ILL Document Delivery

REG-14286731

MDUNMC

NLM -- W1 GE184M (Gen); E-Journal w/ILL access

Mr. Andrew Michaelson
41 Dudley Ct.
Bethesda, MD 20814

ATTN:
PHONE: 301-295-1184
FAX: 206-333-0380
E-MAIL: Andrew.Michaelson@usuhs.mil

SUBMITTED: 2007-12-12 10:14:58
PRINTED: 2007-12-12 12:00:45
REQUEST NO.: REG-14286731
SENT VIA: DOCLINE
DOCLINE NO.: 23888184

REG Copy Journal

TITLE:	GENE
PUBLISHER/PLACE:	Elsevier/North-Holland Amsterdam
VOLUME/ISSUE/PAGES:	1984 Dec;32(3):369-79 369-79
DATE:	1984
AUTHOR OF ARTICLE:	Way JC;Davis MA;Morisato D;Roberts DE;Kleckner N
TITLE OF ARTICLE:	NEW TN10 DERIVATIVES FOR TRANSPOSON MUTAGENESIS AN
ISSN:	0378-1119
OTHER NUMBERS/LETTERS:	Unique ID.: 7706761 23888184 6099322
SOURCE:	PubMed
MAX COST:	$12.00
COPYRIGHT COMP.:	Guidelines
CALL NUMBER:	W1 GE184M (Gen); E-Journal w/ILL access
REQUESTER INFO:	Michaelson, Mr. Andrew
DELIVERY:	E-mail: Andrew.Michaelson@usuhs.mil
REPLY:	Mail:

KEEP THIS RECEIPT TO RECONCILE WITH BILLING STATEMENT
For problems or questions, contact NLM at http://wwwcf.nlm.nih.gov/ill/ill_web_form.cfm or phone 301-496-5511.
Include LIBID and request number.

NOTE:-THIS MATERIAL MAY BE PROTECTED BY COPYRIGHT LAW (TITLE 17, U.S. CODE)

Gene, 32 (1984) 369–379
Elsevier

GENE 1172

New Tn*10* derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition

(Tn*10*; transposon; insertion mutagenesis; *lacZ* fusions vectors; IS elements)

J.C. Way, M.A. Davis, D. Morisato, D.E. Roberts and N. Kleckner*

Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138 (U.S.A.) Tel. (617) 495-4278

(Received May 1st, 1984)
(Revision received September 10th, 1984)
(Accepted September 14th, 1984)

SUMMARY

We describe below several new variants of the tetracycline-resistance transposon Tn*10* which are more useful than the wild-type transposon for many types of genetic and physical analysis of bacteria. These derivatives have one or more of the following new properties: (i) new drug resistance markers; (ii) high transposition frequencies; (iii) removal of the transposase gene to a position outside of the transposing segment; (iv) internal deletions which eliminate the ability of Tn*10* to make adjacent deletion/inversions; or (v) addition of a *trp-lac* operon fusion segment just inside one terminus such that insertion can automatically generate a transcriptional fusion to the interrupted operon. Phage and plasmid vehicles carrying these new elements are described.

INTRODUCTION

The tetracycline-resistance transposon Tn*10* has been widely used as a tool for genetic and physical analyses of bacteria, bacterial plasmids, and bacterial viruses, particularly in *Escherichia coli* and *Salmonella*. We describe below several variants of Tn*10* which are more useful than the wild-type transposon for many types of genetic manipulations. Each of these derivatives (see Fig. 1 and PROCEDURES, section **a**) has one or more of the following new properties.

(1) *kanR*, *camR*, *ampR* or *trpA-lacZ*-fusion genes instead of or in addition to *tetR* genes (see PROCEDURES, section **a**, elements 2, 3, 4, 7, 9, 10, 12, 14).

(2) Transposition frequencies 100 to 1000 times higher than wild type (elements 6, 7, 8, 9, 10, and 11 and 12 under some conditions).

(3) A deletion of one 'inside' IS*10* end which eliminates transposon-promoted TetS deletion/inversions and limits. TetS adjacent deletions of chromosomal sequences on one side of the inserted element (elements 5, 6, 7, 14); or deletion of both 'inside' ends which eliminates all TetS Tn*10*-promoted rearrangements (elements 8, 9, 10, 11, 12). (See Kleckner et al., 1979a and Ross et al., 1979 for descriptions of Tn*10*-promoted rearrangements.)

*To whom correspondence and reprint requests should be addressed.

Abbreviations: bp, base pairs; IPTG, isopropyl-β-D-thiogalactopyranoside; IS, insertion sequence; kb, 1000 bp; λym broth, see PROCEDURES, section **d**4; moi, multiplicity of infection; *ptac*, *tac* promoter, *R* or ^{R}, resistance; Tn, transposon; Xgal, 5-bromo-4-chloro-indolyl-β-D-galactoside; [], indicates plasmid-carrier state; ::, novel joint.

0378-1119/84/$03.00 © 1984 Elsevier Science Publishers

Material may be protected by copyright law (Title 17, U.S. Code)

(4) Removal of the transposase gene to a position outside of the transposing segment so that, once made, insertions of the transposon are stable and incapable of subsequent transposition, deletion or inversion events (elements 8, 9, 10, 11, 12). Unlike insertions of wild-type Tn*10* (Kleckner et al., 1978a), insertions of these transposons are faithfully transmitted during P1 transduction and Hfr or F′ conjugation in *E. coli*.

(5) Addition of a *trpA-lacZ* operon fusion segment just inside one terminus such that insertion of the transposon in appropriate orientation places the *trp* and *lac* genes under transcriptional control of the interrupted operon (elements 10, 14). These constructions are patterned after the Mu d*lac* element (Casadaban and Cohen, 1979; Baker et al., 1983), but are more stable; also, construction 14 uses *tetR* instead of *ampR* as the selective marker.

We also describe derivatives of the 50-kb non-IS-containing F plasmid pOX38 (Guyer et al., 1981) marked with *tetR*, *kanR*, or *trpA-lacZ* genes provided by insertion of stable, function-defective Tn*10* elements.

Each of the Tn*10* variants described below is available as an insertion carried by phage λ, a multicopy plasmid and/or the *E. coli* chromosome. All bacterial, phage and plasmid strains described here are available from us, together with supplementary information about the vehicles, and relevant restriction maps and DNA sequences.

Although some of the approaches discussed here are applicable only to *E. coli* K-12, many are applicable to any organism in which Tn*10* transposes and into which DNA can be transferred by conjugation or transformation. These vehicles can be used for insertion mutagenesis of the bacterial chromosome, of natural or of artificially constructed bacterial plasmids, or bacteriophages. Additional tools for and applications of Tn*10* transposon mutagenesis in *S. typhimurium* LT2 and *E. coli* K-12 are described by Kleckner et al. (1975; 1977); Roth (1981), Chumley (1981), Schmid and Roth (1983), Ciampi et al. (1982), Lam and Roth (1983), Anderson and Roth (1981), and Maurer et al. (1984).

PROCEDURES

(a) Description and origin of Tn*10* elements

The structure of each available Tn*10* variant is drawn to scale in Fig. 1. In some cases (elements 1–7, 11, 12 and 14) only the transposon itself is drawn. In these cases, vehicles carrying the element have been isolated by transposition of the element into a phage or plasmid genome. In the remaining cases (elements 8–10 and 13) a DNA segment containing the construction is diagrammed; phage and plasmid vehicles contain this segment inserted at an appropriate restriction site. Vehicle structures are described briefly below and in the legend to Fig. 1 and described in detail in supplementary material available on request. Transposition frequencies are specified in Table I. The elements shown in Fig. 1 are as follows:

Element 1

Wild-type Tn*10*. See Fig. 2 for partial restriction map.

Element 2

'Wild-type *kan* hopper'. Wild-type Tn*10* with *kan* genes substituted for *tet* genes. A *Hin*dIII-*kanR* segment from Tn*5* has been substituted for Tn*10* material between *Hin*dIII sites at bp 2272 and 7490 of Tn*10* (D.E.R., unpublished).

Elements 3 and 4

'*amp* hopper' and '*cam* hopper', respectively. Composite IS*10* transposons which carry *ampR* or *camR* determinants. These elements also contain IS*10*-Left and IS*10*-Right as inverted repeats having the relative orientation opposite that of the IS*10* elements in Tn*10*. Isolation of both elements is described in Foster et al. (1981). Element 4 was derived directly from element 3 by insertion of a *Pst*I fragment carrying the *camR* genes of Tn*9*. Element 4 has sometimes been referred to previously as TnHACIO.

Element 5

'Tn*10del4*'. A deletion between the *Acc*I and *Bgl*II sites at bp 8964 and 4827 of Tn*10* (Fig. 2 and Foster et al., 1981).

TABLE I

Transposition fre

λ hop assays and
were performed i
λ^R) exactly as de
(pNK474) when t

Element number	Phag num
1	λ561
2	λ110
3	λ634
4	λ940
5	λ775
6	λ840
7	λ1052
8	λ1098
9	λ1105
10	λ1048
11	λ809
12	λ939
13	λ1046
14	λ1045

[a] n.t., not tested; n

Element 6

'Tn*10del4*-HI
del4 (element 5).
transition at bp 7
activity of the tra
abolishes IS*10*-r
ter et al., 1981;
Kleckner, 1983).

Element 7

'High hopper
ment 6) containi
Tn*903* inserted in
(J.C.W., unpubli

Element 8

'*ptac*-transposa
pNK474 (elemen
plasmid's unique
containing a 'min
1983). This mini-

Material may be protected by copyright law (Title 17, U.S. Code)

TABLE I

Transposition frequencies of Tn*10* elements

λ hop assays and construction of λ prophages performed exactly as described in PROCEDURES, section c4. Mating-out experiments were performed in NK5830 (= *Δlac-pro*XIII *recA*56 *arg*$^-$ Nal*R* Rif*R* *su*^{e} [F′ *lacI*^{Q} L8 pro$^+$] with NK6641 (= *Δlac-pro*XIII Sm*R* *thy*$^-$ λ^R) exactly as described in Foster et al. (1981). For elements 11 and 12, IS*10* transposition functions were supplied by a plasmid (pNK474) when the element was present in a prophage, and by a prophage (λ1046) when present in a plasmid.

Element number	Phage number	λ::Tn vehicles		Plasmid vehicles	
		λ-hop Drug*R* colonies per 10^7 infecting phages[a]	Mating-out from prophage Drug*R* exconjugants per ml mating mix (= 10^8 total exconjugants)[a]	Plasmid name	Mating-out from plasmid Drug*R* exconjugants per ml mating mix (= 10^8 total exconjugants)[a]
1	λ561	55	94	pNK81	2.7×10^3
2	λ1104	50		pNK1022	
3	λ634	n.t.	9	—	
4	λ940	2	78	—	
5	λ775	65	241	—	
6	λ840	1200	3730	pNK579	1.6×10^6
7	λ1052	920	6090	—	
8	λ1098	1200	5250 (+ IPTG)	pNK861	3.0×10^6 (+ IPTG)
9	λ1105	10000	n.t.	pNK862	9.5×10^6 (+ IPTG)
10	λ1048	n.t.	n.t.	pNK569	1.3×10^6 (+ IPTG)
11	λ809	n.t.	45000 (w. pNK474 + IPTG)	pNK217	2.1×10^4 (w. λ1046, + IPTG)
12	λ939	n.t.	20000 (w. pNK474 + IPTG)	pNK289	8.4×10^3 (w. λ1046, + IPTG)
13	λ1046	n.a.	n.a.	pNK474	n.a.
14	λ1045	48	192	—	n.a.

[a] n.t., not tested; n.a., not applicable; w., with.

Element 6

'Tn*10del*4-HH104'. A HH104 derivative of Tn*10 del*4 (element 5). The HH104 mutation is a C to T transition at bp 70 of IS*10*-Right which increases the activity of the transposase promoter pIN 100-fold and abolishes IS*10*-mediated multicopy inhibition (Foster et al., 1981; Simons et al., 1983; Simons and Kleckner, 1983).

Element 7

'High hopper *kan-tet*'. Tn*10del*4 HH104 (element 6) containing in addition the *kanR* genes of Tn*903* inserted into the *Bgl*II site at bp 1942 of Tn*10* (J.C.W., unpublished).

Element 8

'*ptac*-transposase mini-*tet*' is derived from pNK474 (element 13, below) by insertion, at the plasmid's unique *Hin*dIII site, of a *Hin*dIII segment containing a 'mini-*tet*' transposon (Morisato et al., 1983). This mini-*tet* segment consists of a 2885-bp *Bgl*II-*tetR* segment from Tn*10* (see Fig. 2) flanked by inverted repeats of a 121-bp *Bam*HI-*Hin*dIII fragment containing the outer 78 bp of IS*10*-Right and an adjacent 43 bp of λ *c*I gene sequence. Constructions similar to this one are also described in Way and Kleckner (1984).

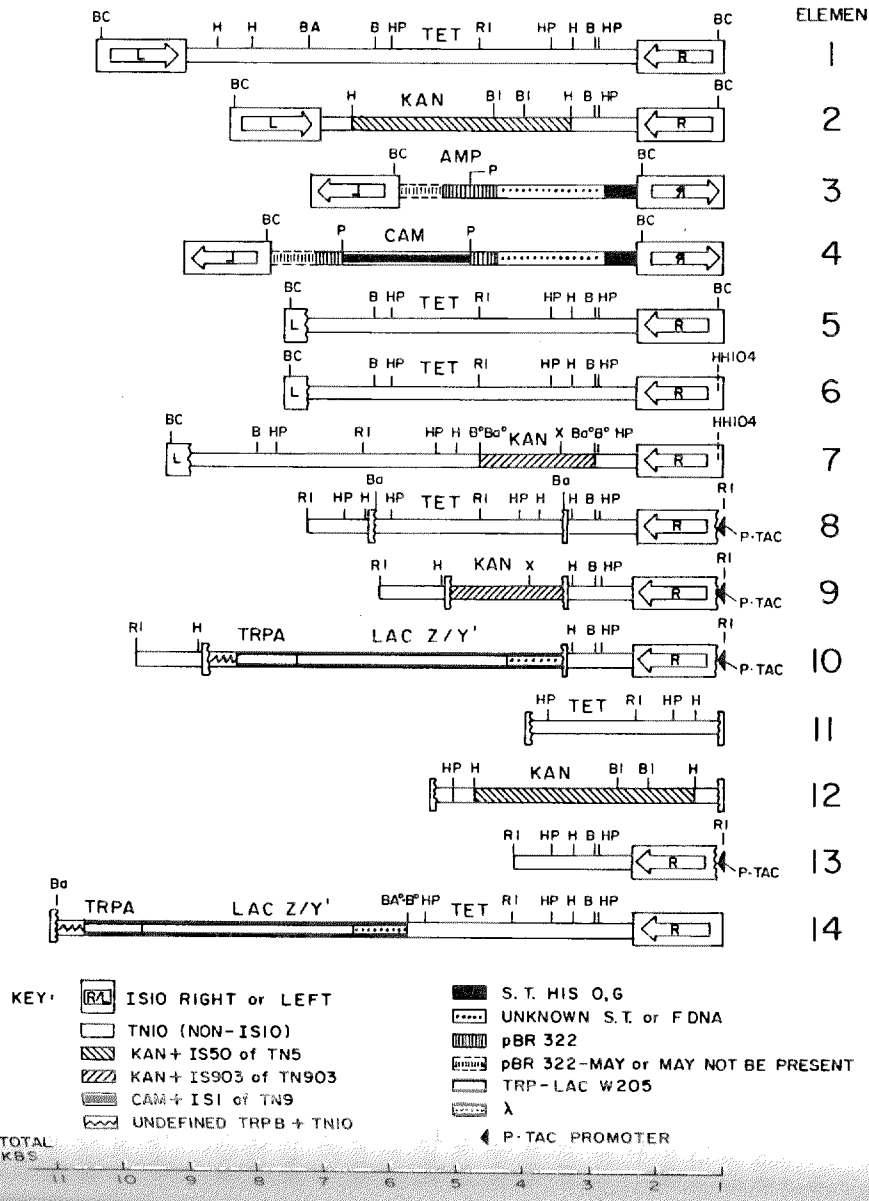
Element 9

'*ptac*-transposase mini-*kan*'. pNK474 (element 13) with a 'mini-*kan*' transposon segment inserted at its *Hin*dIII site. Element 9 is identical in structure to element 8 except that the inserted *Hin*dIII segment contains a 1700-bp *kanR* fragment from Tn*903* between the terminus-containing inverted repeat segments (D.M., unpublished).

Element 10

'*ptac*-transposase mini-*trp-lac*'. pNK474 (element 13) with a 'mini-*trp-lac*' transposon segment inserted at its *Hin*dIII site (Morisato et al., 1983). Like element 9, element 10 is identical in structure to

Material may be protected by copyright law (Title 17, U.S. Code)



ELEMENT	NICKNAME	MARKERS IN TRANSPOSON	PHAGE VEHICLE	PLASMID VEHICLE
1	TNIO	TETR	λ561 & λ370	PNK81
2	KANR–TNIO	KANR	λ1104	PNK1022
3	AMP HOPPER	AMPR	λ634	–
4	CAM HOPPER	CAMR	λ940	–
5	DEL 4	TETR	λ775	–
6	DEL 4 HHIO4	TETR	λ840	PNK579
7	DEL 4 HHIO4 KAN TET	KANR, TETR	λ1052	–
8	PTAC + MINI–TET	TETR	λ1098	PNK861
9	PTAC + MINI–KAN	KANR	λ1105	PNK862
10	PTAC + MINI–TRPLAC	TRPA, LACZ	λ1048	PNK569
11	DEL16 DEL17 TETR	TETR	λ809	PNK217
12	DEL16 DEL17 KANR	KANR	λ939	PNK289
13	PTAC – TRANSPOSASE	NONE	λ1046	PNK474
14	TRPLAC FUSION HOPPER	TRPA, LACZ, TETR	λ1045	–

restriction fragment (8–10, 13) is drawn to scale. When only the transposon is drawn, vehicles carrying the element have been isolated by transposition of the element into a suitable phage or plasmid genome. When a DNA segment is drawn, vehicles contain the indicated DNA segment inserted at an appropriate restriction site. Partial descriptions of the phage and plasmid vehicles are as follows: λ561 is an insertion of wild-type Tn10 in the λ cI gene of λb221cI857Oam29Pam80; λ370 is the same phage except that it carries the UGA261 mutation in gene O instead of Oam29 Pam80, and can be used for making insertions in

Material may be protected by copyright law (Title 17, U.S. Code)

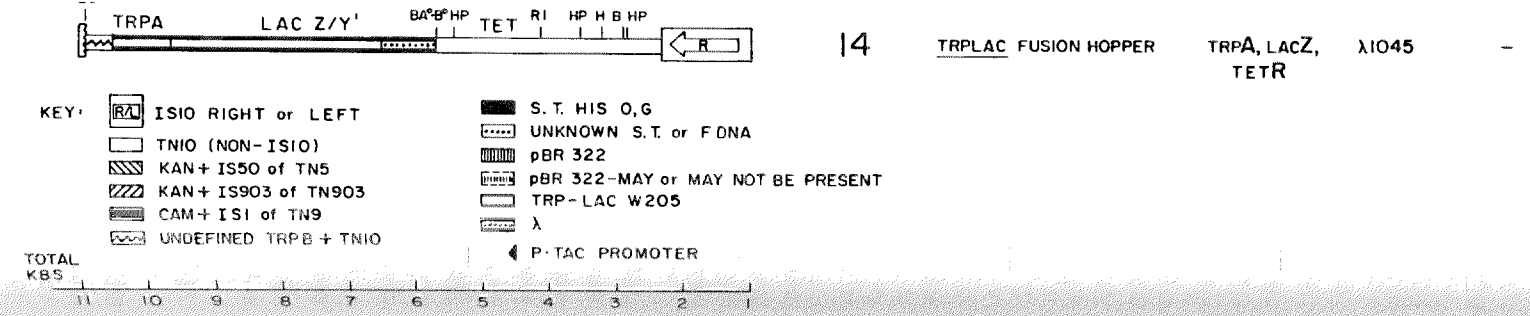


Fig. 1. Structures of transposon derivatives and names of phage and plasmid vehicles carrying those derivatives. Structure of each transposon (1–7, 11, 12, 14) or transposon-containing restriction fragment (8–10, 13) is drawn to scale. When only the transposon is drawn, vehicles carrying the element have been isolated by transposition of the element into a suitable phage or plasmid genome. When a DNA segment is drawn, vehicles contain the indicated DNA segment inserted at an appropriate restriction site. Partial descriptions of the phage and plasmid vehicles are as follows: λ561 is an insertion of wild-type Tn*10* in the λ *c*I gene of λ*b*221*c*I857*O*am29*P*am80; λ370 is the same phage except that it carries the UGA261 mutation (*Ouga*261) in gene *O* instead of *O*am29 *P*am80, and can be used for making insertions in strains carrying amber or ochre nonsense suppressors. λ1104, λ775, λ809 and λ939 are insertions of elements 2, 5, 11 and 12, respectively, in the *hisG* gene of λ*gt7-his c*I857*P*am80 *nin*5. λ634 and λ940 are insertions of elements 3 and 4 between the *P* and *Q* genes of λ*b*221*c*I857*P*am80. λ840 and λ1052 are uncharacterized insertions of elements 6 and 7 into λ*gt7-his* cI857*P*am80*nin*5. λ1045 is an insertion of element 14 between the *P* and *Q* genes of λ in λ *b*221 *c*I857 *P*am80. λ1048 contains in its left arm an insertion of the indicated fragment between *Eco*RI sites 1 and 2 of λ; its right arm is *imm*21 *nin*5 and mutated for *Eco*RI sites 3, 4, and 5. λ1046 is analogous to λ1048. The *Eco*RI fragment cloned into λ is different from that diagrammed for element 13 in that it *also* contains the *TrpLac*1 segment inserted at the unique *Bgl*II site to serve as a genetic marker for the fragment. λ1105 and λ1098 contain in their left arms *Eco*RI fragments inserted between *Eco*RI sites 1 and 2 of λ; the right arms of these phages are *c*I857 *P*am80 *nin*5. For λ1105 the inserted *Eco*RI fragment is that diagrammed for element 9; for λ1098, the inserted *Eco*RI fragment is identical to that diagrammed for element 8 except that there are no *Bam*HI sites at the junctions between the *tetR* and IS*10* terminus segments. Detailed descriptions of and references to phage vehicles are available from us on request. λ561 was constructed in this laboratory from λ171, which is described by Kleckner et al. (1978a). The *Ouga* allele in λ370 was obtained from Ira Herskowitz. λ*b*221 *c*I857 *P*am80 was obtained from M. Lichten and M. Fox. λ1046 and λ1048 are inserts into phage RP167 (Maurer et al., 1980), as are the left arms λ1106 and λ1098. The right arm of λ1098 is derived from λ*b*221 *c*I857 *P*am 80. pBR333 is a deletion between bp 185 and bp 2350 (approximately) of pBR322 (Sutcliffe, 1979); it was obtained from K. Backman. pNK579 is an insertion of element 6 into pBR333 between the *Eco*RI and *Hin*dIII sites. pNK474, 861, 862, and 569 are insertions of the indicated *Eco*RI fragments into the unique *Eco*RI site of a pBR333 derivative having its unique *Hin*dIII site filled in. pNK75 is pBR333 containing a 4500-bp *hisOGD* segment. pNK81 is pNK75 containing the *hisG*9424::Tn*10* insertion introduced by recombination with an F′ *hisG*9424::Tn*10*. pNK1022, pNK217 and pNK289 are altered variants of pNK81. Some of these vehicles are described in Foster et al. (1981) or Morisato et al. (1983). Large open arrows indicate orientations of IS*10*-Right and IS*10*-left.

Material may be protected by copyright law (Title 17, U.S. Code)

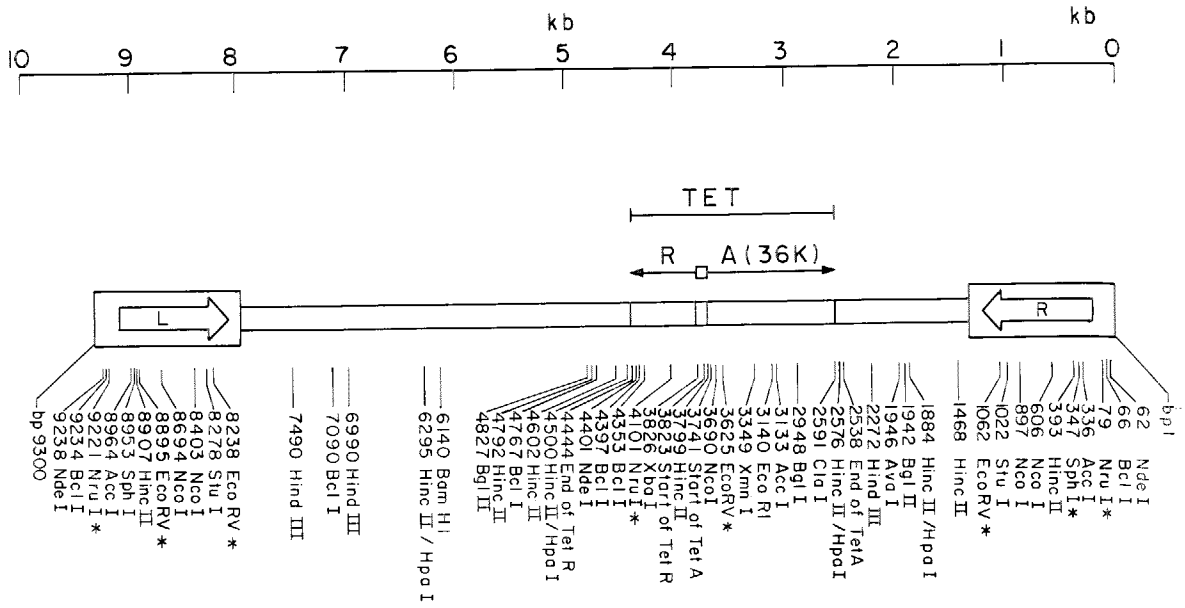


Fig. 2. Selected restriction sites in Tn*10*. Tn*10* includes IS*10*-Right, IS*10*-Left and the tetracycline-resistance determinant, which is composed of two divergently transcribed genes: TetR, the repressor; and TetA, the structural gene for a 36 kDal (K) protein. The positions and orientations of these two genes are indicated by the two correspondingly marked divergent arrows. Information compiled from Kleckner et al. (1978), Halling et al. (1982), Jorgensen and Reznikoff (1979a), Jorgenson et al. (1979), Moyed and Bertrand, (1983), Wray et al. (1981), N.K., unpublished; Hillen and Schollmeier (1983), K. Bertrand, personal communication, and Schollmeier and Hillen (1984). Complete DNA sequences available for all of IS*10*-Right and portions of IS*10*-Left (Halling et al., 1982) and for the region between the *Hin*dIII site at bp 2230 and the *Hinc*II/*Hpa*I site at bp 4464 (K. Bertrand, personal communication; and references above). Numerical positions have been assigned to sites outside of this region for convenience, but are clearly approximations. Numbers begin with IS*10*-Right. Numbers were then assigned to sites within the sequenced *tet* region by designating the *Hin*dIII site as bp 2300 on the map and calculating the positions of sites in that region accoordingly from the DNA sequence of K. Bertrand (unpublished personal communication). Numbers in IS*10*-Left were assigned by assuming the length of the element to be exactly 9300 bp, also clearly an approximation, and assuming that IS*10*-Left contains exactly the same number of bp as IS*10*-Right. Asterisks indicate that Tn*10* contains *Eco*RV and/or *Nru*I sites in addition to those indicated.

element 8 except that in this case the inserted *Hin*dIII segment contains a 6200-bp *trp-lac* W205 fusion fragment, called *TrpLac*1, between the terminus containing inverted repeat segments. The transposon on pNK569 is sufficiently active that transpositions of mini-*trp-lac* into other plasmids or episomes or into phages can often be identified without direct selection by visual screening for LacZ$^+$ on Xgal indicator plates (Miller, 1972). If inserted in the appropriate orientation, mini-*trp-lac* insertions will be transcriptional fusions of *trp* and *lac* genes to the target operon, analogous to those obtained with construction 14.

Element 11

'*del*16 *del*17 *tetR*'. Derived from wild-type Tn*10* by substitution of the 2900-bp *Bgl*II *tetR* fragment for all of the Tn*10* material normally found between the *Bcl*I sites at bp 66 and 9234 of Tn*10* (Fig. 2 and Foster et al., 1981).

Element 12

'*del*16 *del*17 *kanR*'. Constructed from element 11 by insertion of a *Hin*dIII-*kanR* segment of Tn5 at the unique *Hin*dIII site of pNK217 followed by deletion of *tetR* sequences between the *Hpa*I sites at Tn*10* bp 2576 and 4500 (Fig. 2 and Foster et al., 1981).

Element 13

'*ptac*-transposase fusion'. The indicated *Eco*RI fragment contains an *Eco*RI-*Pvu*II *ptac* promoter fragment (Amann et al., 1983) ligated to the 'filled-in' *Bcl*I site at bp 66 of IS*10*-Right. The *ptac* promoter is a hybrid between the *trp* operon and *lac*UV5 promoters. *ptac* is roughly seven times stronger than a *lac*UV5 promoter, and is still repressed by *lac*

Material may be protected by copyright law (Title 17, U.S. Code)

repressor and inducible by IPTG. pNK474 consists of this fragment inserted at the *Eco*RI site of pBR333 *Hin*dIII$^-$ with the *ptac* promoter directed away from the *ampR* gene (Morisato et al., 1983).

Element 14

The '*trp-lac* fusion hopper' was constructed and characterized by J.C.W. The right end of this element is wild-type IS*10*-Right plus the adjacent internal Tn*10* material, including *tetR* genes, out to the *Bgl*II site at bp 4827 of Tn*10* (Fig. 2). Juxtaposed to this *Bgl*II site is *TrpLac*1, the *trp-lac* W205 fusion segment with *Bam*HI ends, and then a properly oriented copy of the 121-bp *Bam*HI-*Hin*dIII IS*10*/cI terminus-containing segment used to construct elements 8, 9, and 10.

The fusion hopper element does give usable transcriptional fusions. Five presumptive fusion hopper insertions in the *lamB* gene were identified as chromosomal insertions of element 14 which conferred resistance to bacteriophage λ but retained a Mal$^+$ phenotype (Hofnung, 1974). In one of these insertions, *lamB*15: : Tn-*trp lac tet*, expression of *lacZ* appears to be under control of the *lamB* promoter, because expression of β-galactosidase, like expression of the wild-type *lamB* gene, is induced by maltose (Table II). We presume that the remaining 4 insertions contain the transposon inserted in the wrong orientation for fusion activity. The β-galactosidase expression in these four strains (Table II) and in strains carrying the transposon at most other chromosomal locations (not shown) is generally about 50 units and is not maltose-inducible. Most of these 50 units are probably attributable to low-level expression from within the fusion segment (R.W. Simons and N.K., unpublished). If β-galactosidase levels are corrected for the 50-unit background activity, the maltose/glucose induction ratio for the *lamB*: : Tn-*trp lac tet* strain is only slightly less than that for a known *lamB-lacZ* gene fusion tested in the same experiment (Table II).

(b) pOX38 plasmids carrying *tet*, *kan*, or *trp lac*

Plasmid pOX38 is a deletion derivative of F which contains no insertion sequences or transposable elements. pOX38 derivatives carrying useful markers have been constructed by insertion of the mini-*tet* element from pNK861 (element 8), of the mini-*kan* element from pNK862 (element 9), and of the mini-*trp lac* element from pNK569 (element 10) (D.E.R., unpublished). The *E. coli* strains carrying these derivatives are NK7379, NK7380 and NK7368, respectively. The host genotype for NK7379 and NK7380 is wild-type W3110; the host genotype for NK7368 is *Δlac-pro*XIII StrR *recA*56 *thy*$^-$ (?) su$^-$ λ^R. All three plasmids are conjugation-proficient. The positions and orientations of the transposon insertions are not known.

TABLE II

Behavior of *lamB*: : Tn-*trp lac tet* insertions

Insertions were isolated in NK5449 (= *Δlac-proX*III NalR *arg*$^-$). Assays for β-galactosidase were performed according to Miller (1972) using minimal A medium supplemented with 0.4% maltose or 0.4% glucose. The bacterial strain carrying the *lamB-lacZ* gene fusion was constructed by and obtained from H. Shuman (unpublished). The strain is MC4100 (= *ΔlacU*169 *araD*139 *strA* B1$^-$) containing gene fusion BZR50/5'.50/42-1.

Exp.	Insertion	Presumed orientation	β-galactosidase activity	
			maltose	glucose
1	*lamB*15::Tn-*trp-lac-tet*	I	1203	96
	*lamB*4::Tn-*trp-lac-tet*	II	53	44
	*lamB*17::Tn-*trp-lac-tet*	II	55	42
2	*lamB*15::Tn-*lac-tet*	I	1309	119
	lamB-lacZ gene fusion	n.a.	273	3

n.a., not applicable.

Material may be protected by copyright law (Title 17, U.S. Code)

(c) Procedural details

(1) Methods for positive selection of tetracycline-sensitive derivatives of TetR strains are described by Bochner et al. (1980) and Maloy and Nunn (1981).

(2) Many strategies can be used to isolate transposon insertions. The choice of strategy depends upon the nature of the target replicon and the available genetic tools. Table III provides a brief summary of the possible combinations of donor vehicles and target replicons with some comments as to feasibility of these experiments, primarily in *E. coli*, with the vehicles described here.

(3) Insertions into conjugative plasmids are generally obtained by a 'mating-out' procedure described in detail by Foster et al., 1981.

(4) Insertions into almost any type of molecule, except λ and other phages, can be obtained using non-replicating, non-integrating, non-killing λ: : Tn vehicles and a 'λ hop' procedure. Most of the vehicles carry amber mutations in the DNA replication genes, and hence are non-replicating only on non-suppressing hosts. However, for wild-type Tn*10* (element 1), a phage vehicle carrying a UGA nonsense mutation in gene *O* is available for making insertions into strains carrying amber or ochre suppressors.

Our standard λ hop procedure is as follows: (a) grow cells to 3×10^8 per ml in λym broth (10 g Bactotryptone + 2.5 g NaCl + 2 g maltose + 1 g yeast extract per liter) or, for slow-growing strains, any rich broth containing 0.2% maltose; (b) concentrate cells ten-fold by centrifugation and resuspension in fresh λym broth; (c) add phage at an moi of 0.3 per cell or as low as feasible; moi's of 10 or 20 may sometimes work; (d) incubate at room temperature for 30 min to allow phage adsorption; (e) incubate for another 90 min at 37°C to allow for transposition and expression of drug-resistance genes; (f) spread directly on λ plates containing 1.25 mM sodium pyrophosphate, to inhibit growth of replication-proficient phages, and the appropriate antibiotic: tetracycline at 15 μg/ml, kanamycin at 50 μg/ml or chloramphenicol at 40 μg/ml. Selection of *ampR* transpositions from a λ infection requires very high levels of antibiotic, preferable carbenicillin, and optimal conditions are not known to us. Selective plates should be incubated at 39°C–42°C for all λcI857 phage vehicles, although 37°C may sometimes work, and can by incubated at any temperature for λ561 and λ370 which contain *c*I: : Tn*10* insertions.

(5) Transpositions of elements from λ into transformable plasmids can be obtained by carrying out a 'λ-hop' experiment (section c4 above) in a strain carrying the desired plasmid, pooling groups of the resulting drug-resistant colonies, isolating plasmid DNA, re-transforming a new non-plasmid-containing strain and selecting for a marker on the transposon and/or a mutation in the plasmid. The DNA isolated in such an experiment contains intact, transformable λ: : Tn DNA along with the desired plasmid DNA. The contaminating λ molecules can result in lysis of the transformed culture, and/or continued

TABLE III

Approaches to Tn*10* transposon mutagenesis in *E. coli*

Transposon vehicle	Target for transposon insertion			
	Bacterial chromosome	Conjugative plasmid	Multicopy plasmid	Virus
Infecting λ	+ + +	+ + +	+ + +	+[a]
Chromosomal insertion or λ: : Tn lysogen	−/+	+ + +	+ + +	+ + +
Conjugative plasmid	−/+	+[c]	+ + +	+ + +
Non-conjugative plasmid	−/+	+ + +	+[b]	+ + +

+ + +, Good approach; +, will work, but with some complications; −/+, requires special vehicle or procedure to select against the donor molecule.

[a] Requires simultaneous infection by two viruses.

[b] Selection of transposition products by transformation complicated by background from simultaneous transformation of both parental plasmids.

[c] The two plasmids must be compatible.

Material may be protected by copyright law (Title 17, U.S. Code)

contamination of plasmid DNA made from the transformants by λ phage. These problems are eliminated if the transformation recipient is λ^R (does not adsorb λ). The strain we use for this purpose is NK7147 = *Δhis su°gal*1, 2⁻ *lac*⁻ *strA*594 r⁻ m⁺ λ^R.

(6) A direct genetic screen for λ*tetR* transducing phages has recently been developed and exploited by Maurer et al. (1984). Also, for transpositions of the *tetR* and *kanR* elements from pNK861 and pNK862 into a turbid-plaque-forming phage, transposition occur at frequencies high enough for λ: : Tn phages to be identified by replica plating: lysates grown on a strain harboring the plasmid are plated and the resulting plaques replicated onto antibiotic-containing plates.

(7) In many but not all strain backgrounds, and depending upon the plasmid copy number and possibly other plasmid properties, multicopy plasmids carrying the Tn*10 tetR* determinant do not confer resistance to high levels of tetracycline (Coleman and Foster, 1981; Moyed and Bertrand, 1983; Chopra et al., 1981). The higher the plasmid copy number, the more severe the problem. However, in a favorable strain background, even very high copy number plasmids confer resistance to 2–5 μg tetracycline per ml. Even in unfavorable situations, TetR transformants can be identified by selecting first for some other plasmid marker, replica-plating or other screening of individual colonies for resistance to very low tetracycline levels, and then preparing DNA from cells on the non-*tet*-selective plates. One particularly permissive strain background is MM294 (Backman et al., 1976).

(8) Lysogens of λ: : Tn phages can be used in three ways: (a) as a source of a self-driven transposon; (b) as a source of a function-defective element which can then be complemented by functions from a plasmid or an IS*10* in the chromosome (see below); or (c) as a source of complementing functions to drive transposition of function-defective elements located on a plasmid or in the chromosome.

Most of the phage vehicles in Fig. 1 can be used directly as λ-hop vehicles (above) because they are defective for replication, repression (at 40°C), and integration. However, these same phages can also be made into integrated prophages using a wild-type λ helper phage. To obtain prophages of the lysogeny-defective vehicles, the desired host strain is grown to 2×10^8 per ml in λ broth as for transposition experiments above and co-infected with a mixture of λ112 and the λ: : Tn phage at multiplicities of 10–15 and 2–5, respectively. The mixture is incubated for 30 min at room temperature and 90 min at 37°C and then diluted and plated on media selective for the appropriate transposon marker. The resulting colonies can be grown up directly and used without further purification to isolate transposon insertions into conjugative plasmids by 'mating-out' procedures or into non-conjugative plasmids by transformation methods.

Two of the phages in Fig. 1, λ1048 and λ1046, are integration- and repression-proficient. Such phages are intended to be used in the prophage state and cannot, in their present form, be used to make insertions by phage infection ('λ hops').

(9) Elements 11 and 12 will only transpose when provided with complementing functions. When these elements are present as λ: : Tn prophages, functions can be provided from pNK474 (element 13), from a Tn*10* or IS*10* (wild type or HH104) insertion elsewhere in the genome, or from λ1046 present as a second prophage genome. The Tn*10* insertion can of course be easily generated in any strain by transposition from the appropriate λ: : Tn phage. We also have available a bacterial strain which is particularly useful for generating stable *kanR* insertions into pBR-derived plasmids or others that are incompatible with pNK474. None of the other vehicles in Fig. 1 is suitable for this purpose. This strain, NK7133, containing insertions of IS*10*--HH104 and Tn*10 del*16 *del*17 *kanR* (element 12) at two different positions in its genome. NK7133 is *Δlac-pro*XIII *arg*⁻, nalR, rifR, su⁻, *thi*⁻, *recA*56, zzz::Tn*10 del*16 *del*17 *kanR*, zzz::IS*10*-HH104-[F′ *lacI*^{Q}*lacPO*L8 *pro*⁺].

REFERENCES

Alton, N.K. and Vapnek, D.: Nucleotide sequence analysis of the chloramphenicol resistance transposon Tn*9*. Nature 282: (1979) 864–869.

Amann, E., Brosius, J. and Ptashne, M.: Vectors bearing a hybrid *trp-lac* promoter useful for regulated expression of cloned genes in *Escherichia coli*. Gene 25 (1983) 167–178.

Auerswald, E.A., Ludwig, G. and Schaller, H.: Structural analysis of Tn*5*. Cold Spring Harbor Symp. Quant. Biol. 45 (1981) 107–113.

Material may be protected by copyright law (Title 17, U.S. Code)

Anderson, P. and Roth, J.R.: Spontaneous tandem genetic duplications in *Salmonella typhimurium* arise by unequal recombination between rRNA (*rrn*) cistrons. Proc. Natl. Acad. Sci. USA 78 (1981) 3113–3117.

Backman, K., Ptashne, M. and Gilbert, W.: Construction of plasmids carrying the cI gene of bacteriophage λ. Proc. Natl. Acad. Sci. USA 73 (1976) 4174–4178.

Baker, T.A., Howe, M.M. and Gross, C.A.: MudX, a derivative of Mud1 (*lac*Ap^R) which makes stable *lacZ* fusions at high temperature. J. Bacteriol. 156 (1983) 970–974.

Beck, E., Ludwig, G., Auerswald, E.A., Reiss, B. and Schaller, H.: Nucleotide sequence and exact location of the neomycin phototransferase gene from transposon Tn*5*. Gene 19 (1982) 327–336.

Bertrand, K.P., Postle, K., Wray, L.V. and Reznikoff, W.S.: Overlapping divergent control expression of Tn*10* tetracycline resistance. Gene 23 (1981) 149–156.

Bochner, B.R., Huang, H-C., Schieven, G.L. and Ames, B.: Positive selection for loss of tetracycline resistance. J. Bacteriol. 143 (1980) 926–933.

Casadaban, M.J. and Cohen, S.N.: Lactose genes fused to exogenous promoters in one step using a Mu-lac bacteriophage: In vivo probe for transcriptional control sequences. Proc. Natl. Acad. Sci. USA 76 (1979) 4530–4533.

Ciampi, M.S., Schmid, M.B. and Roth, J.R.: Transposon Tn*10* provides a promoter for transcription of adjacent sequences. Proc. Natl. Acad. Sci. USA 79 (1982) 5016–5020.

Chopra, I., Shales, S.W., Ward, J.M. and Wallace, L.J.: Reduced expression of Tn*10*-mediated tetracycline-resistance in *Escherichia coli* containing more than one copy of the transposon. J. Gen. Microbiol. 126 (1981) 45–54.

Chumley, F.G.: Rearrangement of the bacterial chromosome by use of transposon genetic homology, in Schlessinger, D. (Ed.), Microbiology—1981. American Society of Microbiology, Washington, DC, 1981, pp. 117–120.

Coleman, D.C. and Foster, T.J.: Analysis of the reduction in expression of tetracycline resistance determined by transposon Tn*10* in the multicopy state. Mol. Gen. Genet. 182 (1981) 171–177.

Daniels, D.L., Schroeder, J.L., Szybalski, W., Sanger, F., Coulson, A.R., Hong, G.F., Hill, D.F., Petersen, G.B. and Blattner, F.R.: Complete annotated lambda sequence, in Hendrix, R.W., Roberts, J.W., Stahl, F.W. and Weisberg, R.A. (Eds.), Lambda II. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1983, pp. 519–676.

Foster, T. Davis, M.A., Robert, D.E., Takeshita, K. and Kleckner, N.: Genetic organization of transposon Tn*10*. Cell 23 (1981) 201–213.

Guyer, M.S., Reed, R.R., Steitz, J.A. and Low, K.B.: Identification of a sex-factor-affinity site in *E. coli* as gamma-delta. Cold Spring Harbor Symp. Quant. Biol. 45 (1981) 135–140.

Halling, S.M. and Kleckner, N.: A symmetrical six-base pair target site sequence determines Tn*10* insertion specificity. Cell 28 (1982) 155–163.

Hillen, W. and Schollmeier, K.: Nucleotide sequence of Tn*10* encoded tetracycline-resistance gene. Nucl. Acids Res. 11 (1983) 525–539.

Hofnung, M.: Divergent operons and the genetic structure of the maltose B region in *Escherichia coli* K12. Genetics 76 (1974) 169–184.

Jorgensen, R.A. and Reznikoff, W.S.: Organization of structural and regulatory genes that mediate tetracycline resistance in transposon Tn*10*. J. Bacteriol. 138 (1979) 705–714.

Jorgensen, R.A., Rothstein, S.J. and Reznikoff, W.S.: A restriction enzyme cleavage map of Tn*5* and location of a region encoding neomycin resistance. Mol. Gen. Genet. 177 (1979a) 65–72.

Jorgensen, R.A., Berg, D.E., Allet, B. and Reznikoff, W.S.: Restriction enzyme cleavage map of Tn*10*, a transposon which encodes tetracycline resistance. J. Bacteriol. 137 (1979b) 681–685.

Kleckner, N., Chan, R.K., Tye, B-K. and Botstein, D.: Mutagenesis by insertion of a drug-resistance element carrying an inverted repetition. J. Mol. Biol. 97 (1975) 561–575.

Kleckner, N., Roth, J. and Botstein, D.: Genetic engineering in vivo using translocatable drug-resistance elements—new methods in bacterial genetics. J. Mol. Biol. 116 (1977) 125–159.

Kleckner, N., Barker, D.F., Ross, D.G. and Botstein, D.: Properties of the translocatable tetracycline-resistance element Tn*10* in *Escherichia coli* and bacteriophage lambda. Genetics 90 (1978a) 427–450.

Kleckner, N., Swan, J.A. and Zabeau, M.: Restriction enzyme analysis of Tn*10* insertions in the immunity region of bacteriophage lambda. Genetics 90 (1978b) 450–461.

Kleckner, N., Reichardt, K. and Botstein, D.: Inversions and deletions of the *Salmonella* chromosome generated by the translocatable tetracycline element Tn*10*. J. Mol. Biol. 127 (1979a) 89–115.

Kleckner, N., Steele, D., Reichardt, K. and Botstein, D.: Specificity of insertion by the translocatable tetracycline-resistance element Tn*10*. Genetics 92 (1979b) 1023–1040.

Kleckner, N.: DNA sequence analysis of Tn*10* insertions: origin and role of 9-bp flanking repetitions during Tn*10* translocation. Cell 16 (1979) 711–720.

Lam, S. and Roth, J.R.: Genetic mapping of IS200 copies in *Salmonella typhimurium* LT2. Genetics 105 (1983) 801–811.

Maloy, S.R. and Nunn, W.D.: Selection for loss of tetracycline resistance by *Escherichia coli*. J. Bacteriol. 145 (1981) 1110–1112.

Maurer, R., Meyer, B.J. and Ptashne, M.: General regulation at the right operator (o_R) of bacteriophage λ, I. o_R3 and autogenous negative control by repressor. J. Mol. Biol. 139 (1980) 147–161.

Maurer, R., Osmond, B.C., Shekhtman, E., Wong, A. and Botstein, D.: Functional interchangeability of DNA replication genes in *Salmonella typhimurium* and *Escherichia coli* demonstrated by a general complementation procedure. Genetics 108 (1984) 1–23.

Miller, J.H.: Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 1972.

Ming, Y., Munson, L.M. and Reznikoff, W.S.: Molecular cloning and sequence analysis of *trp-lac* fusion deletions. J. Mol. Biol. 172 (1984) 355–362.

Mitchell, D.H., Reznikoff, W.S. and Beckwith, J.R.: Genetic fusions defining *trp* and *lac* operon regulatory elements. J.

Material may be protected by copyright law (Title 17, U.S. Code)

Mol. Biol. 93 (1975) 331–350.

Morisato, D., Way, J.C., Kim, H-J. and Kleckner, N.: Tn*10* transposase acts preferentially on nearby transposon ends in vivo. Cell 32 (1983) 799–807.

Moyed, H.S. and Bertrand, K.P.: Mutations in multicopy Tn*10* *tet* plasmids that confer resistance to inhibitory effects of inducers of *tet* gene expression. J. Bacteriol. 155 (1983) 557–564.

Ohtsubo, H. and Ohtsubo, E.: Nucleotide sequence of an insertion element, IS*1*. Proc. Natl. Acad. Sci. USA 75 (1978) 615–619.

Oka, A., Sugisaki, H. and Takanami, M.: Nucleotide sequence of the kanamycin resistance transposon Tn*903*. J. Mol. Biol. 147 (1981) 217–226.

Rothstein, S.J., Jorgensen, R.A., Postle, K. and Reznikoff, W.S.: The inverted repeats of Tn*5* are functionally different. Cell 19 (1980) 795–805.

Roth, J.R.: New genetic techniques using transposable drug resistance elements, in Schlessinger, D. (Ed.), Microbiology—1981. American Society of Microbiology, Washington, DC, 1981, pp. 402–404.

Ross, D., Swan, J. and Kleckner, N.: Physical structures of Tn*10*-promoted deletions and inversions: role of 1400 base pair inverted repetitions. Cell 16 (1979) 721–731.

Sauer, R.T.: DNA sequence of the bacteriophage λ *c*I gene. Nature 276 (1978) 301–302.

Schmid, M.B. and Roth, J.R.: Genetic methods for analysis and manipulation of inversion mutations in bacteria. Genetics 105 (1983) 517–537.

Schollmeier, K. and Hillen, W.: Transposon Tn*10* contains two structural genes with opposite polarity between *tetA* and $IS10_R$. J. Bacteriol. 160 (1984) 499–503.

Simons, R.W., Hoopes, B.C., McClure, W.R. and Kleckner, N: Three promoters near the termini of IS*10*: pIN, pOUT and pIII. Cell 34 (1983) 673-682.

Simons, R.W. and Kleckner, N.: Translational control of IS*10* Transposition. Cell 34 (1983) 683–691.

Sutcliffe, J.G.: Complete nucleotide sequence of the *E. coli* plasmid pBR322. Cold Spring Harbor Symp. Quant. Biol. 43 (1979) 77–90.

Way, J.C. and Kleckner, N.: Essential sites at the termini of Tn*10*. Proc. Natl. Acad. Sci. USA 81 (1984) 3452–3456.

Wray, L.V., Jorgensen, R.A. and Reznikoff, W.S.: Identification of the tetracycline resistance promoter and repressor in transposon Tn*10*. J. Bacteriol. 147 (1981) 297–304.

Communicated by A.M. Campbell.

Material may be protected by copyright law (Title 17, U.S. Code)