Transposable elements

Updated March 29, 2012

Transposable element[a]	code[c]	Vector(s)[d]	Delivery method[g]	Selection marker[k]	marker promoter	Reporter element[l]	Deletion method	Recombined insertion / tag[n]	Other features	Tn-specific primers[o]	CEKG-2 primers[p]	Reference
<KAN-2>	4	(none)	Tpm	kan (Tn903)	Tn903 p_{kan}	none	none	-	-	K	CDE	Epicentre
<DHFR-1>		(none)	Tpm	tp (*dhfr*)	Tn7 p_{dhfr}	none	none	-	-	N/D	N/D	Epicentre
T1 Tn*phoA*		phage λ Tn*phoA*	Transduction	kan	?	*phoA* TL	none	-	-	H	ABC	[1]
T2 Tn*lacZ*		phage λ Tn*lacZ*	Transduction	kan	?	*lacZ* TL	none	-	-	L2	ABC	[2]
T3 Tn*phoA* /in		phage λ Tn*phoA* /in	Transduction	cm	?	*phoA* TL	BamHI/lig	31-codon	-	H	ABC	[3]
T4 Tn*lacZ* /in	a	phage λ Tn*lacZ* /in	Transduction	cm	?	*lacZ* TL	BamHI/lig	31-codon	-	L2	ABC	[3]
T5 IS*phoA* /hah-cm	3	pCM638, pCM665[e]	Conj	cm	?	*phoA* TL	loxP	63-codon, HA, H_6	-	H	ABC	[4]
T6 IS*phoA* /hah-tc		pCM639	Conj	tc	?	*phoA* TL	loxP	63-codon, HA, H_6	-	H	ABC	[5]
T7 IS*lacZ* /hah-cm	1	pIT1	Conj	cm	?	*lacZ* TL	loxP	63-codon, HA, H_6	-	L2	ABC	[6]
T8 IS*lacZ* /hah-tc	2	pIT2	Conj	tc	?	*lacZ* TL	loxP	63-codon, HA, H_6	-	L2	ABC	[5]
T9 ISΩ/hah		pCM1008	Tpm	strep/spec	?	none	loxP	63-codon, HA, H_6	-	N/D	N/D	[6]
T10 IScm/FRT		pCM1767	Tpm	cm	?	none	FRT	35-codon	-	N/D	N/D	[6]
T11 IS*lacZY* /hah-cm		pLG33	Conj	cm	?	*lacZY* TS[m]	loxP	63-codon, HA, H_6	-	L2	ABC	[6]
T12 IS*lacZY*		pLG42, pLG43[f], pLG44[f], pLG49	Tpm[h] (P)[i]	cm	?	*lacZY* TS	none	-	-	L2	ABC	[6]
T13 mTn5*-*lacZ1* -kan	6	pLG48b	Tpm (P)	kan	?	*lacZ* TS	none	-	-	L2	CDI	[6]
T14 mTn5*-*lacZ1* -em		pLG51	Tpm (P)	erm	?	*lacZ* TS	none	-	-	L2	CDI	[6]
T15 ISR6K-em	7	pLG52a, pLG53, pLG55a	Tpm (P)	erm	?	none	none	-	ori_{R6K}	E	ACE	[6]
T16 ISR6K-kan	8	pLG56a	Tpm (P)	kan	?	none	none	-	ori_{R6K}	N	BEF	[6]
T17 IS*Fn* 1[b]	b	pLG61a	Tpm	kan	Fn p_{FTN_1451}	none	none	-	-	K or F1	CDE	[6]
T18 IS*Fn* 2[b]	c	pLG62a	Tpm	kan	Fn p_{FTN_1451}	none	none	-	-	K or F2	CDE or BDE	[6]
T19 IS*Fn* 1/FRT[b]		pLG65a	Tpm	kan	Fn p_{FTN_1451}	none	FRT	out of frame (106 bp)	-	K or F1	CDE	[6]
T20 IS*Fn* 2/FRT[b]	d	pLG66a	Tpm	kan	Fn p_{FTN_1451}	none	FRT	out of frame (58 bp)	-	K or F2	CDE	[6]
T21 IS*gfp-Fn* 2/FRT[b]		pLG67	Tpm	kan	Fn p_{FTN_1451}	*gfp* TS	FRT	out of frame (106 bp)	-	K	CDE	[6]
T22 IS*lacZ-Fn* 2/FRT[b]	f	pLG69, pLG100	Tpm, Conj[j]	kan	Fn p_{FTN_1451}	*lacZ* TS	FRT	out of frame (106 bp)	-	L2 or K	ABC or CDE	[6]
T23 IS*lacZ* -p_{rhaBo}/FRT-tp	e	pLG99	Conj	tp (*dhfr*)	?	*lacZ* TS	FRT	35-codon	p_{rhaB} out, ori_{pMB1}	L2	EKL or OPS[q]	unpub.
T24 IS*lacZ* -p_{rhaBo}/FRT-kan	g	pLG107	Conj	kan (*nptII*)	?	*lacZ* TS	FRT	35-codon	p_{rhaB} out, ori_{pMB1}	L2	EKL or OPS[q]	unpub.
T25 IS*gfp* -p_{rhaBo}/FRT-kan	i	pLG122	Conj	kan (*nptII*)	?	*gfp* TS	FRT	35-codon	p_{rhaB} out, ori_{pMB1}	N/D	N/D	unpub.
T26 ISp_{gro}-tc/LoxP	k	pLG123	Tpm (P)	tc	LVS p_{groES}	none	loxP	76-codon	-	N/D	N/D	unpub.
T28 IS*Fn* 2-*hisD* /FRT[b]	m	pLG125	Tpm (P)	*hisD*	Fn p_{FTN_1451}	none	FRT	out of frame (106 bp)	-	N/D	N/D	unpub.
T29 ISp_{gro}-*hisD* /FRT	n	pLG126	Tpm (P)	*hisD*	LVS p_{groES}	none	FRT	out of frame (106 bp)	-	N/D	N/D	unpub.
HimarBP3	h	pHBurk3	Elec	kan (*nptII*)	?	none	FRT	large (incl. ori_{R6K})	ori_{R6K}	B	BFG	[7]
ISp_{gro}-hyg	j	pMOD-hyg	Tpm (P)	hyg	LVS p_{groES}	none	none	-	ori_{R6K}	Hy	AGH	[9]

Notes:

[a] Each transposable element is identified by both a unique "T" number and a descriptive name. For nucleotide sequences, go to **www.gs.washington.edu/labs/manoil/sequences.htm**

[b] In transposons with "Fn1" designation (T17 and T19), the F. novicida FTN_1451 promoter drives a kanamycin-resistance gene that retains its own translation initiation region. In transposons with "Fn2" designation (T18, T20, T21, T22 and T28), the FTN_1451 promoter drives a translational gene fusion between the native FTN_1451 gene and the kanamycin-resistance o*rhisD* ORF.

[c] Single-character code which designates the transposon in the Manoil Lab nomenclature used for high throughput sequencing of mutant libraries.

[d] Multiple plasmids listed for a given transposon represent distinct constructions and possibly distinct vector sequences or features. For nucleotide sequences and construction notes, go to **www.gs.washington.edu/labs/manoil/sequences.htm**

[e] pCM665 carries the hyperactive allele of the Tn5 transposase gene [8], pCM638 carries a non-hyperactive allele.

[f] pLG43 and pLG44 carry ori R6K as their sole origin of replication.

[g] Tpm, transformation of transposome-transposase complex ("Transposome"). Conj, conjugation. Elec, transformation of plasmid by electroporation. Tpm (P), transposon for transposome assembly can be precisely excised from the vector by PshAI or PvuII digestion as an alternative to amplification.

[h] Transposon end sequences in pLG42, pLG43 and pLG44, while functional, are not perfect matches to the sequences needed for optimal transposome efficiency using hyperactive transposase [8].

[i] Transposon for transposome assembly may be isolated by PshAI digetion from pLG49 but not from pLG42, pLG43 or pLG44.

[j] pLG100 is conjugatable, pLG69 is not.

[k] kan, kanamycin resistance; cm, chloramphenicol; tc, tetracycline; strep/spec, streptomycin/spectinomycin; erm, erythromycin; tp, trimethoprim; hyg, hygromycin/*hisD*, *E. coli hisD* gene (allows growth of histidine auxotrophs on histidinol).

[l] TL, translational fusion; TS, transcriptional fusion.

[m] In T11, the loxP site adjacent to the lacZ gene appears to encode promoter activity which causes *lacZ* expression in *E. coli*.

[n] For some transposons, recombination of insertions in the proper orientation and reading frame produces in-frame internal gene tags after recombination by the deletion method listed (e.g., "35-codon"). For most such tags, partial codons are present at both ends of the defined insertions sequences and are completed by the flanking nucleotides at the insert site. The number of codons or base pairs reported includes those created by the 9-bp target-site duplication produced by Tn5 transposition. Specific features encoded by some tags: HA, hemagglutinin epitope; H_6, hexahistidine.

[o] The set of three transposon-specific primers used for PCR round 1, PCR round 2 and sequencing, respectively [6]. K, primers kan2-211, kan2-145 and kan2-125; H, hah-166, hah-138 and hah-114; L, lacZ-211, lacZ-148 and lacZ-124L; L2, lacZ-211, lacZ-143 and lacZ-124L2; E, erm-204, erm-138 and erm-106; N, nptF-186, nptF-130 and nptF-105; F1, 806b-248, 806b-214 and 806-182; F2, 806c-208, 806-182 and 806-98; B, Burk-160, Burk-135 and Burk-107; Hy, hyg-174, hyg-154 and hyg-107; N/D, not determined. For primer sequences, go to **www.gs.washington.edu/labs/manoil/sequences.htm**

[p] The mixture of three semidegenerate "CEKG-2" primers recommended for PCR round 1 (e.g., "CDE" = CEKG-2C, CEKG-2D and CEKG-2E) [6]. The non-degerate 3' ends (4 or 5 nucleotides) of the chosen primers should not anneal within the transposon between the transposon-specific primer site and the end of the transposon. N/D, not determined. For primer sequences, go to **www.gs.washington.edu/labs/manoil/sequences.htm**

[q] Combination OPS (CEKG-2O, CEKG-2P and CEKG-2S) is recommended for GC-rich genomes.

References:

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6. Gallagher, L. et al. 2007. *Methods Enzymol* 421:126-140.
7. Rholl, D. et al. 2008. *Appl Environ Microbiol* 74(24):7529-35.
8. Zhou, M. et al. 1998. *J Mol Biol* 276(5):913-25.
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