



## Mini-Tn7 transposons for site-specific tagging of bacteria with fluorescent proteins

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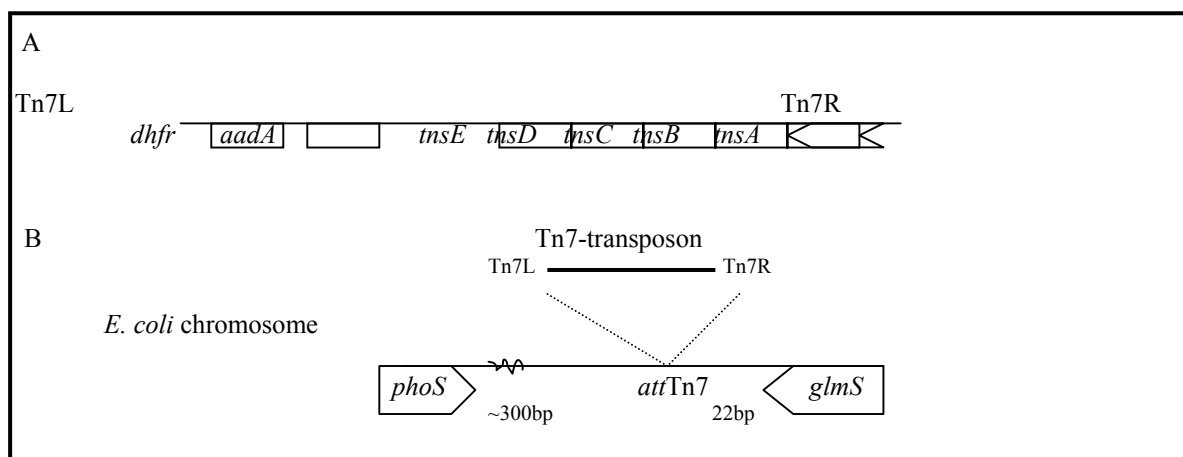
## The Tn7 transposon

The Tn7 transposon was originally discovered by Barth *et al.*, (1976) on the plasmid R483 (IncI $\alpha$ ) as an element carrying the resistance genes trimethoprim (Tm<sup>R</sup>) and streptomycin/ spectinomycin (Sm<sup>R</sup>/ Sp<sup>R</sup>), which could be transposed to other replicons (note; in some earlier papers the Tn7 transposon is named TnC).

The Tn7 transposon is 14 kb. It contains five genes (Fig. 1), involved in the transposition process, and two resistance genes as described above. These genes are flanked by the ends of the transposon, named the left (Tn7L) and the right (Tn7R) end (Barth *et al.*, 1976; Lichtenstein and Brenner, 1982; Rogers *et al.*, 1986). Note; in the literature the names of the ends have not been used consistently. In the following the names used for the ends are as indicated in Fig. 1.

The Tn7 transposition process has been studied intensively in *Escherichia coli* in which Tn7 inserts with high efficiency and unique orientation into one specific location named the *attTn7* site (Lichtenstein and Brenner, 1981; McKnown *et al.*, 1988). This site of insertion is located just downstream of the coding region, in the transcriptional terminator, of the *glmS* gene (Gringauz, *et al.*, 1988). The transposon genes required for specific insertion into the *attTn7* site, are *tnsABCD*, and they function *in trans*. Thus, sequences located in the 3' end of the coding region of *glmS* are recognised by transposase proteins and directing the actual insertion into the *attTn7* site, down-stream of the *glmS* gene (McKnown *et al.*, 1988; Baiton *et al.*, 1993). However, if this site is unavailable the transposon can insert into other sites with low frequency (Rogers *et al.*, 1986; Kubo and Craig, 1990).

The Tn7 transposon recognises a sequence located in the terminal part of the *glmS* gene, but it inserts just downstream of this and thereby it does not disrupt the gene (Gringauz, *et al.*, 1988). The *glmS* gene encodes a glucosamine synthetase, which is required for cell wall synthesis (Volger *et al.*, 1989). It is conserved among many bacteria and therefore Tn7 is likely to have the same specific insertion site in many different bacteria, some have already been tested (Table 1). Detailed information about the Tn7 transposon mechanisms can be found in the literature, two recommendable reviews are Craig (1989) and Peters and Craig, (2001).



**Fig. 1.** Map of transposon Tn7 (A) and its insertion site *attTn7* in *Escherichia coli* (B). The gene *dhfr* encodes dihydrofolate reductase providing trimethoprim resistance and *aadA* encodes adenylyltransferase, which provides resistance to streptomycin and spectinomycin (the figures are redrawn from Craig *et al.*, 1989).

Table 1. Literature overview of bacteria tested for insertion of Tn7

Bacterium	Specific insertion	Test	Sequence of insertion site	Numbers of base pairs downstream of <i>glmS</i> <sup>1</sup>	System tested <sup>2</sup>	Comments	References
<i>Caulobacter crescentus</i>	Yes	Southern	-	-	RP4::Tn7 <sup>3</sup>	9 isolates tested	Ely <i>et al.</i> , 1982
<i>Desulfovibrio desulfuricans</i> G20	Yes	Southern	U46080	28	RK2073::Tn7 <sup>3</sup>	15 out of 16b had specific insertion	Wallet <i>et al.</i> , 1996
<i>Escherichia coli</i>	Yes	Southern	AE000450	22	ColE1::Tn7 <sup>3</sup>	Tested many times by different scientists	Lichtenstein and Brenner, 1981; 1982
<i>Escherichia coli</i> BE6	-	-	-	-	Grinter's system	Stable insertion	Grinter, 1983
<i>Erwinia chrysanthemi</i> EC16	Yes	Southern			Monsanto's system	<i>lux</i> -based reporter system	Shen <i>et al.</i> , 1992
<i>Klebsiella pneumonia</i>	-	-	-	-	-	-	In Caig 1989: Qadri <i>et al</i> personal communication
<i>Methylophilus methylotrophus</i>	-	-	-	-	Grinter's system	Stable insertion	Grinter, 1983
<i>P. fluorescens</i> CHA0	Yes	Southern	-	-	Bao's system	Specific insertion, no effect on growth rate was observed	Højberg <i>et al.</i> , 1999; Zuber <i>et al.</i> , 2003
<i>Pseudomonas</i> DS-S73	Yes	-	-	-	Bao's system	Specific insertion	Koch <i>et al.</i> , 2002
<i>Pseudomonas</i> S108	Yes	-	-	-	Bao's system	Specific insertion	Koch <i>et al.</i> , 2002
<i>Pseudomonas aeruginosa</i> PAC and PAO1161	Yes	Southern	-	-	CAM-OCT::Tn7 <sup>3</sup>	17 of 18 tested were similar	Caruso and Shapiro, 1982

- not tested or described in the article.

<sup>1</sup> Number of base pairs were calculated from the stop codon to the middle base of the five base pairs that are duplicated upon insertion.

<sup>2</sup> Named according to the name of the original tagging system on which the used helper plasmid and delivery plasmids are based.

<sup>3</sup> Wild type Tn7 transposon located at the plasmid.

Bacterium	Specific insertion	Test	Sequence of insertion site	Numbers of base pairs downstream of <i>glmS</i> <sup>1</sup>	System tested <sup>2</sup>	Comments	References
<i>Pseudomonas aeruginosa</i> PAO1	Yes	Southern	-	-	Bao's system	-	Klausen <i>et al.</i> , 2003
<i>Pseudomonas aeruginosa</i> PAOE1A	-	-	-	-	Grinter's system	Stable insertion	Grinter, 1983
<i>Pseudomonas corrugate</i> strain 2140	-	Southern	-	-	Barry's system	Strains was not changed after 4 years in soil	Choi <i>et al.</i> , 2003
<i>Pseudomonas fluorescens</i> 701E1	Yes	Southern	-	-	Monsanto's system	Four isolates tested. Growth not affected, stability tested	Barry, 1986
<i>Pseudomonas fluorescens</i> DR54	Yes	PCR	AJ276127	29	Bao's system		Koch <i>et al.</i> , 2001
<i>Pseudomonas putida</i> KT2440	Yes	PCR, Southern	<sup>4</sup>	25	Bao's system	Growth not affected, many isolates tested, stable and specific insertion	Lambertsen <i>et al.</i> , 2003
<i>Pseudomonas putida</i> GR12-2	Yes	Southern			Monsanto's system		Staley <i>et al.</i> , 1997
<i>Pseudomonas putida</i> R20	No	Southern			Monsanto's system		Staley <i>et al.</i> , 1997
<i>Pseudomonas putida</i> PH6	No	Southern			Monsanto's system		Staley <i>et al.</i> , 1997
<i>Pseudomonas solanacearum</i>	Yes	Southern	-	-	pAS8Rep-1::Tn7 <sup>3</sup>	16 isolates tested, suicide system used	Boucher <i>et al.</i> , 1985
<i>Pseudomonas syringae</i> pv. <i>glycinea</i> PsgR4	Yes	Southern			Monsanto's system	<i>lux</i> -based reporter system	Shen <i>et al.</i> , 1992
<i>Rhodospirillum rubum</i>	-	Southern	-	-	Bao's system	Stable, stability tested for 50 generations	Bao, 1991

<sup>4</sup> Sequenced PCR product of the region between Tn7R and *glmS*: CAGTTGATCAACACCTGACTACCCG

Bacterium	Specific insertion	Test	Sequence of insertion site	Numbers of base pairs downstream of <i>glmS</i> <sup>1</sup>	System tested <sup>2</sup>	Comments	References
<i>Salmonella thyphimurium</i>	-	-	-	-	-	-	In Caig 1989: Mak and Craig, unpub observation
<i>Serratia marcescens</i>	-	-	-	-	-	-	In Caig 1989: Qadri <i>et al.</i> , personal communication
<i>Sphingomonas yanoikuyae</i> B1	Yes	Southern	U37523	93	RKTV14::Tn7	-	Wang and Lau, 1996
<i>Xanthomonas campestris</i> pv. <i>campestris</i> NC PPB 1145	Yes	Southern	-	-	pRK2013:: Tn7	10 tested	Turner <i>et al</i> , 1984

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