ig, b2374-b2375	AE000325	12,877	TACTTACCATGCAGAAAGGA
CAG18468	*nupC510*::Tn*10*	54.1	54.1	2,511,317	>	*nupC*	AE000327	4,976	GGCCTGGCATTCTTCTTCCT
CAG18470	*purC80*::Tn*10*	55.7	55.9	2,595,619	>	*purC*	AE000334	10,116	AACTCAGCTTGCTTTTGCAT
CAG18481	*zfh-208*::Tn*10*	57.5	57.4	2,665,159	>	b2536	AE000340	2,865	GGCCGAGTAGCCAGCTGCCT
CAG18480	*nadB51*::Tn*10*	58.2	58.4	2,708,518	<	*nadB*	AE000344	312	GGCGTAGCGCCAGTGAAAGT
CAG12158	*pheA18*::Tn*10*	58.9	59.0	2,736,076	<	*pheA*	AE000346	3,848	TGCTGAGTGCGGATTAATTT
CAG18642	*zfi-3131*::Tn*10*	59.4	60.9	2,826,375	<	*srlD*	AE000354	6,308	TACCCAGCTTGGTCGCGTAT
CAG12173	*cysC95*::Tn*10*	61.9	62.2	2,886,785	<	*cysI*	AE000360	1,394	AGCAGAGCGTTTCCTGCCGT
CAG12079	*fuc-3072*::Tn*10*	63.2	63.2	2,932,885	>	*fucP*	AE000364	932	CACCTTATATGATCATCGTG
CAG12135	*recD1901*::Tn*10*	63.5	63.6	2,948,961	<	*recD*	AE000365	5,008	CGCGCGTCTGGTTTGCGATG
CAG12168	*zgf-210*::Tn*10*	66.0	66.5	3,082,773	>	*speA*	AE000377	3,072	CACCGTGTATTCGTTACGTT
CAG18472	*nupG511*::Tn*10*	66.9	66.9	3,104,395	<	*nupG*	AE000379	2,008	CGCCCAGCAGCATTGAGAAG
CAG18475	*metC162*::Tn*10*	67.9	67.9	3,150,485	>	*metC*	AE000383	2,934	GGCTGCGTGCTATTTCCCTG
CAG12184	*tolC210*::Tn*10*	68.4	68.5	3,176,389	>	*tolC*	AE000385	6,033	ACCAGTGCGTCCTTGCAGTT
CAG12152	*zgj-3075*::Tn*10*	69.5	69.3	3,215,934	<	*air*	AE000389	1,688	AGCCAGGTGTCCAGTGTCAG
CAG12072	*zha-203*::Tn*10*	70.9	71.8	3,332,800	>	*nlp*	AE000399	6,432	TGCGTAGCTACACTAAACCG
CAG12153	*zhc-6*::Tn*10*	72.1	72.5	3,364,914	>	b3219	AE000401	6,566	GACAACGCGCTTATTCGGCT
CAG12075[f]	*zhe-3083*::Tn*10*	74.7	73.6	3,412,834	>	*acrF*	AE000405	4,993	GACCGTGCAGGATACGGTGA
CAG12071[f]	*zhd-3082*::Tn*10*	73.3	73.9	3,429,813	<	*smg*	AE000407	246	TGCTCAACCTTGAAACTCGT
CAG18456	*zhe-3084*::Tn*10*	74.0	75.5	3,503,687	>	*yhfT*	AE000413	6,756	GGCCAAGCCAGTAAAGAACA
CAG18638[f]	*zhh-21*::Tn*10*	77.8	76.8	3,564,175	>	*glgP*	AE000419	2,951	CGCTAAGCGTGGGCGATGAA
CAG18450[f]	*zhf-5*::Tn*10*	75.8	77.4	3,590,125	>	ig, *ugpB-livF*	AE000421	8,047	GATGGGGCACGGATAAGCGG
CAG18492[f]	*zic-4901*::Tn*10*	82.7	82.5	3,826,553	<	ig, *glts-yicE*	AE000443	259	AGCAAAGCGGGCATTTTAGC
CAG18452[f]	*zhf-3085*::Tn*10*	75.8	82.7	3,837,058	>	*nlpA*	AE000443	10,764	AGCTGCGCCCCCTCGAGTTC
CAG18499	*zid-501*::Tn*10*	83.9	83.5	3,873,001	<	ig, *yidW-yidX*	AE000446	8,448	CGCCTGATATCCCTTTTCAG

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TABLE 1—*Continued*

Strain	Genotype[a]	Map position (min)		Nucleotide position	D[d]	Gene	Accession no.	Length (bp)	Flanking sequence
		Reported[b]	Calculated[c]						
CAG18501	*zie-296*::Tn*10*	84.5	84.7	3,931,475	<	*rbsD*	AE000452	854	CGCTGCGCCCGAGAGGGCTT
CAG18431	*ilv-500*::Tn*10*	85.2	85.2	3,952,161	<	*ilvD*	AE000453	10,898	GGCCAAGTACGTTTTTCACA
CAG18491	*metE3079*::Tn*10*	86.4	86.4	4,010,549	<	ig, *metR-metE*	AE000458	8,157	GACCGG
CAG18496	*fadAB101*::Tn*10*	86.7	86.8	4,025,408	>	*fadA*	AE000460	559	TACATGGCAGGATCTGCGCG
CAG18495	*zih-35*::Tn*10*	87.4	87.6	4,063,253	>	b3872	AE000463	5,333	TGCAGAGCATTAAATTCGAA
CAG18477	*zij-501*::Tn*10*	89.1	89.3	4,143,393	>	*pflD*	AE000469	3,582	CGCGCGGCGGCTACTTCACG
CAG12185	*argE86*::Tn*10*	89.4	89.5	4,151,734	>	*argE*	AE000470	1,041	CGCTTCGTAGTGATAACGTT
CAG18500	*thi-39*::Tn*10*	90.3	90.4	4,192,143	>	*thiC*	AE000473	4,927	TGCTTAACATCTTCTTTATT
CAG12164[f]	*malF3089*::Tn*10*	91.4	91.4	4,241,898	>	*malF*	AE000476	11,030	GGCTTAGCTTTTCATCACCC
CAG18427	*zje-2241*::Tn*10*	94.1	92.8	4,303,986	>	*yjcS*	AE000482	2,055	CGCTGGGCATCGTCAAAATC
CAG18488	*zjd-2231*::Tn*10*	93.7	93.9	4,356,936	<	*cadB*	AE000486	731	TGCTGGGTACTGGTTTAGCA
CAG12073	*cycA30*::Tn*10*	95.5	95.4	4,427,714	<	*cycA*	AE000492	2,708	AGCGAAGTCACTAAAAGATT
CAG12019	*zjg-920*::Tn*10*	96.2	95.8	4,442,377	>	*ytfN*	AE000493	7,248	CGCTTAACCTGAACATTGAA
CAG18462[f]	*zdh-603*::Tn*10*	37.1	95.8	4,446,109	>	*chpS*	AE000494	219	GGCAGAGCGTGGAGGCGCGA
CAG18430	*zji-202*::Tn*10*	98.6	99.0	4,595,002	>	*mdoB*	AE000507	382	TGCTTGTACTCGATTTTTAC

[a] Only the position of Tn*10* is given.
[b] Map position as reported in reference 2.
[c] Map position calculated from the DNA sequence position.
[d] D, direction. The flanking sequence was read clockwise (>) or counterclockwise (<) on the genetic map.
[e] ig, intergenic. Flanking genes or open reading frames are listed.
[f] Strains from this laboratory and the *E. coli* Genetic Stock Center were analyzed.

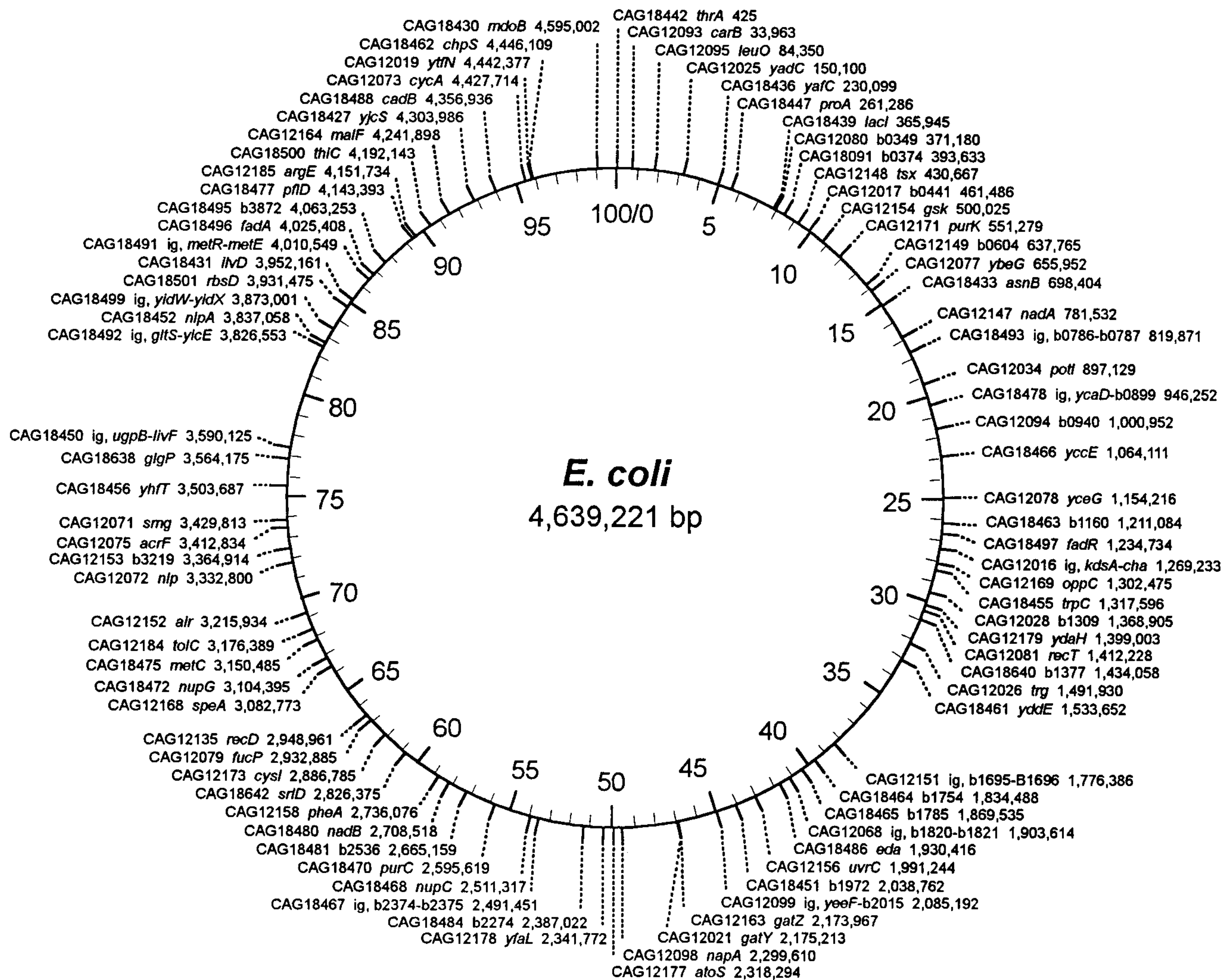

FIG. 2. Positions of Tn*10* insertions on the *E. coli* map. Shown for each strain are its designation, the gene or open reading frame disrupted by the insertion, and the base pair position. Numbering on the inside of the circle is in minutes. ig, intergenic.

were CAG12074 = CAG18465, CAG12159 = CAG18459 = CAG12151, CAG18483 = CAG12080, CAG18498 = CAG18499, and CAG18709 = CAG18456. Each duplicate sequence was confirmed by analysis of strains obtained from the *E. coli* Genetic Stock Center.

Eleven of the Tn*10* insertion sites were in intergenic regions, 51 were in coding regions of known genes, and 26 were in potential open reading frames identified by sequence analysis (3). For the majority of strains, the nucleotide positions of the Tn*10* insertion site fell within 1 min of the position determined by genetic mapping. Six of the sequences differed in map position by greater than 2 min. All strains whose Tn*10* positions differed from the mapped position by greater than 1.5 min were obtained from the *E. coli* Genetic Stock Center and reanalyzed. In most cases, there was agreement between the strains in our laboratory collection and those obtained from the *E. coli* Genetic Stock Center. In several cases, cross-contamination of cultures was evident. In most cases, the mixture was resolved by isolation of single colonies from the cultures. It is not clear whether the positional differences we noted were caused by culture contamination that occurred prior to the distribution of strains to this laboratory and the *E. coli* Genetic Stock Center or some other artifact of the original genetic analysis.

There are two noticeable gaps in this particular collection of transposon-containing strains, each about 5 min long. The gap at 33 to 38 min contains the DNA replication terminus and recombination hot spot sites *dif* and *RhsE*. The gap at 77 to 82 min is bounded by recombination hot spot sites *RhsB* and *RhsA*. Three of the strains with transposons originally mapped to these two gaps (CAG18640, CAG12163, and CAG18462) now contain the transposon at a grossly different location on the chromosome. It seems likely that the failure of the transposons to be maintained at these locations is due to the same features that have led to the characterization of these regions as "recombinationally unstable" (4, 6).

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