

Transposable elements.

Transposable element ^a	Vector ^d	Delivery method ^g	Resistance marker ^j	Reporter element ^k	Deletion method	Internal tag ^m	Tn-specific primers ⁿ	CEKG-2 primers ^p	Reference
<KAN-2>	(none)	Tpm	kan	none	none	none	K	C,D,E	Epicentre ^q
T1	Tn <i>phoA</i>	phage λ Tn <i>phoA</i>	kan	<i>phoA</i> TL	none	none	H	A,B,C	[1]
T2	Tn <i>lacZ</i>	phage λ Tn <i>lacZ</i>	kan	<i>lacZ</i> TL	none	none	L2	A,B,C	[2]
T3	Tn <i>phoA/in</i>	phage λ Tn <i>phoA/in</i>	cm	<i>phoA</i> TL	BamHI/ligase	31-codon	H	A,B,C	[3]
T4	Tn <i>lacZ/in</i>	phage λ Tn <i>lacZ/in</i>	cm	<i>lacZ</i> TL	BamHI/ligase	31-codon	L2	A,B,C	[3]
T5	IS <i>phoA/hah-cm</i>	pCM638, pCM665 ^e	Conj	<i>phoA</i> TL	loxP	63-codon: HA, H ₆	H	A,B,C	[4]
T6	IS <i>phoA/hah-tc</i>	pCM639	Conj	<i>phoA</i> TL	loxP	63-codon: HA, H ₆	H	A,B,C	[5]
T7	IS <i>lacZ/hah-cm</i>	pIT1	Conj	<i>lacZ</i> TL	loxP	63-codon: HA, H ₆	L	A,B,C	This paper ^r
T8	IS <i>lacZ/hah-tc</i>	pIT2	Conj	<i>lacZ</i> TL	loxP	63-codon: HA, H ₆	L	A,B,C	[5]
T9	ISΩ/hah	pCM1008	Tpm	strep/spec	loxP	54-codon: HA, H ₆	CT1, CT28 ^o	N/D	This paper
T10	IScm/FRT	pCM1767	Tpm	cm	FRT	35-codon	CT24 ^o	N/D	This paper
T11	IS <i>lacZY/hah-cm</i>	pLG33	Conj	<i>lacZY</i> TS ^j	loxP	63-codon: HA, H ₆	L2	A,B,C	This paper
T12	IS <i>lacZY</i>	pLG42, pLG43 ^f , pLG44 ^f , pLG49	Tpm ^h or Tpm (PshAI) ⁱ	cm	<i>lacZY</i> TS	none	L2	A,B,C	This paper
T13	mTn5* <i>-lacZ1-kan</i>	pLG48b	Tpm (PshAI)	kan	<i>lacZ</i> TS	none	L2	C,D,I	This paper
T14	mTn5* <i>-lacZ1-em</i>	pLG51	Tpm (PshAI)	erm	<i>lacZ</i> TS	none	L2	C,D,I	This paper
T15	ISR6K-em ^b	pLG52a, pLG53, pLG55a	Tpm (PshAI)	erm	none	none	E	A,C,E	This paper
T16	ISR6K-kan ^b	pLG56a	Tpm (PshAI)	kan	none	none	N	B,E,F	This paper
T17	IS <i>Fn1</i> ^c	pLG61a	Tpm	kan ^{Fn}	none	none	K or F1	C,D,E	This paper
T18	IS <i>Fn2</i> ^c	pLG62a	Tpm	kan ^{Fn}	none	none	K or F2	C,D,E or B,D,E	This paper
T19	IS <i>Fn1/FRT</i> ^c	pLG65a	Tpm	kan ^{Fn}	none	FRT	K or F1	C,D,E	This paper
T20	IS <i>Fn2/FRT</i> ^c	pLG66a	Tpm	kan ^{Fn}	none	FRT	K or F2	C,D,E	This paper
T21	IS <i>Sgfp-Fn2/FRT</i> ^c	pLG67	Tpm	kan ^{Fn}	<i>gfp</i> TS	FRT	none	C,D,E	This paper
T22	IS <i>lacZ-Fn2/FRT</i> ^c	pLG69	Tpm	kan ^{Fn}	<i>lacZ</i> TS	FRT	L2 or K	A,B,C or C,D,E	This paper

^aEach transposable element is identified by both a unique "T" number and a transposon name. **Click on the transposon names for complete transposon sequences.**

^bTransposon is derived from plasmid pMOD-3 (Epicentre) and carries an R6K origin of replication.

^cIn T17 and T19, an endogenous *F. novicida* promoter (for FNU0649, the *F. novicida* orthologue of *F. tularensis* Schu4 gene omp26 (FTT1542c)) drives a kanamycin resistance gene that retains its own translation initiation region; In T18, T20, T21 and T22, the *F. novicida* promoter drives a translational gene fusion between the native *F. novicida* gene and the kanamycin resistance ORF.

^dMultiple plasmids listed for a single transposon represent different construction histories. The transposons they carry are functionally equivalent, though minor sequence differences may exist within the transposon sequences. Other elements of the plasmids may also differ. **Click on vector names for complete sequences and construction histories.**

^eThe transposase gene in pCM665 is a more active derivative of the one in pCM638 [6].

^fpLG43 and pLG44 carry the R6K origin as their only replication origin.

^gTpm, transposon-transposase complex ("Transposome") transformation; Conj, conjugation; Tpm (PshAI), transposon can be precisely excised by PshAI digestion prior to transposome assembly.

^hTransposon end sequences in pLG42, pLG43 and pLG44, while functional, are not perfect matches to the hyperactive sequence needed for optimal transposome efficiency [6].

ⁱThe "Tpm (PshAI)" delivery method is a feature of pLG49 but not of pLG42, pLG43 or pLG44.

^jkan, kanamycin resistance; cm, chloramphenicol; tc, tetracycline; strep/spec, streptomycin/spectinomycin; erm, erythromycin; kan^{Fn}, kanamycin driven by a *Francisella novicida* promoter.

^kTL, translational fusion; TS, transcriptional fusion.

^lIn T11, the loxP site adjacent to the *lacZ* gene appears to contain promoter elements that are active in *E. coli*.

^mFor some transposons, insertions that are in the proper orientation and reading frame can be converted by the deletion method shown into small internal gene tags. For most tags, partial codons are present at both ends of the defined insertion sequences and are completed by the flanking nucleotides at the insert site. The number of codons shown for each tag includes those created by the 9-bp duplication produced by Tn5 transposition. Specific features encoded by some tags: HA, hemagglutinin epitope; H₆, hexahistidine.

ⁿThe set of three primers used for round 1 PCR, round 2 PCR and sequencing, respectively (see Fig. 3B and protocol in text), are: K, primers kan2-211, kan2-145 and kan2-125; H, primers hah-166, hah-138 and hah-114; L, primers lacZ-211, lacZ-148 and lacZ-124L; L2, primers lacZ-211, lacZ-148 and lacZ-124L2; E, primers erm-204, erm-138 and erm-106; N, primers nptF-186, nptF-130, nptF-105; F1, primers 806b-248, 806b-214, 806-182; F2, primers 806c-208, 806-182 and 806-98. **Click on column headings for primer sequences.**

^oThree-step mapping with transposon-specific primers (see previous note) has not been done for T9 and T10. The individual primers listed may be used for one-step sequencing out of the transposons.

^pFor round 1 PCR, a mixture of 3 "CEKG-2" primers (semidegenerate primers with short defined sequences at their 3' ends; see protocol in text) is used. Primers must be chosen whose defined 3' ends (4 or 5 nucleotides) do not anneal within the transposon-specific primer site and the end of the transposon. Suggested mixtures of primers CEGK-2A, B, C, D, E, F, G and H are listed. **Click on column headings for primer sequences.** N/D, not determined.

^qEpicentre Biotechnologies, www.epicentre.com.

^rThaipisuttikul, I., personal communication.

References:

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