Transposable Element Tools for Microbial Genetics

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INTRODUCTION

Transposable elements are discrete DNA segments that can repeatedly insert into a few or many sites in a host genome (56). Transposition occurs without need for extensive DNA sequence homology or the *rec* gene functions required in classical homologous recombination. Transposable elements have proven to be superb tools for molecular genetics: (i) as insertion mutagens, resulting in stable loss-of-function mutations that are easy to map and manipulate; (ii) as sources of selectable markers that facilitate mapping, localized mutagenesis, in vitro cloning, and construction of recombinant strains; (iii) as agents for generating replicon fusions, deletions, and inversions; (iv) as portable restriction sites for in vitro DNA manipulation, gene mapping, and large-scale genome mapping; (v) as portable regions of homology for generating replicon fusions, deletions, duplications, and inversions by homologous recombination; (vi) as vectors for moving virtually any gene or sequence to fixed or random sites in the genome; (vii) as carriers of reporter genes for analyzing the control of gene expression, detecting genes that are expressed only under particular circumstances, protein localization, and localizing domains in membrane-spanning proteins; (viii) as carriers of transcriptional terminators for analyses of operon organization and transcriptional and translational regulation; (ix) as carriers of regulated promoters for generating conditional mutations; (x) for in vivo cloning; and (xi) as mobile primer binding sites for sequencing cloned DNAs.

Many uses of transposable elements were first developed and exploited in laboratory strains of *Escherichia coli* and *Salmonella typhimurium* (official designation, *Salmonella enterica* serovar Typhimurium), but the approaches and also the elements are well suited for use in clinical or other natural isolates and in many other bacterial species (17–19, 45a, 69, 176, 176a, 194a, 198). This chapter reviews uses of some transposable elements (Table 1) that are valuable in *E. coli* and *S. typhimurium* and aspects of their transposition behavior that contribute to their utility as tools. Chapter 124 provides a general review of transposable elements, with emphasis on biochemical mechanisms. Additional information can be found elsewhere (32, 199, 254).

Transposable elements are diverse in size, structure, specificity of insertion, mechanism of transposition, and regulation of movement and may have several phylogenetic origins. The simplest elements, designated insertion sequence (IS) elements, generally contain only a transposase gene flanked by sites on which transposase and host factors act in moving the element. Most IS elements are less than 2 kb in size. *E. coli* K-12 contains at least eight distinct IS elements, none of which are related to the one IS element found in *S. typhimurium* LT2, although some are present in other *S. typhimurium* strains and in many clinical and environmental isolates of *E. coli* (38, 103; see chapter 111).

More complex transposable elements, designated transposons, contain genes encoding auxiliary traits such as antibiotic resistance, heavy metal resistance, or virulence determinants, in addition to genes and sites needed for transposition. Many transposons, such as Tn*5*, Tn*9*, and Tn*10*, are composites, consisting of direct or inverted repeats of an IS element bracketing the auxiliary gene(s), while others, such as Tn*3* and γδ (Tn*1000*), do not contain functional IS elements.

Temperate integrating bacteriophages such as Mu and lambda (λ) are more complex than typical transposons in containing genes for DNA replication, phage development, and cell lysis, as well as the genes and sites needed for transposition (integration). Although mechanistic considerations now lead many to consider λ distinct from "true" transposable elements (see chapter 124), we include it for historical and

operational reasons: transposable elements are defined as "specific DNA segments that can repeatedly insert into a few or many sites in the genome" (56). Furthermore, the ability of λ to insert into many different sites made it very important for in vivo gene cloning before in vitro recombinant DNA methods were available.

Despite the variety of structures, the minimum requirements for transposition are quite simple: the sites of action of transposition proteins (in many cases inverted repeats of only 18 to 38 bp at each end of the element), the cognate element-encoded and element-specific transposases, and one or more specific host factors. The simplicity of these requirements has permitted the engineering of many special-purpose derivatives of naturally occurring transposable elements. The most useful elements for molecular genetics in *E. coli* and *S. typhimurium* include transposons Tn*3* (Amp^r) and the closely related γδ element (no resistance), Tn*5* (Kan^r), Tn*10* (Tet^r), and phages Mu and λ (no resistance).

TABLE 1 Structure and properties of commonly used wild-type transposons[a]

Transposon (IS element)	Marker(s)[b]	Size (kb)	Comments (reference[s])[c]
Tn*3* family			Duplication, 5 bp. Inverted repeat, 35–38 bp. No IS element. Transpose predominantly replicatively, generating cointegrates after intermolecular transposition (Fig. 1B) (intramolecular transposition generates an inversion or deletion in one step). Cointegrates are resolved by an element-encoded resolvase acting on the *res* site or by host recombination enzymes. Transposase and resolvase act in *trans*, so the *tnpA* and *tnpR* genes can be in other vectors. Transposition is frequent into plasmids but rare into the chromosome. Transposition is preferentially into sites that are more A+T-rich than the surrounding DNA, but there is no obvious consensus sequence (112, 130, 253). Insert more randomly than other transposons in most plasmid DNA targets, usually without hot spots, even in target DNA of heterogeneous or high G+C-rich base composition (24, 25, 277). However, occasional hot spots (292) and cold regions, which contain no or very few insertions (178, 245, 304), are found.
Tn*3*/Tn*A*	amp	5	Inverted repeat, 38 bp. Tn*3*-related elements found in many naturally occurring Amp^r plasmids. Cannot be used with most pBR322-related vectors due to transposition immunity caused by an end of Tn*3* left in during plasmid engineering. Essentially identical to Tn*1*, Tn*2*, and other Amp^r transposons (collectively, Tn*A*). Tn*3* and γδ resolvases are interchangeable (253).
γδ/Tn*1000*	Unknown	6	Inverted repeat, 35 bp. A component of the *E. coli* F factor; responsible for most F-mediated chromosome and plasmid transfer (Fig. 1B) (18, 25, 119, 120, 291, 304).
Tn*1721*	*tet*	11.1	Inverted repeat, 38 bp. Member of Tn*21* subgroup of Tn*3* family. Transposase is specific for this group. Tn*1721* is a compound element with three functional ends. Its smaller component, which uses the internal end, is fully functional but does not contain the *tet* gene. Transposes at high frequency to plasmids (6, 112).
Tn*5* (IS*50*)	*kan, ble, str*	5.7 (IS*50*, 1.5)	Duplication, 9 (rarely 8 or 10 bp; see reference 212). Inverted repeat of IS*50*, 8 of 9 bp, but about 19 bp needed for transposition. IS*50* elements inverted and both can move, but only IS*50*R encodes transposase. Transposase acts efficiently only in *cis.* Transposition is regulated by an inhibitor protein produced from a second transcriptional start and an internal in-frame translational start in the *tnp* gene and also by transposase instability. Transposes efficiently to the chromosome and plasmids. Inserts into dozens of sites in most genes, although there are some hot spots. G·C pairs are usually present at each end of the duplication in hot spots but less often in non-hot spots. Otherwise, there is no consensus target sequence. Transposase mutants that are *trans* acting (80) or hypertransposing (234, 305) have been reported. Tn*5* and IS*50* transposition more frequent in Dam^- than in Dam^+ cells because of increased transposase synthesis and increased activity of the I end (29, 78, 171, 233).

Tn*7*	*tmp, str/spc*	14	Duplication, 5 bp. Inverted repeat of more than 30 bp at each end needed for transposition. Transposes to a single site in the *E. coli* chromosome with very high frequency and to secondary sites with lower frequencies. Useful for introducing genes to a single chromosomal site (72).
Tn*9* (IS*1*)	*cam*	2.6 (IS*1*, 0.8)	Duplication, 9 (rarely 8) bp. Inverted repeat of IS*1*, 18 of 23 bp, but about 28 bp needed for transposition. Both IS elements in the same orientation and functional. Transposase acts efficiently only in *cis*. Transposase is encoded by two overlapping ORFs in different phase. Transposition is controlled by ribosome slippage frequency and by binding of product of first ORF (*insA*) to its promoter in one end of IS*1* (62a, 243). Transposes with high specificity, probably equivalent to or higher than that of Tn*10* (55, 196, 314). RecA-mediated crossovers between the directly repeated IS*1* elements remove *cam* and one IS element from Tn*9* insertions. IS*1* elements in the *E. coli* K-12 chromosome (103) lessen its utility for genetic analysis.
Tn*10* (IS*10*)	*tet*	9.3 (IS*10*, 1.3)	Duplication, 9 bp. Inverted repeat of IS*10*, 23 bp, but about 70 bp needed for transposition. IS*10* elements inverted; only IS*10*R encodes transposase, but both can move. Transposase acts efficiently only in *cis* but will act in *trans* when hyperproduced. Transposition is regulated by a *trans*-acting RNA inhibitor of *tnp* gene translation. Tends to insert into just one or a few sites per typical gene, preferentially at sites that match a weak consensus sequence (GCTNAGC). The choice of hot spots is also influenced by the flanking DNA. Tn*10* and IS*10* transposition more frequent in Dam^- than in Dam^+ cells because of increased transposase synthesis and increased activity of the I end. Mutant transposase alleles that increase the efficiency and decrease the specificity of Tn*10* insertion have been isolated and used in some engineered Tn*10* elements (14, 15, 157, 158, 187a).
Phage Mu	imm^{Mu}	37.5	Duplication, 5 bp. Transposes with high frequency: conservatively to the chromosome after infection and replicatively during lytic growth. Wild-type Mu not recovered in multicopy plasmids, but mini-Mu derivatives insert into plasmids and the chromosome. Insertion is quite random in many regions but sometimes exhibits pronounced regional or site specificity (61). Inserts preferentially into sites near the control regions of repressed genes (300). The 5-bp target consensus sequence is N, T/C, G/C, G/A, N (113, 201, 202, 216, 282).

[a]None of the elements described here are present in *S. typhimurium.* Multiple copies of IS*1*, the IS element of Tn*9*, are present in *E. coli* K-12. γδ is present in the *E. coli* K-12 F factor and in some K-12 sublines, including DH5a, DH10B, and MG1043. γδ is absent from others, including AB1157, C600, CBK884, HB101, JM109, MG1655, RR1, and W3110 (18, 120; F. LaBanca and C. M. Berg, unpublished data).
[b]Resistance determinants: *amp*, ampicillin; *ble*, bleomycin; *cam*, chloramphenicol; *kan*, kanamycin, neomycin, or G148 (in eukaryotes); *str/spc*, streptomycin and spectinomycin; *str*, streptomycin; *tet*, tetracycline; *tmp*, trimethoprim.
[c]Duplication refers to the short direct duplication of target-site DNA flanking the insertion that is generated upon transposition. The inverted repeats refer to the short inverted sequences at the termini of IS elements and transposons that are essential for transposition and constitute sites at which transposase and host factors act. ORFs, open reading frames.

SOME USEFUL HISTORY

Phage Mu, the first bacterial transposable element to be recognized, is a temperate phage that attracted attention because it occasionally generated mutations during lysogenization (285, 289). Mu was correctly interpreted as a prokaryotic analog of the eukaryotic transposable controlling elements that McClintock had discovered nearly 20 years earlier (194). The first use of Mu as a genetic tool was in the study of genes needed for lysine and diaminopimelic acid synthesis (51).

In the next dozen years other classes of transposable elements were discovered and their value as tools for genetic analysis was also immediately evident. IS elements were found a few years after Mu to be the cause of novel spontaneous mutations that were often associated with loss of function of adjacent genes but that mapped genetically as point mutations, that could revert spontaneously, and that were not suppressible or stimulated to revert by mutagens (147, 250). The initial characterizations of IS elements included

recombining chromosomal *gal* mutant alleles into λ*gal*$^+$ transducing phage and determining the DNA content of the resultant phage particles. Many *gal* mutant phage exhibited higher-than-normal buoyant densities in CsCl equilibrium gradients, indicating that they contained additional DNA. Electron microscope heteroduplex studies of several such phages demonstrated two different types of insertions (called IS*1* and IS*2*) in *gal* DNA (94, 138, 269). Thus, although it was clear that IS elements could cause mutations, IS elements could not yet be easily harnessed for molecular genetics because they were not marked genetically and no method for selecting their movement was known.

A few years later, phage λ was found to insert into many secondary chromosomal sites in strains that lacked the primary λ attachment site (255, 256). Aberrant excision of λ prophages at these sites yielded specialized transducing phages that carried chromosomal genes from one or the other side of the prophage (244, 255). This allowed the in vivo cloning of many different segments of the *E. coli* chromosome in λ phage vectors before in vitro cloning methods became widely available.

Several transposable elements carrying antibiotic resistance genes ("transposons") were recognized almost simultaneously in the mid 1970s as natural components of R-factor (antibiotic resistance) plasmids by their ability to insert into other DNA molecules (11, 31, 98, 108, 129, 131, 159, 167) (Table 1). For example, the Kan^r Tn*5* element was discovered during an attempt to exploit λ integration and aberrant excision, described above, to clone resistance genes from R-factor plasmids. However, the segment containing *kan* mapped as a simple insertion at different sites in different isolates, indicating that the λ-*kan* phage had arisen by translocation, not by the expected λ integration adjacent to *kan* and aberrant excision (31).

The resistance genes in transposons made it easy to select for their transposition (e.g., from λ or P22 phage vectors to the *E. coli* or *S. typhimurium* chromosome), to isolate insertion mutations, and to begin analyzing transposition mechanisms. Interest in the resistance transposons was also high because, along with selection pressure caused by antibiotic usage in medicine and agriculture, their existence helped explain the disturbing spread of antibiotic resistance in bacteria.

Recombinant DNA methods became widely available a few years after the discovery of resistance transposons, and their resistance genes were frequently incorporated into plasmid cloning vectors (e.g., *amp* from Tn*3, kan* from Tn*5* or Tn*903,* and *cam* from Tn*9*). The synergism between transposon-based in vivo and recombinant DNA-based in vitro approaches continues to this day in the numerous sophisticated special-purpose derivatives of several transposons (Tables 1 and 2), in the development of suicide approaches and vector systems for delivering transposons to target DNAs (Table 3), in the use of their selectable genes in a variety of applications, and in the development of transposon-based systems for in vivo and in vitro cloning (Table 2 and below).

TABLE 2 Useful engineered transposons[a,b]

Transposon	Comments
A. With reporter genes for transcriptional (operon, type I) fusions[c]	
MudI1 (*amp; lac* reporter)	Tnp$^+$. Defective for plaque formation but can be complemented by helper phage. 37 kb. The first in vitro-engineered transposable element (60). Many smaller Mu derivatives with other special features have been constructed (see below and references 19 and 113).
λ*plac*Mu50 series (*imm*$^{\lambda}$ or *imm*^{21}; *kan*[in some members];*lac* reporter	Tnp$^+$. Hybrid phage contain the Mu ends and Mu transposase genes and the λ genes and sites for replication and packaging. These phage insert with the near randomness of Mu but are maintained as lysogens or grow lytically as λ phage. These phage have been used primarily for isolating operon fusions and specialized transducing phage that contain regions next to sites of insertion (in vivo cloning) (47). Also derivatives with ′*lacZ* reporter genes designed for generating protein fusions (see B, below).
mTn3Cm (*cam; lac* reporter)	Tnp$^-$. Complemented by *tnpA* gene in *trans. cam* is expressed in both *Neisseria gonorrhoeae* and *E. coli.* Contains P1-specific recombination site, *loxP,* instead of *res;* resolves in P1 *cre*$^+$ recipient strain. Delivered from transposon-free derivative of the F factor, pOX38. 6.1 kb (46).
Tn*1737* series (*cam, kan,* or *str/spc; lacZ* reporter)	Tnp+. Derivatives of Tn*1721,* a distant member of the Tn*3* family, with distinct transposase and resolvase genes. 9.8–11.1 kb. Delivered from a temperature-sensitive conjugative RP4 derivative, a pBR322 derivative, or a single-copy F′ plasmid (293).
Tn*5lac* (*kan; lac* reporter)	Tnp$^+$. Developed for delivery to *M. xanthus* from a P1 vector because *M. xanthus* does not support P1 growth. Can also be used in *E. coli* by transposition from λ or P1 phage vectors (173).

Transposon	Comments
Mini-Tn*5*-UT series (*kan; lacZ, xylE*, or *luxAB* reporter)	Tnp$^-$. Complemented by *tnpA* gene in *cis*. This vector also carries the replication origin of R6K and the transfer origin of RP4. It is transfer proficient but replication defective because it carries the *pir* mutation. It must be maintained in an *E. coli* strain that carries the R6K *pir*$^+$ gene in a λ prophage and serves as a suicide transposon donor when mated into recipient strains that carry the *pir* mutation. Also derivatives for forming protein fusions (see B, below). 3–5 kb (84, 85)
Mini-Tn*5* (*kan; lacZ-tet* bicistronic reporter)	Tnp$^-$. Complemented by *tnpA* gene in *cis*. Member of the mini-Tn*5*-UT series containing a *lacZ-tet* bicistronic reporter, which is used to identify genes transcribed only in stationary phase. Old Kan^r colonies resulting from transposition are screened for β-galactosidase production. Positive colonies are streaked on tetracycline medium and screened for tetracycline sensitivity. About 8 kb (82). Also derivatives with *′lacZ* reporter genes designed for generating protein fusions (see B, below).
Mini-Tn*10* no. 111 (*kan; lacZ* reporter)	Tnp$^-$. Complemented by *tnpA* gene in *cis*, downstream of P*tac* promoter in donor plasmid. 4.8 kb (158). Larger Tnp$^+$ derivatives (301).
Tn*5-lux* (*tet* or *kan; lux* reporter)	Different constructs are Tnp$^+$ or Tnp$^-$. *lux* reporter genes from *Vibrio* spp. (84, 121, 307).
Tn*4431* (*tet; lux* reporter)	Tnp$^+$. Delivered on a narrow-host-range suicide vector or a temperature-sensitive F derivative. 15 kb (252, 271).
Tn*5*-VB32 (*tet; kan* reporter)	Tnp$^+$. Used to study gene expression in *Caulobacter crescentus* (13).
Tn*1736* series (*tet* or *kan; cam* reporter)	Tnp$^+$. Derivatives of Tn*1721*. Similar to Tn*1737* series (above). 6.8–8.4 kb (293).

B. With reporter genes for translational (protein, type II) fusions[c]

MudII301 (*amp; ¢lac*)	Tnp$^+$. Defective for plaque formation but can be complemented by helper phage. 35.6 kb (59). Many additional Mu derivatives with other special features have been constructed (19, 113).
λ*plac*Mu1 (imm$^\lambda$; *′lac*)	Tnp$^+$. Hybrid phage that transposes like Mu but grows lytically and packages like λ (48, 49). Also derivatives with *lacZ* reporter genes designed for operon fusions (see A, above).
mTnLac (*amp; ¢lac*)	Tnp$^-$. Derivative of Tn*3*. Complemented by *tnpA* gene in *trans*. Resolution via engineered phage P1 *loxP* sites rather than *res* sites. Delivered from F-factor derivative that lacks other IS elements. 4.6 kb. Also derivatives with genes selectable in *Saccharomyces cerevisiae* (248).
Mini-*Tn10′lacZ* no. 112 (*kan; ¢lacZ)*	Tnp$^-$. Complemented by *tnpA* gene in *cis*. Present in plasmid or λ suicide vector. 4.9 kb (158).
Tn*phoA* (*kan; ϕphoA*)	Tnp$^+$. Tn*5* derivative used to detect genes for proteins that are membrane bound, periplasmic, or secreted. Can be used to map domains of transmembrane proteins [see Protein Fusions (Type II)]. 7.7 kb (190, 191). Delivered from a λ phage suicide vector, the plasmid suicide vector, pJM703.1, or a Tet^r IncP conjugal plasmid vector pRT291, which is cured by incompatibility (150, 190, 265).
Tn*phoA-lac* (*kan* or *tet; ′lacZ*)	Tnp$^+$ and Tnp$^-$. Tn*5lacZ* elements with homology at ends to ends of Tn*phoA*. Used for exchange of *phoA* and *lacZ* genes by homologous recombination, to identify secreted versus cytoplasmic proteins, and to assess domain structure of membrane-bound proteins. 5–8 kb. Carried on a λ suicide vector. Some are Tnp$^+$ (for new transpositions) and others are Tnp$^-$ (for recombination with preexisting element) (189, 306).
Mini-Tn*5phoA* series (*kan; ′lacZ* or *′phoA*)	Tnp$^-$. Complemented by *tnpA* gene in *cis*. Members of mini-Tn*5*-UT series (see A, above). 3 kb (84, 85).
Mud*phoA* (*kan, ′phoA*)	Tnp$^+$. For mutagenesis of *Legionella pneumophila* (5).
mTn*3CmPhoA* (*cam; ′phoA*)	Tnp$^-$. Complemented by *tnpA* gene in *trans*. *cam* is expressed in both *Neisseria* and *E. coli*. Derived from mini-Tn*3*Cm(above) (46b).
Tn*5*ORF*lac* (*tet; ′lacZ*)	Tnp$^+$. Carried in a pBR322-related plasmid. Used in papillation assay to find mutations affecting Tn*5* transposition (168) and to examine mechanism of Tn*5*-mediated deletion formation (146).
Mini-Tn*10′kan no. 114* (*erm; ′kan*)	Tnp$^-$. *tnp*$^+$ is present in *cis* downstream of P*tac* promoter in donor plasmid. 2.2 kb (158).
Mini-Tn*5* (*kan; ′lacZ-tet*)	A member of the mini-Tn*5*-UT series in which the *′lacZ-tet* bicistronic reporter operon is used to identify genes transcribed only in stationary phase (see A, above) About 8 kb (82).
TnMax (*cam* or *erm; ′phoA;* fd *ori*)	Tnp$^-$. Complemented by *tnpA* gene in *cis*. Tn*1721* derivative. Phage fd vegetative replication origin in transposon. No origin in vector; a λ prophage in the host chromosome. fd replication genes required for survival. Several versions (122).

C. Containing outward-facing promoters

Tn*5tac1* (*kan;* P*tac*)	Tnp+ Outward-facing P*tac* (synthetic *lac* promoter). For IPTG-regulated turn-on/turnoff transcription of adjacent genes ; for generating conditional mutations (see As Mobile Promoter Elements). Delivered from Pbr322 or λ suicide vector. 4.6 kb (66, 209).
Mini-Tn*5-trc* (*kan;* P*trc*)	Tnp$^-$. Complemented by *tnpA* gene in *cis* in the pUT suicide vector plasmid. P*trc* (synthetic *lac*) promoter for regulated transcription of adjacent genes, as with Tn*5tac1*. 2.5 kb. Also contains restriction sites between P*trc* and the transposon end for cloning genes downstream of P*trc;* one variant contains a *lacZ* gene downstream of P*trc* for detecting putative transcription terminators in segments cloned between the promoter and *lac* (83, 85).
Tn*5*seq1 (*kan;* PT7 and PSP6)	Tnp$^+$. Outward-facing T7 and SP6 phage promoters. 3.2 kb. Originally for transposon-based DNA sequencing; promoters can also be used for in vitro or in vivo transcription of adjacent genes (208).
Mini-Mu-*tac* (*kan;* P*tac*)	Tnp$^+$. Outward-facing *tac* promoter as in Tn*5tac1*. 7 kb (109).

Transposon	Comments
Mini-Tn*10*-108 (*kan;* P*lac*)	Tnp$^-$. Complemented by *tnpA* gene in *cis*. Present in plasmid or λ suicide vector. Transcription across ends of element may be constitutive, even in cells containing *lac* repressor, since upstream *kan* gene is transcribed in the same direction as P*lac*. 1.9 kb (158).
Tn*1735* series (*cam, kan,* or *str;* P*tac*)	Tnp$^+$. Derivatives of Tn*1721*. Similar to Tn*1737* series (above). 8.4–9.8 kb (293).
Tn*5virB* (*kan;* P*tac*)	Tnp$^+$. Outward-facing P*virB*. For acetosyringone-regulated turn-on of transcription of adjacent genes. Derived from Tn*5tac1* and designed for generating nonpolar insertions in *vir* genes of *Agrobacterium tumefaciens* but could have wider applications. Delivered from λ suicide vector. 5 kb (74).
Tn*5*-P-out (*tet, neo,* or *gen; npt* or *tac*)	Tnp$^-$. Complemented by *tnpA* gene in *cis*. Delivered from mobilizable suicide vector for mutagenesis of non-*E. coli* strains (262).
D. For large-scale genome mapping	
Tn*5oriT* (*kan; oriT*)	Tnp$^+$. Contains transfer origin of RK2. Insertion into chromosomes and plasmids yields Hfr-like and F-prime-like derivatives, respectively, in presence of helper RK2. Delivered from pBR322 in *polA* strain that cannot support pBR322 replication. 6.5 kb (311).
Tn*5*-mob (*kan; oriT*)	Tnp$^+$. Equivalent to Tn*5oriT* but with transfer origin of RP4. Used to mutagenize gram-negative bacteria (259). Derivatives with different resistance markers (262).
Tn5pfm1 (*kan, cam;* restriction sites)[d]	Tnp$^-$. Complemented by *tnpA* gene in *cis*. Contains sites for infrequently cutting restriction endonucleases (*Swa*I, M.*Xba*I-*Dpn*I, *Pac*I, *Not*I). Delivered on an R6K-derived conjugal suicide plasmid. Used for physical mapping by PFGE (see For Genome Mapping) (309).
Tn*10*dCamMCS (*cam;* restriction sites)[d]	Tnp$^-$. Complemented by *tnpA* gene in *cis* downstream of P*tac* promoter. Delivered on a pBR322-derived plasmid. High transposase levels result in multiple insertions. Insertions that cause auxotrophy are separated from the donor plasmid and other chromosome insertions by P1 transduction. Contains sites for infrequently cutting restriction endonucleases (*Not*I, *Nhe*I, *Dra*I, *Xba*I, *Sfi*I). Used for physical mapping by PFGE (see For Genome Mapping) (128, 218).
Tn10*d*RCP (*cam, erm, kan,* or *tet;* rare cutting polylinker) series[d]	Tnp$^-$. Complemented by altered-target-specificity *tnpA* allele (*ats-1*) in *cis*. Contains sites for infrequently cutting restriction endonucleases, including *Avr*II, *Eag*I, *Mlu*I, *Nhe*I, *Not*I, *Pac*I, *Sfi*I, *Spe*I, and *Xba*I. Carried on a 2.9-kb *Bacillus* plasmid that cannot replicate in gram-negative bacteria. Delivered to *E. coli* by electroporation or transformation. Can be used for physical mapping by PFGE (see Genome Mapping) (40a, 187a, 238a). New versions in this series contain additional sites for I-*Ceu*I and I-*Sce*I endonucleases (Mahillon et al., personal communication).
MiniTn*10* (*aac1, cam,* or *kan;* pBluescript polylinker) series[d]	Tnp$^-$. Complemented by altered-target-specificity *tnpA* allele (*ats1, ats2*) in *cis*. Contains sites for infrequently cutting restriction endonucleases, including *Eag*I, *Mlu*I, *Not*I, *Xba*I. The R6K-based vector is conjugation proficient, but replication deficient in strains lacking the R6K *pir* gene (5a).
mTnCmNS (*cam;* restriction sites)	Tnp$^-$. Derivative of Tn*3*. Complemented by *tnpA* gene in *trans*. Contains sites for infrequently cutting restriction endonucleases (*Nhe*I, *Spe*I). Derived from mTnCm (above). Used for physical mapping of *Neisseria gonorrhoeae* by PFGE (see For Genome Mapping) (46a).
E. For in vivo cloning	
λp*lac*Mu series	Hybrid phage that transpose like Mu but grow lytically and package like λ, as described in A, above. Aberrant excision yields specialized transducing phage analogous to λ*gal* and λ*bio* (see A and B, above) (47–49).
MudII4042 series (*cam; ′lac; ori*)	Tnp$^+$. Used for in vivo cloning of DNA segments between pairs of insertion sites (see Vectors for In Vivo Cloning). MudII4042 contains the multicopy replication origin of plasmid p15 and *′lacZ* reporter gene for generating protein fusions. 16.7 kb (116). Many smaller mini-Mu derivatives with different replication origins and resistance markers and with or without *lac* reporter genes or outward-facing promoters have been constructed (113, 115, 117). Also useful for generalized transduction and localized mutagenesis of fragments smaller than those carried by P1 (295).
F. For DNA sequencing[e]	
Tn*5*seq1 (*kan;* PT7 and PSP6)	Tnp$^+$. Outward-facing T7 and SP6 phage promoters. Designed for transposon-based DNA sequencing. 3.2 kb (208).
Tn*5supF* (*supF*)	Tnp$^-$. Complemented by *tnpA* gene in *cis*. For insertion mutagenesis and sequencing of λ phage clones, in which selection for drug resistance is not feasible and which often are too large to be packaged after insertion of larger transposons. *supF* suppressor tRNA gene can be selected during λ lytic growth (antibiotic resistance genes cannot be). Delivered from mini-F-factor vector that contains *tnp* gene. 264 bp (219). Variants differing in subterminal sequences used as primer binding sites are ~300 bp (149). Insertions in *E. coli* DNA in λ clones can be recombined into the chromosome. They are obtained by infecting cells carrying plasmid p3 [*kan, tet*(Am), *amp*(Am)] and selecting for suppression (Tet^r, Amp^r) (220).
Mini-Tn*10supF* (*supF*)	Tnp$^-$. Complemented by *tnpA* gene in *cis*. Contains terminal inverted repeats of 70 bp. ~400 bp (158). Analogous to Tn*5supF*.
Mini-γδ-1 (*kan; res*)	Tnp–. Complemented by *tnpA* gene in *trans*. Derivative of γδ (Tn*1000*). For insertion mutagenesis and sequencing of DNAs cloned in nonconjugative plasmids such as pUC19. Resolvase (*tnpR*) provided in *trans* from a compatible plasmid or from the chromosome of strains carrying γδ. Several variants with multiplex primer binding sites. 1.8–1.9 kb. Delivered from F-factor plasmid derivative that lacks other IS elements (pOX38).

Transposon	Comments
	Useful for marker exchange. Unlike Tn*3*, can be used with standard cloning vectors that contain a Tn*3* end (24, 25).
Mini-γδ-plex series (no selectable marker; *loxP*)	Tnp$^-$. Complemented by *tnpA* gene in *trans*. Derivative of γδ. Each element contains the *lac* universal and reverse primer sites and a pair of multiplex "tag" sites for mapping and sequencing. Delivered from F-factor derivative that lacks other IS elements. 260 bp. Use P1 *loxP* site for cointegrate resolution (Weiss and Gesteland, personal communication).
m-Tn*3* FO α (*amp;* f1 *ori; lacZ*α)	Tnp$^-$. Complemented by *tnpA* gene in *trans*. When inserted in correct orientation in f1 *ori*-containing plasmidand cell is infected with helper phage, single-stranded nested deletion products are packaged, yielding single-stranded templates for one-stranded sequencing from the transposon end. mTnUFO has yeast *URA3* gene but no *lacZ*α (76).
AT2 (*tmp*)	Yeast retrovirus-like transposon Ty1-derived artificial transposon for in vitro insertion mutagenesis and sequencing of cloned DNAs. In vitro transposition requires only a specific 4-bp sequence at each end of a linear transposon (prepared by *Xmn*I digestion of transposon-carrying plasmid DNA) and integrase-containing virus-like particles prepared from yeast. *tmp* (dhfr) gene is from Tn*7*. 864 bp (87).
G. Transposon-based vectors for in vitro cloning and in vivo nested-deletion formation[f]	
pAA3.7X (*amp; tet; galKT*) (Tn*9*, *cam*)	Tnp$^+$. Deletions arise by IS*1* or Tn*9* (to give Cam^s) intramolecular transposition. Only deletions that extend in one direction are viable; the reciprocal products lack a replication origin and a selectable gene. Counterselectable *galKT* genes (Table 3) are between intact Tn*9* (initial versions) or defective Tn*9* (later versions) and cloning site (*tet*). Plasmid, cosmid, or phagemid vector derivatives (2, 3, 217). Not widely used because Tn*9* transposition is very nonrandom and only one DNA strand can be accessed in a given clone.
pDUAL, pDelta (*tet; kan; sacB; rpsL;* γδ [*amp* in some versions]) (Fig. 4)	Tnp$^-$. Complemented by *tnpA* gene in *trans*. Deletion factory cosmid cloning vectors for selecting sets of nested deletions that extend either clockwise or counterclockwise from a γδ end. Deletions arise by γδ intramolecular transposition and can be recovered in both clockwise and counterclockwise directions because the plasmid replication origin is between transposon ends and is retained in each deletion derivative. One counterselectable gene (*sacB* or *rpsL*) (Table 3) is between each γδ end and the cloning site. One selectable gene (*tet* or *kan*) is between each counterselectable gene and the cloning site to select against deletions that extend beyond the cloned fragment (see Fig. 4 and Transposon-Based Vectors for In Vitro Cloning). This structure allows for deletions that extend into the cloned fragment in either direction to be selected. Versions with different replication origins and different restriction sites. 8–10 kb (296, 297; C. M. Berg et al., unpublished data).
pMM251 (Tn*3*, *amp*)	Tnp$^-$. Complemented by *tnpA* gene in *trans*. Deletions arise by Tn*3* intramolecular transposition. Only deletions that extend in one direction are viable; the reciprocal products lack a replication origin. Counterselectable p_L-*kil* gene is between Tn*3* and the cloning site (281).
H. Other useful elements	
Tn*5*-"R" series (*amp, cam, gen, tet, tmp,* or *str*)	Tnp$^+$. Delivered on a temperature-sensitive replication mutant of a broad-host-range conjugative plasmid(R388), which makes them useful for mutagenizing diverse bacterial species. 3.9–7.5 kb (242).
Mini-Tn*5res* series (*kan; res; xylE*)	Tnp$^-$. Complemented by *tnpA* gene in *cis*. Contains direct repeats of 140-bp "multimer resolution sites" (*res*) from plasmid RP4. *res* is used for site-specific recombination bracketing selectable (Kan^r) and visual (XylE$^+$) markers. Even transient expression of the resolvase gene from a nonreplicating suicide vector introduced by conjugation results in excision of the selectable marker segment by recombination between these sites. This permits multistep strain constructions involving the programmed deletion of segments between pairs of transposon insertions and the maintenance of mutations created by transposon insertion, with loss of the original selectable marker. Some versions contain *lac* or *lux* reporter genes (172).
Tn*5-rpsL* (*kan, rpsL*)	Tnp$^+$. Counterselectable *rpsL* gene (Table 3) permits plasmid curing and selection of deletions in strains with chromosomal insertion. 7.7 kb (276). A derivative containing the R6K replication origin has been used for in vivo cloning (see above) (275).
Mini-Tn*5*-UT series (*cam, kan, str/spc, tet, ars, bar,* or *mer*)	Tnp$^-$. Complemented by *tnpA* gene in *cis*. Numerous derivatives in R6K transfer-proficient, replication-defective suicide vector (see A and B, above, and Table 3). Nonantibiotic resistance markers are safe for release in genetically engineered microorganisms. Contain a rare-cutting restriction endonuclease (*Not*I) cleavage site (81, 84, 85, 135).
Mini-Tn*5*-tag (*kan*) series	Derivatives of mini-Tn*5*Km2, a member of the mini-Tn*5*-UT series (above) that contain different 20-bp tags for multiplex screening (134b).
Mini-Tn*10*-LOF series (*cam, kan, str/spc, tet, ars, bar,* or *mer*)	Tnp$^-$. Complemented by *tnpA* gene in *cis*. Derivatives in R6K transfer-proficient, replication-defective suicide vector (Table 3). Marked with antibiotic or nonantibiotic resistance and a rare-cutting restriction endonuclease (*Not*I) cleavage site for recloning (84, 85, 135).
Tn*10* Tek (*kan*); Tn*10* Tes (*str*)	*kan* and *str* are placed at different sites in the Tn*10 tet* gene in these transposons. Genome rearrangements (inversions and deletions) can be obtained by recombination between Tn*10* Tek and Tn*10* Tes elements at different locations and selected as Tet^r. Used to probe functional organization of the genome. 11–12 kb (100).
Tn*5*-24 (*amp,* T4 gene 24)	Tnp$^+$. For insertion mutagenesis of T4 phage with mutation in T4 gene 24. Insertions selected by complementation (310).
Mini-Tn*10*-cm, RSVneo (*cam*)	Tnp$^-$. Complemented by *tnpA* gene in *cis*. Designed for insertion into P1 clones and selection in transgenic animals where RSVneo encodes G418 resistance (273).
Mini-Tn*10*-cm, RSVneo (*cam; loxP*)	Tnp$^-$. Complemented by *tnpA* gene in *cis*. Designed for insertion into P1 clones and the generation of deletions by site-specific recombination between the *loxP* site in the transposon and the *loxP* site in the P1

Transposon	Comments
	vector (273).
Tn*5*-V (*kan; ori* pSC101)	Tnp$^+$. Used to mutagenize *M. xanthus*, in which pSC101 does not replicate. Insertions are cloned directly by restriction digestion of genomic DNA, self-ligation, and transformation of *E. coli* (102).
Tn*5*-PV (*kan; ori* RK2; T-DNA borders)	Tnp$^+$. Contains the origins of replication and of transfer from the broad-host-range plasmid RK2 (but not the replication genes) and the T-DNA borders from *Agrobacterium* Ti plasmid. Designed for shuttling between *E. coli* and *A. tumefaciens* and the introduction of plasmids containing a Tn*5*-PV insertion into plant genomes (165).
Omegon-Km (*kan; ori* pBR322)	Tnp$^-$. Complemented by *tnpA* gene in *cis*. IS*1* based. Carried on pJF350, a narrow-host-range conjugative plasmid (93).
Tn*5*MAP (*kan; cos;* I-SceI)	Tnp$^+$. Provides a unique (18-bp I-*Sce*I recognition site) cutting site for linearizing plasmid DNA and generating a nonpalindromic 4-bp overlap. Designed for generating plasmid restriction maps by partial digestion with other enzymes and probing each restriction digest ladder. Carried on ColE1 (137). Tn*5cos*, which lacks the I-*Sce*I site, is linearized using λ terminase (315).
MupAp1 (*amp*)	Tnp$^+$ and proficient for lytic growth without helper Mu phage. The first in vivo-engineered Mu phage (by Tn*3* insertion and spontaneous deletion). 37.5 kb (175).
Mud-P22 P, Mud-P22 Q (*cam*)	Phage P22 containing ends of Mu in place of P22 *att* site and *int* gene. Inserts into the *S. typhimurium* chromosome with near randomness of Mu when complemented with transposase gene. P22 induction leads to in situ replication and then unidirectional packaging of 2–3 min of amplified chromosomal sequences starting from the P22 *pac* site. Preexisting Mu insertions can be converted into hybrid elements that have the *pac* site in either orientation (P and Q) to package from the insertion in either direction. 36.4 kb (312). A set of mapped Mud-P22 insertions (16) has proven to be useful for constructing restriction maps (180, 308).

[a]The parent transposons are described in Table 1, and delivery strategies are described in Table 3. More extensive listings and descriptions of older transposon derivatives are in references 17, 19, 113, 158, and 265.
[b]Selectable genes and other special features are in parentheses. Antibiotic resistance determinants as in Table 1, footnote *b*, and as follows: *erm*, erythromycin. Nonantibiotic resistance determinants: *ars*, arsenite; *bar*, bialaphos; *mer*, mercury; *tmp*, trimethoprim. Reporter genes: *lacZ*, β-galactosidase; *lux*, luciferase; *phoA*, alkaline phosphatase. Counterselectable genes (Table 3): *rpsL* (*strA*), confers streptomycin sensitivity on *rpsL* (Str^r) strain; *sacB*, confers sucrose sensitivity on *E. coli* and many other gram-negative species. Other: imm$^{\lambda}$ and imm^{21}, immunity to phages λ and 21, respectively; *loxP*, phage P1 recombination site, acted on by Cre protein; *ori*, replication origin; *oriT*, transfer origin; P*tac* and P*trc*, engineered *lac* promoters; PT7 and PSP6, promoters from phages T7 and SP6, respectively; *res*, resolution site, acted on by transposon-specific resolvase; *supF*, tRNA suppressor gene; Tnp$^+$, transposase proficient.
[c]Reporter genes lacking promoters are indicated as reporter, while reporter genes that also lack translation start sites are preceeded by a prime.
[d]Wild-type Tn*5* and Tn*10* also contain rare restriction sites that have been exploited in PFGE analysis (180, 193, 308).
[e]Any element can be engineered to provide unique mobile source of primer binding sites for sequencing cloned DNAs (see For Cloning) While naturally occurring elements in the Tn*3* family contain unique DNA sequences internal to their 35 to 38-bp terminal repeats, compound transposons such as Tn*5*, Tn*9*, and Tn*10* do not because they contain two copies of their respective IS elements. Engineered derivatives can have unique sites starting 19 bp (Tn*5*) or 70 bp (Tn*10*) from the outside ends.
[f]Nested deletions formed by intramolecular transposition.

TABLE 3 Genes and strategies potentially useful for transposon delivery[a]

Gene/property	Comments (reference[s])[b]
A. Conditional replication defects in vector	
pSC101^{ts}	Temperature-sensitive replication (41, 123).
F^{ts}	Temperature-sensitive replication. pML31^{ts} contains the replication, but not the transfer, region of the F factor (92). pUS53 is a derivative with the RP4-specific *mob* region (271).
RP4^{ts}	Temperature-sensitive replication. RP4 is a broad-host-range conjugative plasmid (293).
R388^{ts}	Temperature-sensitive replication. R388 is a broad-host-range conjugative plasmid (242).
pWV01^{ts}	Temperature-sensitive replication. pWV01 is a broad-host-range *Lactococcus* plasmid. The mutant is thermosensitive above 35°C in *Lactococcus lactis*, *Bacillus subtilis*, and *E. coli*. Has been used to deliver Tn*10* to *B. subtilis* (187).
R6K *pir*	Essential R6K replication gene of this broad-host-range plasmid placed in host genome (164, 200), converting R6K *pir* into a suicide vector in strains lacking the *pir* gene. Different *pir* alleles allow plasmid replication to levels of between 15 and 250 copies per cell (110, 223). *pir* alleles in the chromosome can be moved to other *E. coli* strains by P1 cotransduction with linked markers (195).
M13mp	Phage that contain amber mutations in gene II cannot replicate in nonsuppressing host (42).
fd *ori*	fd *ori* requires fd gene 2 protein, which is cloned in host strain (122) (see TnMax, Table 2).
P1 *rep*	Essential P1 replication gene cloned on another plasmid (215).
RK2 *rep*	Essential R6K replication gene of this broad-host-range plasmid placed in host genome (165).
B. Mutations in host affecting vector replication	
polA	Replication of ColE1-based plasmids is PolA dependent (118, 154).
rep	M13 cannot replicate in *E. coli rep* mutants. Selection for antibiotic resistance marker of

Gene/property	Comments (reference[s])[b]
	clones that carry *E. coli* DNA enriches for transposition to other replicons. M13-free recombinants selected by deoxycholate resistance (195, 270).
recD	Plasmids pBR322, pACYC184, and pSC101 are unstable when grown in a *recD* strain in minimal medium (36).
dnaA	Replication of pSC101 is *dnaA* dependent (101, 127).
C. Host range for vector replication[c]	
pHT1030	Propagates in *Bacillus subtilis*, not in *E. coli* (187a).
pME290	Propagates in *P. aeruginosa* but not in *E. coli* (89, 143).
ColE1	Propagates in most enteric, but not in most nonenteric, species (95, 246, 261, 274).
p15A	Propagates in *E. coli* but not in *Rhizobium meliloti* (249).
P1	Propagates in *E. coli* but not in *M. xanthus* (174).
D. Plasmid incompatibility	
Incompatibility	Superinfection with a plasmid from the same incompatibility group carrying a different resistance marker is widely used to displace the resident plasmid. For examples, see references 150, 190, 265, and 286.
E. Counterselectable markers	
adh$^+$	Encodes acetaldehyde dehydrogenase, confers sensitivity to 2-chloracetaldehyde (73).
ccdB$^+$	The F-factor *ccdB* gene product is cytotoxic in the absence of the *ccdA* product in Gyr$^+$ strains, but not in *gyrA* strains (35a).
galE$^+$ or *galU*$^+$	*galE* and *galU* strains are Gal^s because they accumulate galactose-1-phosphate, which is toxic. Resistance due to *galK* mutants, which cannot metabolize galactose (see references 198, 199, and 268). Used to select for Tn*9*- or IS*1*-mediated deletions (2, 3).
gpt^{+d}	Encodes guanine-xanthine phosphoribosyltransferase; confers sensitivity to 6-thioguanine in *E. coli* and in mammalian cells (89, 205).
ksgA^{+d}	Encodes an rRNA methylase; confers sensitivity to kasugamycin (132).
lacY^{+d}	Encodes lactose permease; confers sensitivity to tONPG (orthonitrophenyl-β-D-thiogalactoside) (205a).
pheS^{+d}	Encodes a tRNA gene, confers sensitivity to *p*-fluorophenylalanine, a phenylalanine analog, in *E. coli* (133, 149a, 260).
relF$^{+,\ hok+,\ gef+}$	*E. coli* and phage genes that encode a family of small polypeptides. When overproduced, these peptides cause cell death due to collapse of the membrane potential (107, 224). Have been fused to a number of strong regulatable promoters (12, 70, 145, 162, 203).
rpsL (*strA*)$^{+d}$	Encodes a ribosomal subunit gene, confers sensitivity to streptomycin (77, 176, 260, 264, 274, 287). Used to select IS*1*- and γδ-catalyzed deletions (225, 297, 298).
sacB$^+$	*Bacillus* gene that encodes levansucrase, which converts sucrose to levan, causing cell lysis and death in *E. coli* and some other gram-negative and gram-positive bacteria that lack sucrose degradative enzymes. Originally used to "entrap" IS elements (106). Also used in suicide vectors and to isolate Tn*5*- and γδ-mediated nested deletions (54, 140, 144, 148, 226, 236, 246, 260, 288, 297, 298). When sucrose is absent, *sacB* is not harmful to *E. coli* (288, 297, 298) unless overexpressed (221, 273). Extent of sucrose sensitivity conferred by *sacB* is strain and medium dependent (41).
tet (Tn*10*)	Select against *tet*$^+$ (Tet^r) by penicillin enrichment (97), fusaric acid or quinaldic acid resistance (40, 43, 188), or chlortetracycline or 5a,6-anhydrotetracycline resistance (204, 266). Tn*10* *tet*$^+$ confers a lower level of tetracycline resistance when in a multicopy plasmid (not true for the related pBR322 *tet* gene) and can be used to select against plasmids that contain *tet* (21, 195).
thyA (TD)$^+$	Encodes thymidylate synthetase and confers sensitivity to trimethoprim and related compounds. *thyA* mutants are obtained by selection for trimethoprim resistance in the presence of thymine (267) (selection in the absence of thymine yields *folA* mutants [151]). Used to select for γδ-catalyzed deletions (298).
ush$^+$	Encodes UDP-sugar hydrolase and 5′-nucleotidase. Confers sensitivity to 5-fluorouracil plus 5-AMP to *upp ush* mutants (52).
E. coli HB101 *rcsB*$^+$	*rcsB* is involved in colonic acid production. Overproduction of the gene product is lethal to HB101 but not to JM109 or C600 (7).
*E. coli Eco*RI *r*$^{+m-}$	pSCC31 contains the *Eco*R1 endonuclease gene downstream from λ p_L and kills *r*$^-$ *m*$^-$ λ lysogens unless the gene is inactivated (65).
λ *c*I-*tet*	*c*I repression of *tet* expression. Tet^s unless *c*I is inactivated (39, 203a, 238).
λ cI-*kan*	*c*I repression of *kan* expression. Kan^s unless *c*I is inactivated (228).
λ-*kil*	p_L-*kil* gene under the control of λ *c*I857 allele. Lethal at 42°C unless the *kil* gene is inactivated (281).
P22 *mnt-tet*	*mnt* repression of *tet* expression. Tet^s unless *mnt* is inactivated (181, 313).
λ gene *S*	Causes lysis of *E. coli* cells when IPTG is present. λ lysis gene *S* under control of *lac* promoter (227).
ϕX174 gene *E*	gpE induces cell lysis in *E. coli.* Gene *E* under control of *lac* promoter (134, 134a).
DT (diphtheria toxin)	*E. coli* cells expressing DT are killed at pH 5 but not at pH 7 (214).

Gene/property	Comments (reference[s])[b]
F. Miscellaneous strategies	
Mobilization	Selection for transposition of replicatively transposing elements by cointegrate formation between conjugative and nonconjugative plasmids (Fig. 1B). Originally exploited for mutagenesis of cloned genes in pBR322 by wild-type γδ, which is resident in the *E. coli* F factor (119). pOX38, a deletion derivative of the F factor lacking all transposable elements (120), is now often used to deliver engineered derivatives of members of the Tn*3* family (24, 46, 148, 291, 293, 304).
Restriction	P1::Tn*5* from *E. coli* r^- m^- to *S. typhimurium* r^+ m^+ (C. M. Berg, unpublished data).
P1 size	Addition of Tn*5* (or any large transposon) makes the entire transposon-containing P1 genome too large to package in one phage head. Since P1 packages DNA by the headful mechanism, each P1::Tn*5* genome is incomplete, instead of terminally redundant. This makes it unable to circularize on single infection. It is viable at high multiplicity of infection (>2 phage per cell) because a complete genome can be reconstituted by recombination between permuted partial genomes. However, P1 derivatives that can package the entire genome arise by internal deletions of nonessential P1 sequences (173).

[a]Many of these genes or strategies have been used in transposon delivery (Table 2); others have been used to select against a vector or strain in different applications and could be adapted for transposon delivery (see also chapter 139).
[b]Antibiotic resistance determinants as in Tables 1 and 2, footnotes *b*, and as follows: *gen*, gentamicin.
[c]Any narrow-host-range vector can be used as a suicide vector in a nonpermissive host.
[d]Mutant allele, which confers resistance, is recessive to the wild-type sensitive allele.

TRANSPOSITION MECHANISMS AND PRODUCTS IMPORTANT FOR TRANSPOSON USE

The structures of transposition products and the mechanisms of transposition are important in the use of transposons as molecular genetic tools. Early studies of the intermolecular transposition products of several different transposons revealed simple insertions of the element, free of vector DNA. The finding of such simple insertions after Tn*5* and Tn*10* transposition, plus the precedent provided by phage λ integration, led us to propose a conservative (nonreplicative) transposition model. In this model the element is separated from vector DNA by a double-strand break at each end. It is inserted into target DNA without being replicated, and the linearized vector is lost (Fig. 1A) (26, 28). Evidence of an alternative, replicative, transposition mechanism was provided by the finding of cointegrates consisting of vector and target DNAs joined by direct repeats of the element (Fig. 1B) with certain mutant derivatives of Tn*3*. Further studies showed that cointegrates are the primary products of Tn*3* transposition, that they are normally broken down very efficiently by a transposon-encoded, site-specific recombination enzyme called resolvase, or more slowly by RecA-mediated homologous recombination, and that the mutants had lesions in either the resolvase gene or the resolution site (130, 253). Phage Mu was found to generate cointegrates and other rearrangements by transposition during lytic growth, although it does not encode a function corresponding to resolvase (62, 201).

In the replicative transposition model that was proposed to account for the findings with Tn*3* and Mu, single-strand nicks at each element end allow the joining of element and target sequences, followed by copying of the element from the free 3′ ends of target DNA (9, 111, 251; see chapter 124). The impact of the unexpected cointegrate structures and the appeal of a single transposition mechanism led some to assume in the early 1980s that all transposition was replicative (see references 103, 125, 155, 156, and 316). However, the insertion of Mu DNA after infection, like Tn*5* transposition, seemed to be conservative (4, 177), and Tn*9* (IS*1*) was found to make both simple insertions and cointegrates (37, 185). Accordingly, it seemed that the choice between conservative and replicative mechanisms might be based on whether transposition was initiated with double- or single-stranded cuts at each transposable element end to give conservative and replicative tranposition, respectively (30, 37). Widespread acceptance of two modes of transposition, either conservative or replicative, depending on the element or the conditions, began to emerge in the mid-1980s partly because of additional evidence of conservative transposition after Mu infection (124) and new studies indicating that Tn*10* and IS*903*, like Tn*5*, transpose conservatively (160, 302). The assumption by some in the early 1980s that there is a single replicative mechanism should be

kept in mind when evaluating literature from that period.

While intermolecular transposition yields simple insertions or cointegrates (Fig. 1), intramolecular transposition yields deletions and inversions (see below). These rearrangements are rare but can be recovered efficiently by selecting for loss of a gene that is near the transposon or that is between the ends of the two IS elements of composite transposons (2, 3, 97, 158, 231, 288, 297, 298).

Intermolecular Transposition

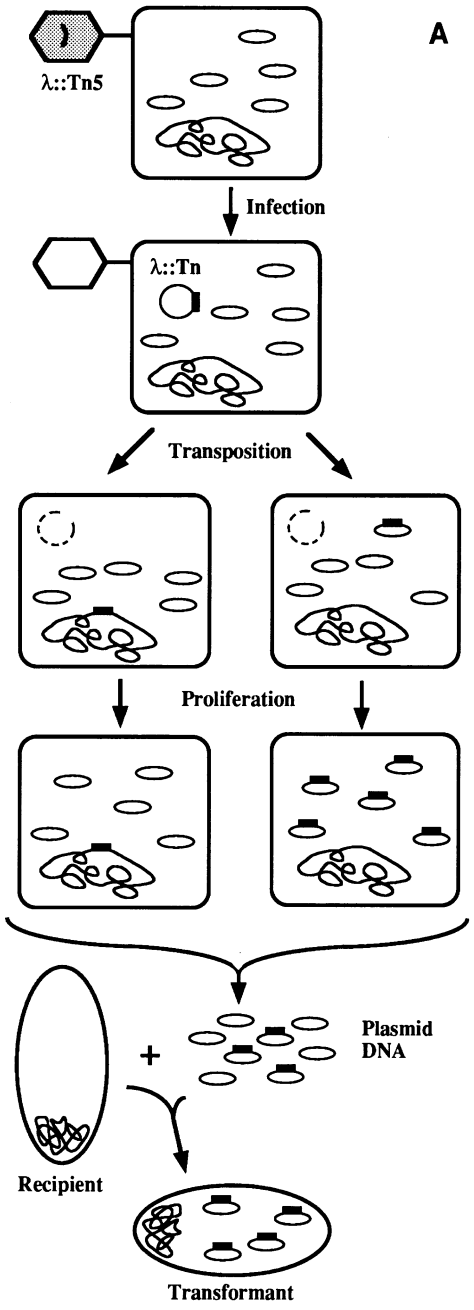
The very first studies of IS elements and transposons showed that transposition is rare, with most such elements moving at frequencies in the range of 10^{-7} to 10^{-4} per cell, regardless of whether the host is *rec*+ or *recA*. Hence, much of the power of transposon-based methods depends on the ability to select transposition products—separating them from the many potential target DNAs that had not sustained transposition and also from the original donor DNAs or cells. Good selectable markers are essential, and antibiotic resistance genes have been used most often. Genes for heavy metal resistance, for biosynthetic or other metabolic enzymes, or for suppressor tRNAs have also been valuable in a number of instances (Tables 1 and 2; see chapter 139 for additional markers). An interesting exception to the general need for selectable markers in the transposon is provided by the strategy for selecting γδ transposition from the conjugative F factor to a nonconjugative plasmid (Fig. 1B).

As described above, the product of conservative intermolecular transposition is a simple insertion (Fig. 1A), while the product of replicative transposition is a cointegrate that can be resolved to yield also a simple insertion product (Fig. 1B). However, two types of conservative transposition yield structures that resemble cointegrates but that should not be mistaken for them: (i) "inverse transposition" (63)(described below) and (ii) "dimer transposition," in which transposition from a dimer that contains two transposon copies (and thus four IS elements for composite transposons such as Tn*5*) yields a product that contains vector sequences, as well as transposon DNA. However, unlike true cointegrates, these transposition products do not contain the entire donor molecule; the size of the donor fragment that is transposed depends upon which pair of IS elements participated in the transposition event (28).

Inverse Transposition (63). Inverse transposition, which involves the inside, rather than the outside, ends of IS elements in composite, conservatively moving transposons such as Tn*5* and Tn*10*, was discovered in studies of integrative suppresson (211) by a Tn*10*- containing R-factor plasmid (63). Most suppression events resulted from insertion of the R factor into the host chromosome, thereby allowing the R factor to take over chromosomal replication. The inside ends of the IS*10* elements mediate inverse transposition so that the DNA segment that moves is the entire R factor, except the Tetr determinant. In such inverse transpositions the central portion of the transposon corresponds to the "vector." Thus, inverse transposition, like replicative transposition, can fuse different replicons, but the products differ from true cointegrates (generated by replicative transposition) in lacking the DNA from the central portion of the transposon and in not having the transposon duplicated (63, 207, 241).

Intramolecular Transposition

Transposition from one site to another in the same DNA molecule (intramolecular transposition) is mechanistically equivalent to intermolecular transposition, but the consequences are quite different and generally less familiar. Intramolecular transposition yields an inversion or a deletion product in a single step, independent of whether the element moves by a conservative or a replicative mechanism. The structures of intramolecular transposition products depend, however, on the number of twists between the donor and target sites and on whether transposition was conservative or replicative. In conservative


A
λ::Tn5
Infection
λ::Tn
Transposition
Proliferation
Plasmid
DNA
Recipient
Transformant

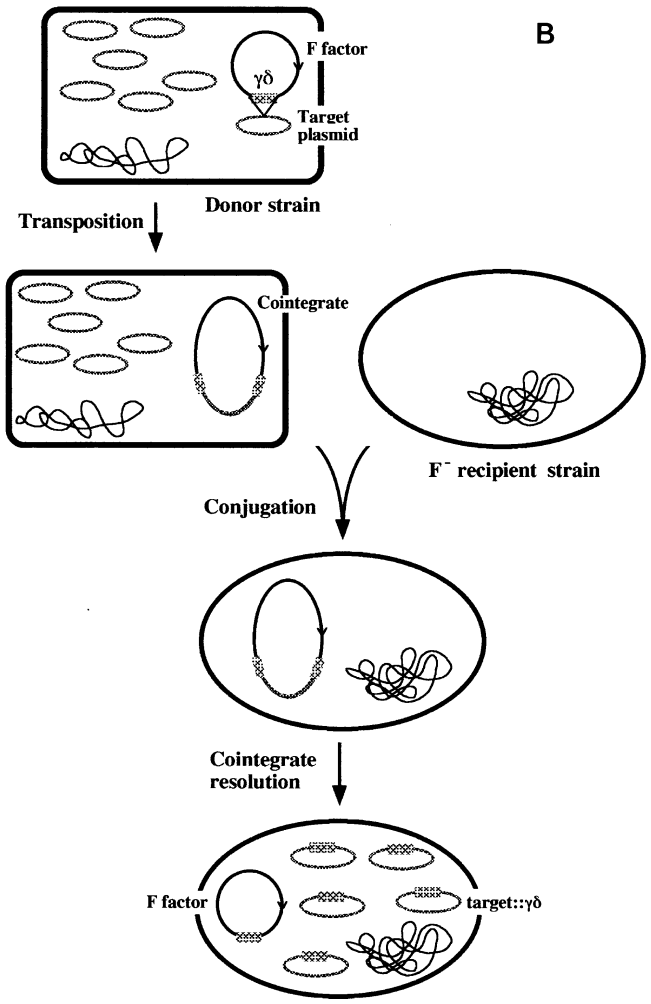


FIGURE 1 Formation of simple insertions by conservative and replicative termolecular transposition. (A) Conservative transposition of Tn5 from λ to the bacterial chromosome on the (left) or to a Plasmid(right).Transposition is obtained by selecting for kan^r colonies after infection with a λ phage that carries Tn5 and lacks the phage attachment site. Plasmids containing insertions are obtained by extracting DNA and transforming a new host. The linearized donor fragment is presumed to be destoryed during transposition (26, 28, 29, 33). (B) Replicative transpositiion of λδ from the F factor to a nonconjugative plasmid. The cointegrate product is resolved in a second recombination step (either site specific or generalized) (251, 253). Cointegrates formed between F and the garget nonconjugative plasmid are selected by conjugal transfer (119). The *E.coli* F factor, contains IS elements as well as λδ but λδ is themost active in this selection. A derivative of the F factor, pOX38, that is deleted for its naturally occurring transposable elements, including λδ (120), is used to deliver engineered λδ derivatives (24, 25). kept in mind when evaluating literature from that period. While intermolecular transposition yields simple insertions or cointegrates (Fig. 1), intramolecular transposition yields deletions and inversions (see below). These rearrangements are rare but can be recovered efficiently by selecting for loss of a gene that is near the transposon or that is between the ends of the two IS elements of composite transposons (2, 3, 97, 158, 231, 288, 297, 298).

intramolecular transposition, which is a form of inverse transposition, the inside ends of the element participate and the DNA between the transposon ends is lost (Fig. 2), while in replicative intramolecular transposition no intermediate corresponding to a cointegrate is formed (Fig. 3) and there is no resolution step.

In both conservative and replicative transposition, the formation of a deletion versus an inversion depends on the relative orientation of donor and target sites. Deletions arise when there is an odd number of twists between the donor and target sites (Fig. 2 [left] and 3 [left]), and inversions arise when there are no or an even number of twists between the sites (Fig. 2 [right] and 3 [right]). The simple expectation that deletions and inversions with endpoints in a target gene should be equally frequent is borne out for replicative γδ transposition (298) but not for conservative Tn*5* transposition (288), where deletions were severalfold more frequent than inversions, with the excess probably occurring by abortive transposition (146, 288).

It should be noted that, regardless of the frequency with which they arise, deletions always far outnumber inversions among recombinant products if loss of gene function is selected. This is because transposition to sites either in or beyond the counterselected gene results in loss of function in deletion derivatives, whereas only transposition to sites in the gene results in loss of function in inversion derivatives (288, 298).

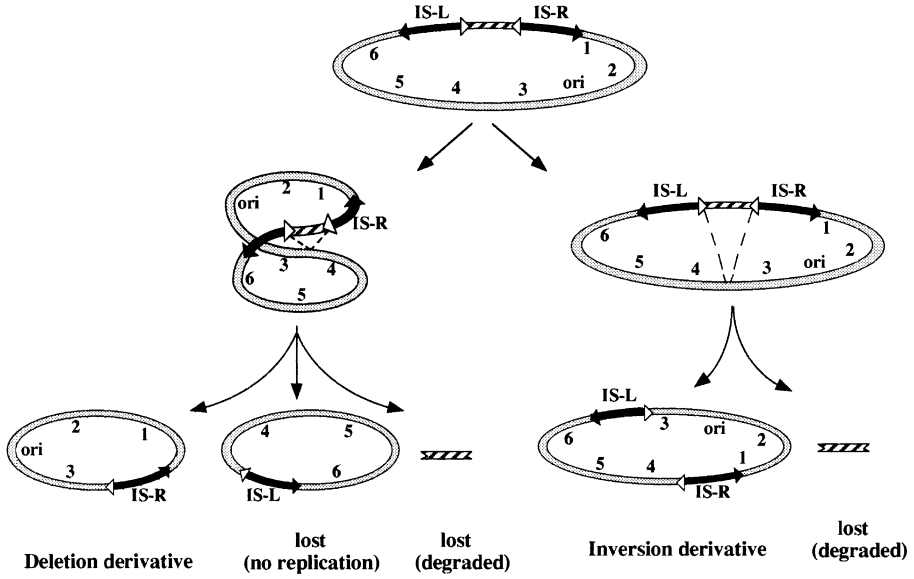


FIGURE 2 Formation of deletion and inversion products by conservative intramolecular transposition. The inner, rather than the outer, IS element ends of these composite elements are involved in this intramolecular inverse transposition. The "vector" (marked by diagonal lines) is not found in transposition products. One of the two expected reciprocal deletion products is generally inviable because it lacks a replication origin. Inversion derivatives are smaller than the parent molecule by the size of the donor fragment (228; C. Chin and C. M. Berg, unpublished data).

Conservative Intramolecular Transposition. If the outer ends of composite transposons such as Tn*5* and Tn*10*, or of their IS components, participate in intramolecular transposition, linear, inviable products are expected. Therefore, findings of additional transposed copies of Tn*5* or Tn*10* or one of their component IS elements in a chromosome that already carries a copy of the element probably reflect transposition from

one chromosome to its sibling chromosome—that is, an intermolecular event (as has been adduced for IS*10*-adjacent deletions [237a]). However, if the inner transposon ends participate in transposition, deletions and inversions result (15, 19, 158, 288). This inside-end intramolecular transposition is equivalent to inverse intermolecular transposition (63) (described above). In both situations the central segment of the transposon serves as the vector and is linearized and lost. It should be noted that the single inversion product retains both IS elements, while each of the two predicted deletion products retains one IS element; in both cases the transposition product(s) is smaller than the parent clone by the size of the interstitial vector fragment (Fig. 2) (19, 288).

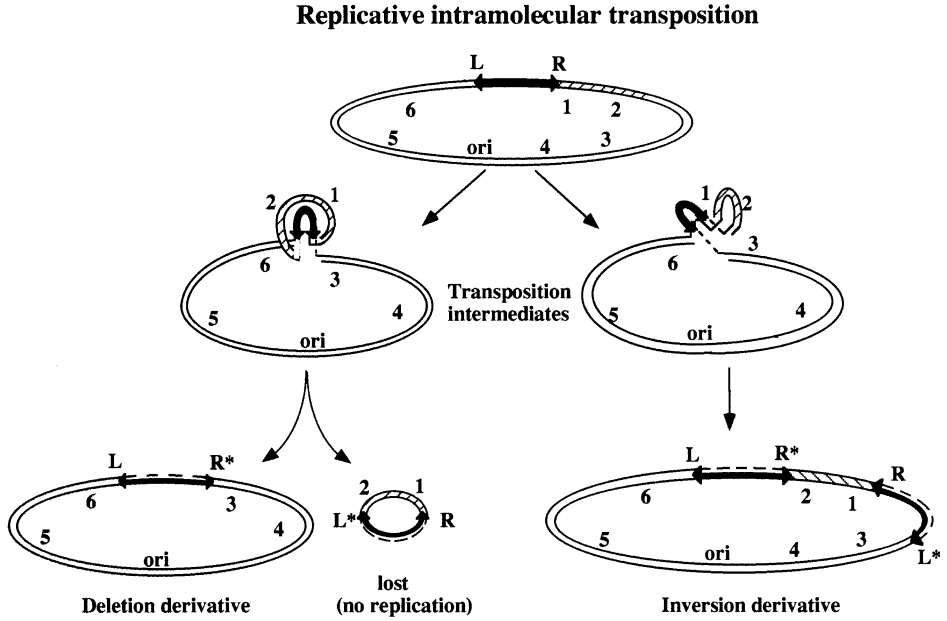


FIGURE 3 Formation of deletion and inversion products by replicative intramolecular transposition. The transposon is duplicated and no DNA is lost during the transposition process, although one of the expected deletion products is generally inviable because it lacks a replication origin. Inversion derivatives are larger than the parent molecule by the size of the transposon (298, 303).

The lucky circumstance that the functional *tet* gene of Tn*10* can be selected against (Table 3) led to the widespread use of Tn*10* to isolate deletions and other rearrangements adjacent to the insertion site (43, 97, 188). Rounds of Tn*10* insertion and selection for loss of Tet^r have been used repeatedly to inactivate a series of genes in the construction of some laboratory strains. In these cases, one or both IS*10* elements are expected to be retained after each conversion of a Tet^r to a Tet^s strain (Fig. 2). The retention of IS*10*, which was not always understood, can cause problems when Rec^+ strains with a history of Tn*10* insertion mutagenesis and Tet^s selection are used. For example, one study of a plasmid that happened to contain a hot spot for IS*10* insertion revealed that the host strain retained three active IS*10* elements, one from a Tn*10*-mediated deletion event and a pair from a Tn*10*-mediated inversion event, each associated with prior Tn*10* mutagenesis and selection for Tet^s (44). In another study, most mutants selected for constitutive expression of the *udp* gene resulted from rearrangements mediated by a previously unrecognized IS*10* element in the host (I. Brikum, A. Mironov, and D. E. Berg, unpublished data). Fortunately, chromosomal IS*10* elements appear to be stable in *recA* strains (214a; J. P. O'Neil, P. J. L. Morris, and G. Bogosian, personal communication), even though Tn*10* (IS*10*) transposition in other assays (e.g., from phage vectors) is RecA independent (157).

Replicative Intramolecular Transposition. In replicative transposition the entire element is duplicated and no DNA is lost. Consequently, inversion products generated by replicative transposition are larger than the parent molecule by the size of the transposon (39, 291, 298, 303). In the deletion mode, two circular products should be produced, each containing a copy of the element, and their net size should also be larger than that of the parent molecule by the size of the transposon (Fig. 3). With the exception of Tn*9,* most elements known to undergo replicative transposition are not compound elements: they contain only one pair of ends capable of undergoing transposition.

The reciprocity of deletion products produced by either replicative or conservative transposition has not yet been examined at the molecular level because one of the two products is usually selected against or lacks a replication origin.

TARGET SPECIFICITY

Transposable elements differ greatly in insertion specificity, with none moving completely randomly. Most move at relatively low frequencies (~10^{-7} to 10^{-4}) and as readily to the chromosome as to plasmids. However, members of the Tn*3* family, including γδ and Tn*1721,* transpose poorly into the chromosome, even though they insert readily and more randomly than most other elements into plasmid DNAs (130, 253). Insertions of wild-type Mu and its large engineered derivatives, in contrast, are generally not recovered in multicopy plasmids. This is probably due to selection against multiple copies of particular Mu genes, not to an inherent feature of the Mu transposition mechanism, because insertions of small mini-Mu elements into plasmids are easily obtained. Tn*7* and phage λ insert preferentially into just one chromosomal site. However, when their primary chromosomal sites are deleted, each of these elements is found to insert at numerous secondary sites, albeit at lower frequencies (72, 255, 256).

Most elements generate short direct duplications of a sequence at the target site that is 5 bp long in the cases of Mu, Tn*3*, γδ, and Tn*7* and that is usually 9 bp long in the cases of Tn*5*, Tn*9*, and Tn*10* (phage λ inserts by site-specific recombination). How insertion sites are chosen is complex and different for each element. Except for Tn*7* and λ, specific target sequences may often be less important than properties of the intracellular DNA milieu, including supercoiling, transcription, and methylation (Table 1) (29, 136, 157, 300).

Tn*3* and γδ transpose most randomly to most plasmid targets, with relatively few hot spots or cold regions. Tn*5* and Mu exhibit intermediate specificity for plasmid or chromosomal targets, with a majority of insertions into random sites, but some hot spots. Tn*9* and Tn*10* transpose nonrandomly, with most insertions into just one or a few hot spots per target gene (Table 1). However, reduced-specificity Tn*10* transposase alleles (14) have been incorporated into several mini-Tn*10* delivery systems (5a, 187a; J. Mahillon, C. K. Rode, and C. A. Bloch, personal communication) (Table 2).

The choice of transposon is affected by the purpose of the project at hand. Almost any transposon can be useful if just one or a few insertions in any given gene is needed. However, when a high density of insertions is needed in certain applications, e.g., to provide primer-binding sites for DNA sequencing, then one of the more randomly transposing elements should be used.

POLARITY AND DISTAL GENE EXPRESSION

Most IS element and transposon insertions in an early gene in an operon prevent transcription of distal genes from the operon promoter because of transcriptional stop signals or polarity caused by long stretches of untranslated mRNA (103). Some insertions appear incompletely polar, with distal genes being expressed constitutively. This expression can be due to the following: (i) outward facing promoters in the transposon and transcription across its end (Tn*3* stimulates distal transcription by read-through from the transposase gene [130], both Tn*5* and Tn*10* stimulate distal transcription due to outward-facing promoters near their

termini [35, 68, 184, 294], and the inside end of IS*50*L [Tn*5*] stimulates distal transcription by read-through from the promoter used for expression of the *kan ble str* resistance operon in Tn*5* [27, 152]); (ii) new promoters formed by insertion (some elements contain potential "–35" sequences close to their ends and can form new promoters when they insert near potential "–10" sequences in target DNA); and (iii) internal promoters in the operon (transposons have been useful for ordering genes in operons and for detecting significant internal promoters [e.g., references 22 and 23] when element-associated promoters are low in activity [35, 68, 294]).

The turn-on of distal gene expression is not always due to transposon promoter activity: new insertions of IS*1* and IS*5* cause most of the mutations allowing expression of the cryptic *bgl* operon in *E. coli* K-12. These elements seem to act at a distance by providing an enhancer function, rather than a new promoter. This may be because of an effect on DNA supercoiling that allows the normally silent promoter to function either directly or by facilitating cyclic AMP receptor protein binding (103, 232). Conversely, clones containing *Helicobacter pylori groES* and *groEL* (chaperone protein) genes were found to acquire an upstream insertion of IS*5* during propagation in *E. coli*, apparently because the IS*5* insert decreased the normal high-level expression of these cloned genes, which was deleterious in *E. coli* (280). A complementary position effect has been found with a cloned *avtA* gene, in which some γδ insertions that knocked out *avtA* expression were downstream of the structural gene. $AvtA^+$ revertants turned out to carry dimeric plasmids, probably reflecting changes in the local supercoiling by dimerization and its effects on gene expression (179; C. M. Berg and L. Liu, unpublished data).

STABILITY OF TRANSPOSON INSERTION MUTATIONS: EXCISION AND REVERSION

Two aspects of transposable element stability are important when using transposons as genetic tools: frequency of loss and the frequency of movement to new sites. Each of these parameters can be tested easily by using insertions in biosynthetic or catabolic genes. The results indicate that chromosomal insertions are quite stable, with revertants generally occurring at frequencies of less than 10^{-8}. In addition, revertants (or transductants to prototrophy) of a strain carrying Tn*5* or Tn*10* in a biosynthetic gene are almost always Kan^s or Tet^s, respectively, and devoid of all transposon sequences. This indicates that the elements rarely move to a second site in the genome (29, 157). The very few cases where Tn*5* or Tn*10* (or one of their respective IS elements) is found at a second genomic site may be due to prior intermolecular transposition between sibling chromosomes (or partially replicated chromosomes) because, as described above, these elements are not replicated during transposition and loss of the vector sequences would be lethal in intramolecular transposition.

Many of the current generation of engineered elements can transpose from their delivery vector with high efficiency but are then crippled for further movement because they depend on a transposase gene cloned in the vector or another plasmid (Table 2). However, many older, well-characterized insertions continue to be used, so secondary movement must still be considered.

Several cases of strains containing IS*50* or IS*10* at new sites have been found in Rec^+ cells (44, 126; I. Brikun, A. Mironov, and D. E. Berg unpublished data). This accumulation of transposed elements is not found in RecA cells (214a; O'Neil et al., personal communication). It may be enhanced by prolonged storage of strains in room temperature stabs and probably on plates on the benchtop as well (8) but is generally not seen with freshly isolated insertion mutations or after transduction of an insertion mutation into a fresh strain. Strains that carry the transposon or component IS element at new sites also generally retain the complete element at its original site.

Most mutations due to insertion of Tn*5* or Tn*10* revert by loss ("excision") of the element. Revertants are generally Kan^s or Tet^s, respectively, and lack transposon sequences. In true revertants the element plus one copy of the 9-bp target sequence that had been duplicated during insertion must be lost in order to restore the ancestral gene sequence. These events depend on the inverted repeat structure but not the transposase functions of the elements and are not correlated with transposition to new sites. Rather, they are

ascribed to mistakes in DNA replication: slippage of the growing DNA strand between the first and second copy of the 9-bp direct repeat, which may be triggered by hairpin formation between complementary sequences of the inverted repeats and then failure to copy the entire element. No actual DNA cleavage need be involved, nor is the excision process related to transposition per se. The following lines of evidence support this interpretation. (i) Insertions early in an operon are usually polar, and revertants selected for distal gene expression are often partial revertants, still mutant in the target gene. For example, in strains with Tn*5*, Tn*10*, or Mu insertions in the *lacZ* gene, selection for revertants in the downstream *lacY* gene yields many partial revertants that are antibiotic sensitive and have lost much, but not all, of the Tn or Mu element but have not regained *lacZ* function (26, 45, 50). Upon selection for $LacZ^+$, some partial revertants give rise to true revertants: in these the initial deletion involved only part of the element (50, 91, 99, 239). Partial revertants that can give rise to true revertants are relatively common for Tn*10* insertions, probably because there is a 24-bp direct repeat near each outside end of Tn*10* that can serve as a substrate for slippage during replication. (ii) The frequency of Tn*5* or Tn*10* excision depends on the insertion site, even within a single gene (91), and is enhanced by certain mutations in DNA repair or recombination genes (182, 183). (iii) The excision of Tn*5* and Tn*10* is more frequent in F^+ than in F^- cells and is strongly stimulated by moving the mutant allele from the chromosome to an F′ plasmid. This excision-enhancing effect of F is exerted primarily in *cis*. It appears to depend on F-factor transfer between cells, probably because F transfer involves single-stranded DNA intermediates (which can form hairpin substrates that promote slippage during replication) (34).

Mutations due to Mu insertion are seen to revert even less frequently than those due to Tn*5* or Tn*10* insertion, whether selected for relief of polarity or for restoration of function of the mutated gene. This probably reflects the absence of long inverted repeats at the Mu termini and also the large size of Mu DNA. In fact, Mu insertion mutations are only seen to revert under conditions of partial induction of special Mu prophages that are themselves mutant in genes needed for full lytic development, and such reversion requires the MuA transposase protein (50). Mu excision may also reflect errors in DNA replication, in which slippage of the nascent DNA strand is triggered by MuA protein binding to Mu ends (206).

Insertions of Mu are also extremely stable if the Mu prophage is completely repressed. However, even partial induction results in cycles of Mu transposition to new sites and concomitant chromosomal rearrangement, reflecting the replicative nature of Mu transposition (Fig. 3). In the case of bacterial strains lysogenic for the widely used Mu*cts*62 phage, which encodes a temperature-sensitive repressor, such partial induction is evident during brief periods at 37°C. Transposition to new sites is not observed, however, with engineered elements that lack a transposase gene (19, 113).

TRANSPOSON DELIVERY SYSTEMS

The strategy used to select for transposition depends on the target strain; on whether the target is the bacterial chromosome, a phage, or a plasmid; and, in some cases, on whether the element transposes conservatively or replicatively. Usually, a suicide vector that cannot survive is used (Table 3). In the last few years transposon-vector systems have been engineered that contain the transposase gene outside of the transposon ends (Table 2), so that the transposition product is stable. For phage and plasmid targets, a suicide vector is not required since transfer of the target replicon to a new host is sufficient to select for transposition. Strategies for transposon delivery include the following.

(i) For chromosomal targets, the transposon is usually delivered on a phage (usually λ for *E. coli* K-12 and P22 for *S. typhimurium* LT2) or plasmid suicide vector. Mu is itself a phage, and its DNA can be packaged into infectious virus particles, or Mu can be transferred on a plasmid. Bacteria containing chromosomal insertions are selected simply by their ability to grow in the presence of the antibiotic under conditions in which the donor vector cannot persist. Tn*5*, Tn*10*, Mu, and their derivatives are most often used for mutagenesis of the *E. coli* and *S. typhimurium* chromosomes. In contrast, Tn*3*, γδ, and related elements seem unable to insert efficiently into chromosomal DNA.

(ii) For phage targets, the transposon moves from a chromosomal or plasmid vector. Transposon-containing phage are recovered by packaging and infection of a new host and are selected after lysogenization or during lytic growth, depending on the marker used.

(iii) For plasmid targets, the transposon can be delivered from various heterologous vectors, as long as the transposition products can be selected. Common strategies include the following: (a) use of a suicide phage or heterologous suicide plasmid vector as the transposon donor and selection for the transposon marker (e.g., Fig. 1A); (b) extraction of plasmid DNA from pools of cells after transposition and transformation or electroporation, selecting for the transposon marker (e.g., Fig. 1A); (c) selection for hyperresistance after transposition from the chromosome to a high-copy-number plasmid; and (d) selection for mobilization of a normally nontransmissible target plasmid by a conjugative transposon-containing donor (e.g., the F factor) to form a cointegrate (e.g., Fig. 1B).

Phage used as suicide vectors usually have defects in integration or replication (26, 31, 159, 161). For example, transposition of Tn*5* (*kan*) into a plasmid or the *E. coli* chromosome is readily obtained by selecting for Kan^r colonies after infection with a λ phage that carries Tn*5* and lacks the phage attachment site (*attP*) (Fig. 1A). Lytic (and potentially lethal) phage replication is blocked by nonsense mutations in phage replication genes and the use of nonsuppressing host strains. Alternatively, replication is blocked by λ repressor synthesized by the infecting phage or by a resident prophage. Most transductants recovered under these conditions contain simple insertions of Tn*5* in the genome and lack λ sequences. Equivalent strategies are used with transposon-containing derivatives of temperate phage P22 in *S. typhimurium.* In addition, λ suicide vectors can be used in *S. typhimurium* strains carrying a cloned constitutive allele of the *E. coli* λ receptor gene *lamB* (88).

A variety of suicide strategies have been useful with plasmid vectors (Table 3), including the following: (i) use of a mutant plasmid unable to replicate at high temperature; (ii) use of a mutant plasmid that lacks an essential *trans*-acting replication gene and an engineered host strain carrying this gene in the chromosome (genes and sites for DNA transfer during conjugation are generally included in such vectors, enabling their high-efficiency transfer to target cells in simple bacterial matings); (iii) use of a donor plasmid with a narrow host range in terms of replication but usually a broader host range in terms of conjugation and DNA transfer; (iv) use of a donor plasmid that replicates in gram-positive but not in gram-negative bacteria, and introduction into *E. coli* by transformation or electroporation; (v) use of a second incompatible plasmid to block replication of the transposon donor; and (vi) inclusion in the vector plasmid of a gene whose product is lethal to the cell under particular selective conditions.

Counterselectable genes have been used in suicide vectors and for a number of other purposes: to select for intramolecular transposition (discussed below), to select for the introduction of mutant alleles from cloned DNA to the bacterial chromosome (called marker exchange, allelic replacement, or gene targeting) (24, 41, 220, 226, 236, 239a, 240), to recover IS elements from the genome by “entrapment” (86, 106, 228, 247, 260), to cripple plasmids in genetically engineered microorganisms that are released into the environment (230), and for in vitro cloning (77, 133). A number of counterselectable genes are described in Table 3 and in chapter 139.

SPECIAL USES OF TRANSPOSONS

Once the utility of naturally occurring transposons for generating simple, polar, easily selected, loss-of-function mutations was established and recombinant DNA methods became available, many variants of these constructs were engineered to facilitate and extend molecular genetic analysis in laboratory, clinical, and environmental strains of *E. coli, S. typhimurium,* and many other bacterial species. Some of these elements and their uses are described here and in Table 2.

As Mobile Reporter Elements

Operon Fusions (Type I). The first of the special engineered transposons, Mud1 (60), was the prototype of a diverse collection of elements that provide sensitive mobile reporters of transcription and, in later constructs, of translation and protein localization (Table 2). The use of these elements has revolutionized the study of genetic regulatory mechanisms and protein-membrane interactions and led to the discovery of many previously unknown genes. Mud1 is a derivative of phage Mu with an inward-facing promoterless *lacZ* gene near one end. Insertion of Mud1 in an operon in the correct orientation results in *lacZ* transcription from the operon promoter and hence β-galactosidase synthesis (this is a "type I" or "operon fusion") (60). The transcriptional regulation of the target genes is monitored by colony color on indicator medium or by a sensitive and easy enzyme assay. In an early implementation of this approach, a number of different genes whose transcription is induced in response to DNA damage were identified by screening a few thousand colonies containing new Mud1 insertions on medium under normal and damage-inducing conditions (96, 153). Some of these genes were previously known (e.g., *uvrA*, *uvrB*, and *uvrC*), but others were new. This approach was extended to identify genes that are turned on by shifting to medium of high osmolarity, shifting cultures to high temperature, etc. It also proved valuable in identifying new genes, identifying new functions for previously known genes, and studying the expression of genes whose products are difficult to assay (258, 265).

Reporter insertions can also be used to select mutations that alter expression of the reporter gene, thereby facilitating studies of gene regulation. The power of the promoter-probe approach has been extended by the construction of many derivatives of Mu, Tn*3*, Tn*5*, Tn*10*, and Tn*917* (for gram-positive bacteria) with a variety of reporter genes. These include *lac; uidA* (*gusA*) (β-glucuronidase), a useful reporter gene in plant and animal cells (104); *lux* (luciferase), which is more sensitive than *lac; cam* (chloramphenicol transacetylase); and *kan* (aminoglycoside phosphotransferase) (see Table 2). *cam* and *kan* encode antibiotic resistances and allow direct selection, rather than just visual screening, for expression.

Protein Fusions (Type II). Transposons with reporter genes that are missing the start sites for translation, as well as transcription initiation, are valuable for many purposes. To obtain mutants that express a hybrid fusion protein, the transposon must be in the correct orientation and reading frame (one-sixth of the insertions in a structural gene). The hybrid protein must also be in the proper milieu for expression: β-galactosidase (*lacZ*) is a cytoplasmic protein, while alkaline phosphatase (*phoA*) is active only if it has traversed the cytoplasmic membrane. Consequently, elements with one of these reporter genes can be used to identify the cellular compartment to which the target protein is localized. For transmembrane proteins, specific (surface exposed versus cytoplasmic) domains can also be identified (265).

A truncated *phoA* gene, lacking the signal sequence segment, as well as the promoter and the translational start site, was first used as a reporter for protein localization in a plasmid cloning vector (139). Soon thereafter, a comparable Tn*5* reporter transposon, called Tn*phoA*, was developed. Tn*phoA* insertions result in $PhoA^+$ phenotypes only in cases of in-frame insertion into genes for secreted proteins or in gene segments encoding surface-exposed domains (integral membrane protein genes) (190, 191). As with β-galactosidase, alkaline phosphatase can be scored by colony color on medium with a chromogenic substrate, as well as by a sensitive and easy direct enzyme assay.

In addition to its original use for probing protein localization and conformation, Tn*phoA* has been of use in identifying genes in pathogens that are important for interaction with the host, since many proteins involved in colonization or virulence are surface exposed or secreted (150). For example, in one study, one-tenth of *S. typhimurium* colonies selected for a Pho^+ phenotype after random Tn*phoA* insertion were avirulent in mice (197a). This strategy is thought to sample only a subset of these genes, however, in part because it selects for genes that are strongly expressed on bacteriological medium (see references 176a and 176b).

Construction of Mud*phoA* has permitted the *phoA* fusion strategy to be extended to species such as *Legionella pneumophila*, in which Tn*5* transposition is rare and Mu transposition is quite efficient (5).

The complementary properties of β-galactosidase (active only in the cytoplasm) and alkaline phosphatase (active only if transported) were exploited to develop a strategy for examining membrane protein topology based on the activities of these two enzymes in sibling strains carrying Tn*phoA* and Tn*5lacZ* (gene fusion) elements at exactly the same site in a target gene. This was accomplished by constructing pairs of Tn*phoA* and Tn*5lacZ* elements with different antibiotic resistance determinants and homology at their ends, to allow replacement of one by the other by homologous recombination (189, 306).

Transposon tagging to analyze protein location has been extended with a Tn*5* derivative containing a 5′-truncated *bla* (β-lactamase) gene instead of *phoA*. This element is useful in allowing in-frame insertions in genes for surface-exposed and secreted proteins to be selected directly by ampicillin resistance, rather than by screening for colony color (284).

As Mobile Promoter Elements

Transposons can be engineered to provide mobile promoters to turn on and also to turn off expression of adjacent genes. A Tn*5* derivative called Tn*5tac1* was constructed to allow isolation of insertion mutations with conditional mutant phenotypes. This element contains an isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible outward-facing promoter (which is regulated by the *lacI* repressor gene), as well as the Tn*5* transposase and kanamycin resistance genes (66). Tn*5tac1* can be delivered to the *E. coli* genome from a λ suicide vector (66) and to nonenteric bacteria from a plasmid suicide vector (71). Tests with representative Tn*5tac1* insertions showed that, in addition to standard insertion mutations, two kinds of conditional mutations were found: mutations whose effects are repaired by the addition of IPTG and are due to polarity in the absence of transcription from the element and mutations whose effects are only observed in the presence of IPTG and are due to IPTG-induced transcription. Transcription-induced mutant phenotypes were found for insertions upstream of the target gene, due to excess transcription (66), and for insertions downstream of the target gene, due to interference with normal transcription (209, 210).

A mini-Tn*5* derivative, analogous to Tn*5tac1*, containing an outward-facing *lacI*-regulated promoter (P*trc*) has been constructed recently (83). This transposon is in the pUT broad-host-range conjugal suicide vector (135) that is useful for mutagenizing diverse enteric and nonenteric gram-negative bacteria.

For Multiplex Screening

It is appropriate to reemphasize here that many strains of *E. coli* and *S. typhimurium* are important pathogens and that transposon-based searches for potential pathogenicity determinants have been very useful. However, an elegant strategy for screening many insertion mutants simultaneously was recently developed to overcome the cost and impracticability of testing hundreds or thousands of insertion strains in animals, one at a time (134b). This method, termed “signature-tagged mutagenesis,” entails mutagenesis of the pathogen en masse with a set of mini-Tn*5* elements that contain thousands of different random oligonucleotide tags, arraying individual mutant colonies, and screening for tags that are missing after passage of the pathogen through an animal host. These missing tags represent mutants that could not proliferate in vivo. This strategy has been implemented with *S. typhimurium*, and a number of genes needed for infection of mice were identified (134b). This transposon-based multiplex tag approach should also be valuable for many other screening, mapping, cloning, and sequencing purposes. A complementary multiplex insertion and PCR process to identify genes required under specific growth conditions has been described for yeast (266a). It could readily be applied to bacteria.

For Genome Mapping

Transposable elements greatly facilitate genetic and physical chromosome mapping at several levels. For genetic mapping, the resistance transposons provide selectable markers at known sites that facilitate conjugational and transduction mapping of nearby genes (17, 19, 161). This is particularly well illustrated by a set of isogenic *E. coli* strains containing both Tet^r and Kan^r insertions every approximately 1 min along the chromosome at positions that are known with respect to both the *E. coli* genetic map and the Kohara library of ordered λ phage clones (263; see appendix to chapter 109). A rapid two-step mapping procedure was implemented with these strains. In the first step, any new mutation is located to a 5- to 15-min region of the chromosome by Hfr mapping with a set of Hfr strains carrying either Tn*10* (*tet*) or Tn*10* (*kan*) about 20 min from their respective origins of transfer (see chapters 127 and 137). In the second step, the mutation is localized to a 1-min region by P1 transduction, using a subset of the insertion mutant strains as donors. This strain collection is available from the *E. coli* Genetic Stock Center. A less complete, but still very useful, set of insertions is available from the *Salmonella* Stock Center (see chapter 141).

Large-scale genetic mapping has also been aided by the construction of Tn*5* and mini-Mu derivatives containing plasmid origins of conjugational transfer (*oriT*). Insertion of these *oriT* transposons into the chromosome allows them to serve as origins of Hfr-like and F′-like transfer and thus allows efficient mapping of these chromosomal insertions and other markers by conjugation (229, 259, 311). Homologous recombination between a transposon on a fertility plasmid and a homologous transposon in the chromosome has also been used for conjugational mapping (67).

Physical mapping of genomic DNA by pulsed-field gel electrophoresis (PFGE) (see chapter 138) has also been facilitated by using transposon insertions. Standard PFGE analysis involves digesting genomic DNA with enzymes, such as *Not*I, that cut the DNA infrequently. The relatively few large fragments are separated by PFGE, and hybridization to probes for specific mapped genes is used to align the restriction fragments. Rare restriction sites in wild-type transposons Tn*5* and Tn*10* (180, 193, 238a, 265a, 308; Mahillon et al., personal communication), as well as additional sites engineered into mini-Tn*5* and mini-Tn*10* derivatives (128, 309), have been exploited by PFGE. A comparison of the PFGE restriction fragment profiles of strains containing insertions at known locations with the profile from the nonmutant parent or from a related strain reveals which parental fragment has been replaced by two fragments and also the approximate position of the insertion relative to one fragment end or the other.

This use of transposons containing rare restriction sites has been extended to the study of comparative genome content and organization of different isolates of the same species. This has been implemented with *E. coli* by moving mapped *E. coli* K-12 insertions to the same sites in clinical *E. coli* isolates by P1 transduction and selecting for the transposon marker. Comparison of the PFGE restriction fragment profile of the clinical isolate with that of *E. coli* K-12 helped locate and define differences in the physical maps; some of the genes unique to one or the other strain are likely to be involved in host colonization or disease (40a; Mahillon et al., personal communication).

Insertions can be used to anchor PCR for generating hybridization probes. For PCR, a second primer binding site within a few kilobases of the insertion has to be identified or created. This site can be created by using a PCR strategy, such as (i) inverse PCR (53, 213, 235); (ii) PCR between a transposon and naturally occurring “REP” (repetitive) sequences (279); or (iii) vectorette or “cassette ligation” PCR, in which a double-stranded oligonucleotide containing a unique primer binding site is ligated to restriction-digested DNA (142, 237). These PCR fragments can be used to probe Southern blots of ordered clone libraries, allowing rapid physical mapping.

With the recent development of long-distance PCR protocols that amplify cloned DNA fragments of up to 50 kb from phage λ templates (10, 64), it may soon be possible to map chromosomal insertions directly if they are within 50 kb of known markers.

For Mapping Cloned Fragments

Transposons have been exploited to localize genes in large cloned fragments and are particularly valuable when insertion in the gene can be detected by phenotype and then mapped by restriction analysis (17, 19, 79, 119), although insertions outside genes sometimes exert "position effects" (179).

A method for generating nested deletions in one direction in DNAs cloned in very large capacity (70 to 100 kb) P1 phage vectors (221, 222, 272) has been developed. This is based on the presence in P1 clones of the P1 *loxP* site for efficient site-specific recombination in the presence of Cre protein. Transposition of a *loxP*-containing mini-Tn*10* element into Cre^+ cells that contain a P1 clone and recombination between the two *loxP* sites generate sets of nested deletions in which DNA between the insertion and vector *loxP* sites is deleted (273). This system can be used to isolate deletion derivatives that contain the vector replication origin but not the reciprocal product lacking a replication origin. Nonetheless, this nested deletion strategy is particularly attractive for dissecting P1 clones, in which the DNA is too large for easy mapping of simple insertions.

Transposons can also be used to generate detailed restriction maps of the cloned fragment, in much the same way that they are used in generating PFGE (above) or "dropout" maps (described below).

For Sequencing Cloned DNA

Transposons provide mobile and easily mapped binding sites for universal PCR and DNA sequencing primers. They can give access to all regions of large cloned target DNAs without the need for (i) shotgun subcloning and attendant high-redundancy sequencing or (ii) extensive primer walking, which is slow and costly at present. In addition, transposons can be used to sequence DNA that contains repeated sequences. Both simple insertions, which allow sequences to be read from each transposon end (reading one strand in each direction), and transposon-mediated nested sets of deletions can be used to great advantage. The most effective transposon-based strategy for a given DNA segment depends on the size of the DNA segment and the vector in which it is cloned.

For plasmid clones, which are generally less than 10 kb, any element that inserts into many target sites and contains unique DNA sequences close to each end can be used (1, 179, 208). In cases of elements such as wild-type Tn*5* or Tn*10* with long inverted repeats, effective sequencing depends on separating the left and right inverted repeat, e.g., by digestion with a restriction endonuclease that cuts within the transposon and electrophoresis or subcloning (90). The best-developed method for transposon-based plasmid sequencing, however, exploits γδ and its relatives, in large part because of the many sites per gene in which these elements can insert and the simple conjugation method for obtaining insertions (Fig. 1B) (19, 76, 119, 299).

In vitro transposition of synthetic transposons is also very promising, as illustrated by AT-2, an "artificial transposon" based on the yeast Ty1 transposon (a retrovirus-like element). The addition of AT-2 DNA and integrase-containing "virus-like particles" specific for Ty1 to a target plasmid and then transformation of *E. coli* and selection for the transposon marker yield insertions throughout the cloned target DNA (87).

In all of these cases, insertions to be used for sequencing in each direction from any site of insertion can be chosen on the basis of map position, which can be determined easily by restriction endonuclease or probe mapping (277) or by PCR with primers specific for transposon ends and appropriate vector sequences (219). These approaches can be automated for large-scale sequencing (20, 192; R. Weiss and R. Gesteland, personal communication).

For DNAs cloned in most λ phage vectors, it is not possible to select for transposon insertions by using resistance markers because these phage cannot lysogenize *E. coli* and resistance to an antibacterial agent cannot be selected during lytic growth. This problem is overcome with Tn*5supF*, a 300-bp derivative of Tn*5* marked with a suppressor tRNA gene (149, 170, 219). Tn*5supF* insertion into λ can be selected by

plaque formation on one of two hosts: a standard nonsuppressing *E. coli* strain for vectors with amber mutant alleles of essential phage genes or a *dnaB*(Am) *E. coli* strain (the *dnaB* product is essential for λ growth) for vectors lacking amber mutations, such as those used for the Kohara *E. coli* library (163).

For large cosmid clones the effort needed to map many individual insertions makes a simple insertion strategy less appealing. In contrast, with nested deletions made in such clones by transposition, the position of the transposon end, and thus the region accessed, is indicated simply by the size of the plasmid remaining after the transposition event. The transposon-based cloning vectors described in the following section (2, 3, 20, 217, 296, 297) were developed for DNA sequencing and mapping.

For Cloning

In Vitro Cloning of Insertions. Transposons can facilitate in vitro gene cloning in several ways.

(i) They provide resistance markers and an array of known restriction sites that can be inserted into or next to a gene and used to clone that gene (detailed restriction maps of useful natural and genetically engineered transposons can be obtained from public domain databases or from references cited in Tables 1 and 2).

ii) Insertions in a gene can be used to clone DNA to one or both sides of the insertion, depending on the restriction enzyme(s) used. In clones that contain DNA from both sides of the insertion, the wild-type allele can be recovered in the plasmid clone by homologous recombination with the chromosomal allele. This is facilitated by using a transposon, such as Tn*5*-*rpsL*, that carries both counterselectable and selectable markers (276). In addition, for insertions of elements in genes with selectable phenotypes, the wild-type allele can be obtained directly from the insertion mutant plasmid by selecting for function, if the element can undergo precise excision. Tn*916* is especially useful for recovering revertants because it undergoes a high frequency of precise excision in *E. coli* (57, 105).

iii) Transposons that have been engineered to contain a plasmid replication origin can be used directly for in vitro cloning of segments bracketing the insertion. However, because insertion of a functional plasmid replication origin into the chromosome is often deleterious, this strategy is most effective when the origin does not function in the strain to be mutagenized. For example, Tn*5*-V, which contains the plasmid pSC101 origin of replication, can be used to mutagenize *Myxococcus xanthus*, in which pSC101 does not replicate. Insertions were cloned directly by restriction digestion of genomic DNA, self-ligation, and transformation of *E. coli* (102). Another derivative, Tn*5*-PV, has the origins of replication and of transfer but not the replication genes from plasmid RK2 (165). The replication origin in Tn*5*-PV insertions in RK2-free strains is nonfunctional; hence, this transposon can insert into the chromosome and be used to clone adjacent segments when the self-ligated genomic DNA mixture is transformed into a strain carrying RK2 replication genes.

Generally, the restriction endonuclease(s) used for cloning insertion mutations is one that does not cleave the transposon, but if DNA to only one side of the insertion is sought, an enzyme that cuts the transposon could be used.

Vectors for In Vivo Cloning. As noted above, an early, and important, in vivo cloning method involved λ specialized transducing phages from aberrant excision of prophages inserted at dispersed secondary sites (244, 255). Other phage-based in vivo cloning strategies are discussed below.

(i) One strategy involves λ-Mu hybrid phage (λp*lac*Mu) that use Mu DNA homology to direct their integration by homologous recombination into Mu prophage (Mu inserts into more sites than λ), followed by aberrant excision and packaging into λ heads (58, 166, 186). More recently, this approach was greatly simplified to eliminate the homologous recombination step: other λ-Mu hybrid phage have been constructed that insert with the randomness of Mu, but replicate and package DNA as λ phage (47, 49, 290).

(ii) Another strategy involves Mu-P22 hybrid phage that insert into the *S. typhimurium* chromosome

with the randomness of Mu. Upon induction, they replicate from the P22 origin in one direction and package sequential headfuls of DNA, starting from the P22 *pac* site. Therefore, chromosomal segments corresponding to a few percent of the genome to one side of the inserted phage are recoverable in high yield from any single lysogen (312).

(iii) The most generally useful approach to in vivo cloning involves small engineered derivatives of Mu (114, 116) and the Mu-related *Pseudomonas aeruginosa* phage D3112 (75). These phage replicate by transposing to different sites and they package DNA by the headful mechanism. Consequently, small derivatives of these phage also package a segment of adjacent host DNA, which sometimes contains part or all of a second phage copy (because of intramolecular replicative transposition). Such transducing phage give rise to clones containing the interstitial DNA by transduction of a Rec^+ recipient and homologous recombination between the Mu ends. DNAs that do not contain a second Mu end cannot circularize.

Mini-Mu and D3112 derivatives contain phage ends, essential phage genes, sites that are essential for plasmid replication, and a selectable resistance determinant, but not a full set of morphogenetic genes, and thus require a helper phage to form phage particles. Some are small enough to package up to 34 kb of adjacent DNA into Mu or D3112. Many derivatives carrying various antibiotic resistance determinants and plasmid replication origins are available. Some of these engineered mini-Mu and mini-D3112 elements contain a promoterless *lac* reporter gene, a plasmid origin of transfer, a *tac* promoter, a λ *cos* site, and/or a phage T7 promoter (see Table 2 and reference 113).

In vivo mini-Mu cloning has some advantages over traditional in vitro methods for those species in which it can be used: the cloned piece is always colinear with the parental chromosome (there is no risk of obtaining chimeric clones), and the fragment endpoints are determined by Mu insertion, which is more random than cleavage by any one restriction enzyme (even a 4-bp cutter such as *Sau*3A), so that clones with a wide array of fragment endpoints can be obtained (299).

(iv) A recently described transposon, Tn*5*-*rpsL ori*R6K (Table 2), contains the crippled R6K origin and has been used to clone by P1 generalized transduction and recircularization by illegitimate recombination in a RecA cell expressing the R6K *pir* (replication) gene (275).

Transposon-Based Vectors for In Vitro Cloning. Several cloning vectors have been constructed with transposons or transposon ends, which are used to generate nested deletions in the cloned fragment by intramolecular transposition. The deletion endpoints can be used to map and sequence the cloned DNA. The first implementation of a transposon-based strategy for making nested deletions in cloned DNA, which was also the first demonstration of transposon-based sequencing, involved the use of a vector containing Tn*9* and *galKT*$^+$ counterselectable genes between the transposon and the cloning site (*galE* strains are galactose sensitive [Table 3]). Most Gal^r derivatives of strains carrying this plasmid arise by intramolecular transposition (either replicative or conservative) into or beyond *galKT*, producing deletions that extend into target DNA from a transposon end (2, 3, 217). The Tn*9*-based vectors have two disadvantages: Tn*9* transposition is very nonrandom and deletions can be isolated in only one direction, so that two clones with oppositely oriented fragments are needed to access both strands of the cloned DNA.

The disadvantages of Tn*9*-based deletion vectors were addressed in a family of γδ-based "deletion factory" vectors called pDUAL or pDELTA (Fig. 4): (i) in these vectors, γδ intramolecular transposition is quite random, so nested deletions with near random distributions of endpoints can be obtained; and (ii) the vector was engineered so that deletions extending in either direction into the cloned fragment can be selected. This ability to isolate nested deletions in each direction, and thereby access each strand without recloning, is based on two features: (i) incorporating a plasmid replication origin within the synthetic γδ element, so that each of the two complementary circular DNAs generated by replicative transposition (deletion mode) (Fig. 3 [left]) is replication proficient; and (ii) placing different counterselectable genes (*sacB* and *strA;* sensitivity to sucrose and streptomycin, respectively [Table 3]) on each side of γδ. In addition, one selectable gene is placed on each side of the cloning site to increase the efficiency of selecting deletions that extend from either γδ end to sites within, rather than beyond, the cloned DNA segment (20,

296, 297). With these features, sets of nested deletions can be selected in either direction by using sucrose-tetracycline medium for clockwise deletions or streptomycin-kanamycin medium for counterclockwise deletions (Fig. 5). The selection against one counterselectable marker and for one selectable marker yields colonies with plasmids whose nested deletions extend in the selected direction for various distances into the cloned target DNA. After transposition, one transposon end always abuts a deletion endpoint, and the unique sequence just inside the transposon end can serve as a "universal" primer binding site for sequencing adjacent cloned DNA. All target regions can be sequenced by using a pair of universal primer binding sites engineered into γδ . Outward-facing SP6 and T7 promoters just within the γδ ends serve as sites for universal primers and also for riboprobe synthesis after γδ-mediated deletion formation (Fig. 4). pDUAL-1 (297) and pDELTA-1 (296) contain the pUC19 high-copy-number replication origin and *amp* gene but different unique restriction sites. pDUAL-3 contains an amplifiable low-copy-number replication origin to allow greater stability of segments not clonable in high-copy-number vectors (C. M. Berg, X. Liu, G. Wang, X. Xu, and D. E. Berg, unpublished data).

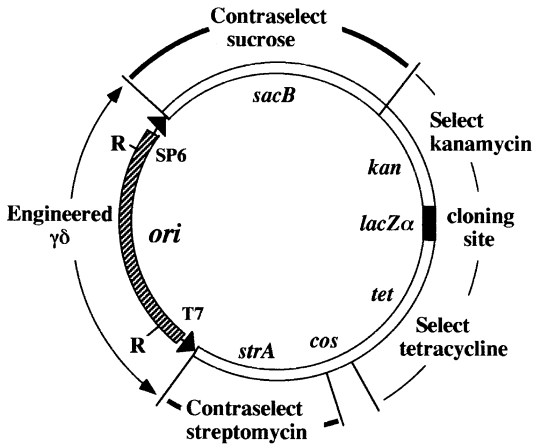


FIGURE 4 Generic pDUAL or pDELTA cosmid cloning vector for generating nested deletions in vivo. These vectors contain a synthetic γδ transposon containing 40 bp of inverted repeat copies of the δ end of γδ bracketing SP6 and T7 promoter/primer binding sites, rare restriction sites (R), a replication origin, and, in some cases, an amp gene. The counterselectable genes, sacB and strA (rpsL), immediately outside the transposon ends are used to select for deletioins that extend in either direction from a transposon end. The selectable genes kan and tet are between the counterselectable genes and the cloning site and are used to select against deletions that extent through the cloned fragment into vector sequences. pDUAL-1 (297) and its commercialized relative, pDELTA-1 (296), contain the replication origin and amp from pUC19 between the γδ ends but differ in their restriction sites. pDUAL-3 (C. M. Berg, unpublished data) contains an emplifiable R1 replication origin and no amp gene.

Uses of nested deletions to generate restriction maps and for sample sequencing. Restriction maps of cloned fragments in vectors such as pDUAL can be generated with relative ease by aligning restriction data from single-enzyme digestions of the parent clone and of a small set of nested deletion derivatives, as depicted in Fig. 5. Consideration of the restriction fragments from the parent clone in comparison with those that drop out in the deletion derivatives permits the fragments and restriction sites to be ordered (174a). These deletion derivatives can also be used to obtain "sample sequence" information at known sites in the clone, so that regions of interest can be readily identified (169).

FUTURE PROSPECTS

Transposable elements continue to be superb as tools for mutational analysis and in vivo genetic engineering in laboratory strains of *E. coli* and *S. typhimurium,* in a diverse array of other prokaryotes, and in eukaryotes (18, 19, 32). Improved transposons and delivery systems continue to be developed for an expanding array of organisms. The next few years should bring even greater use of transposons: in direct genome analysis, especially in conjunction with long-distance PCR (10, 64); in mapping and sequencing >80-kb fragments cloned in large-capacity bacterial vectors (P1, BAC, and PAC) (141, 257, 272); in robust in vitro transposition systems (e.g., reference 87); and in primer walking with preformed oligonucleotide libraries (278, 283).

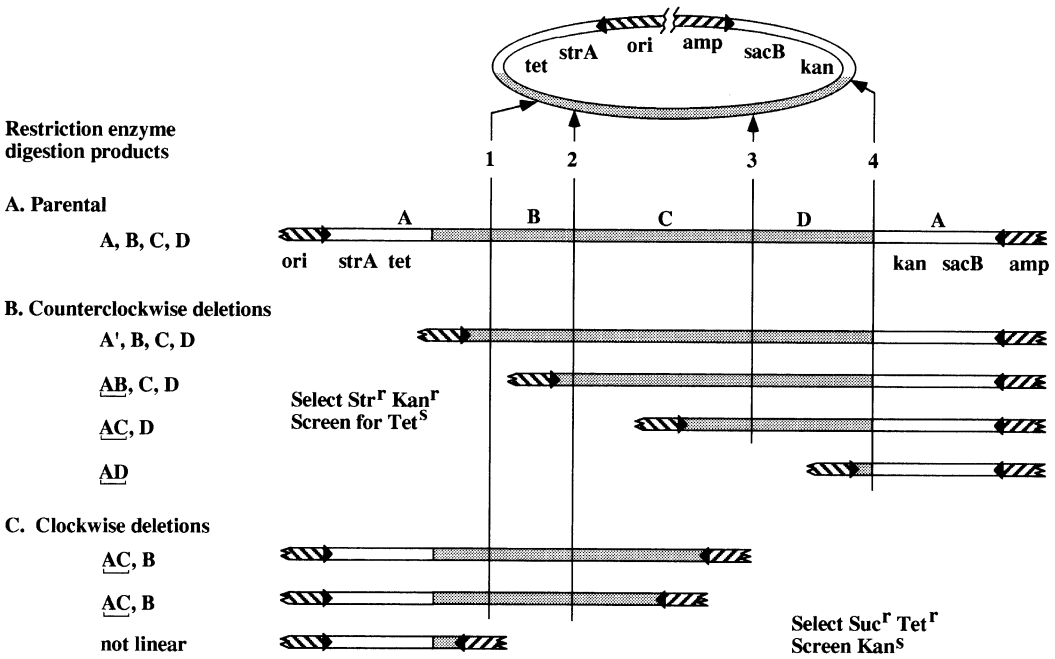


FIGURE 5 Expected restriction fragment profiles of pDUAL clone deletion derivatives. The four restriction sites (vertical lines 1 to 4) divide the clone into four fragments (A to D). Restriction sites 1 to 3 are in the cloned fragment; site 4 is at the cloning site. One fragment (A) contains both vector and cloned DNA; the other fragments. A′ indicates a truncated fragment;underline indicates fusion fragments. (A) Linearized parent plasmid. (B and C) Deletion derivatives.Every γδ- generated deletion reduces the size of the vector component (A), and most deletions generate fusion fragments (letters underlined on the left) between the remainder of the A fragment and one of the restriction fragments in the cloned portion. Note that the transposition target site (deletion endpoint) can be determined approximately by the size of the deletion plasmid and more accurately by the restriction fragment pattern (dropout mapping; 174a).

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