Facts about the mini-Tn*7* transposon system as a tool for tagging

Based on the Tn*7* transposon, different mini-Tn*7* systems have been developed (Grinter, 1983; Barry *et al*., 1986). One of these systems, based on constructs described in Bao *et al* (1991); Højberg *et al*., (1999) and Koch *et al*., (2001) is described here. But all mini-Tn*7* transposons have common features, which provide the advantages making this system a useful tagging tool.

Important characteristics of the Tn*7*-transposon, which are maintained by the mini-Tn*7* transposons (see also the document "The Tn*7* transposon"):

- The Tn*7* transposon inserts as one copy into one chromosomal site (in most of the studied bacteria, see Table 1 in "The Tn*7* transposon" for specific information and see later in this document for test of tagged strains).

- The Tn*7* transposon insertion site is specific and located downstream of the coding region of the gene *glmS*.

- The Tn*7* transposon inserts in one orientation.

- The mini-Tn*7* transposon specific inserted sequences are stably maintained during growth.

- The mini-Tn*7* transposon insertions into this specific site do not seem to affect bacterial performance or growth. Of course, included sequences affecting the bacterium may be carried by the mini-Tn*7* transposon.

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Guidelines for using the mini-Tn*7* tagging system

The mini-Tn*7* tagging system presented here is based on the constructs developed by Koch *et al*., (2001), which are based on constructs described in Højberg *et al*., (1999) and Bao *et al*. (1991).

Sequences inserted by the mini-Tn7 transposon

The DNA sequences that are inserted by the mini-Tn*7* transposon system are: the sequences located on the delivery plasmid between the transposon ends, Tn*7*L and Tn*7*R (see Fig. 2). The delivery plasmid is some times also named the carrier plasmid.

Delivery plasmids

The delivery plasmids presented here are all pUC19 derivatives, which can replicate in *E. coli* and other *Enterics*, but for example not in *Pseudomonas.* The plasmids carry antibiotic resistance and other markers specified by the DNA located between Tn*7*L and Tn*7*R as well as resistance to ampicillin. In addition, the plasmids contain a *mob* region, which can be used for mobilising the plasmid from one bacterium to another by the RP4/ RK2 transfer genes, e.g. located on the plasmid pRK600. Constructed delivery plasmids of this series are shown in the document:"Mini-Tn7 delivery plasmids", see also Lambertsen *et al*., 2003.

Helper plasmid

For insertion of the mini-Tn*7* transposon the Tn*7* transposase genes (*tnsABCDE*) are needed and these are located on a second plasmid, the helper plasmid (Bao *et al*., 1991; see Fig. 2). In addition, this plasmid encodes resistance to ampicillin, it carries *mob* like the delivery plasmids and it contains the R6K origin and can only be maintained in a bacteria strain expressing the π-protein (replication factor).

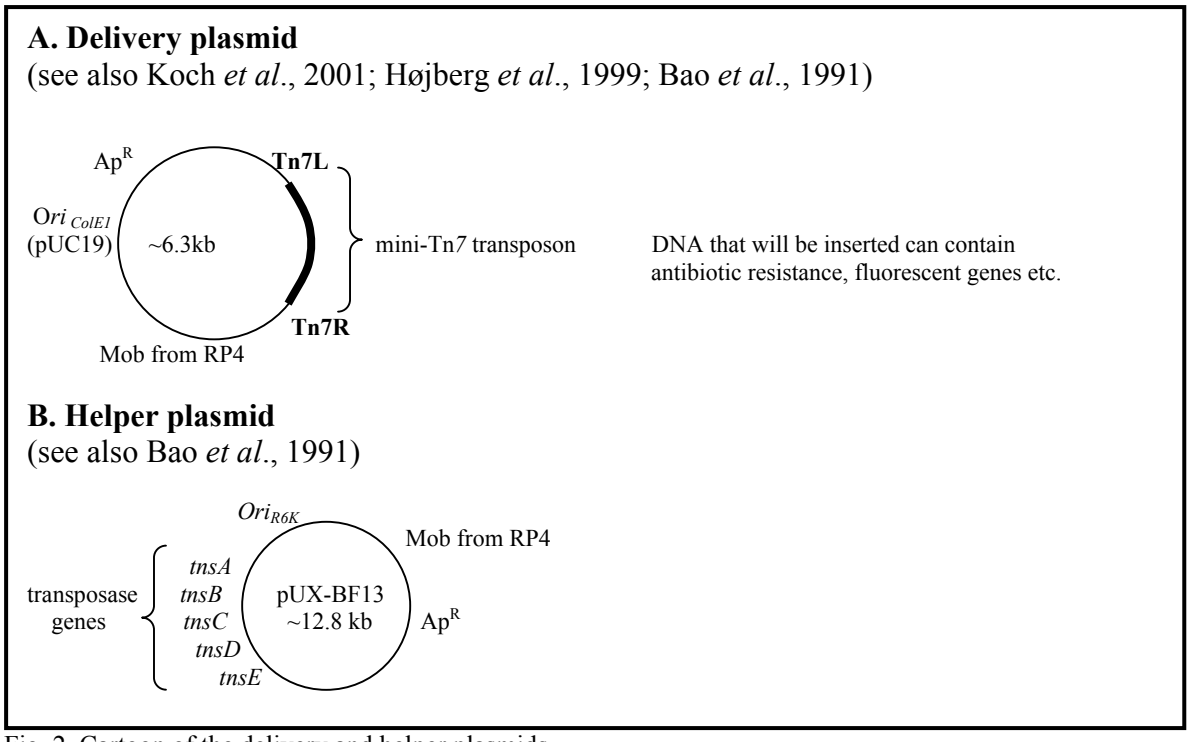

Fig. 2. Cartoon of the delivery and helper plasmids.

Principle of the system for tagging a bacterium

To tag a bacterium both the delivery plasmid and the helper plasmid must be introduced into and be present in the bacterium at the same time. When that is the case, the transposase genes will be expressed and the mini-Tn*7* transposon (the DNA flanked by Tn7L and Tn7R) will be transposed into a specific site at the chromosome. The bacterium will maintain the transposed and inserted sequence stably, whereas the two plasmids will be lost from the bacterium, when the bacteria grow, if the plasmids are unable to replicate in the bacterium being tagged.

Introduction of plasmids and tagging of a bacterium

There are two methods whereby the two plasmids can be introduced into the bacterium, which is going to be tagged: mobilisation or transformation. The two methods are described below.

Method 1: Mobilisation, by a four-parental conjugation

1) Grow the four bacteria (a, b, c and d) over night at appropriate temperature, in medium with appropriate antibiotics.
   a. bacterium that is going to be tagged
   b. bacterium with helper plasmid (pUX-BF13, grow with 100 ug/ul ampicillin)
   c. bacterium with delivery plasmid
   d. bacterium with the plasmid that can mobilise the other plasmids (ex. pRK600, grow with 6 ug/ml chloramphenicol).

2) Mix washed samples of the four cultures (for example 50 ul of each) and put them as one big spot on a non-selective plate, ex. an LB-plate or filter the bacterium onto a 0.2 μm polycarbonate filter (this increases the plasmid transfer frequency and thereby also the tagging frequency) and place that on an LB-plate and incubate at 30 °C over night or at another temperature, depending on which bacteria is tagged.

3) Select a tagged bacterium by transferring cells from the LB-plate or the filter. Dilute the cell-sample and spread on selective plates, where *E. coli* can not grow. ex. minimal citrate medium with appropriate antibiotics. Incubate over night at 30 °C. Remember also to plate a negative control and test for growth of the bacterium that has not been tagged on the selective plate. The antibiotic concentration for selective growth of tagged bacterium depends very much on the actual bacterium being tagged.

4) Re-streak single colonies to single colonies on selective plates. Then test the insertion by for example PCR (described below).

Method 2: Electroporation

1) Purify samples of the helper plasmid and the delivery plasmid.

2) Prepare electro-competent cells of the bacterium that is going to be tagged as recommended for that bacterium.

3) Introduce both plasmids (approximately 1-2 ug of each) into the bacterium by electroporation as recommended for that bacterium.

4) Grow the cells for 2-3 hours at 30 °C and spread samples of the culture on selective plates (remember also to plate a negative control, test growth of the bacterium that has not been tagged on the selective plate). Grow over night at 30 °C - the temperature depends on the bacteria being tagged.

5) Re-streak some single colonies to single colonies on selective plates. Then test the insertion by for example PCR.

Test of the tagged bacterium

After selective growth on plates only the bacteria that are tagged with the mini-Tn*7* transposon inserted into the chromosome should grow. In cases, where the mini-Tn*7* transposon contains a visual marker of course this can also be inspected.
To ensure that the mini-Tn*7* transposon has inserted into the specific attTn*7* site located downstream of the *glmS* gene, a PCR should be performed on a cell sample of the tagged bacterium.
PCR is performed with a primer that anneals to the inserted DNA and a primer that anneals to the 3'end of *glmS* (chromosomal site close to insertion site of the transposon (see Table 2, for primers that have been used to test Tn*7* insertion with). We usually do PCR on colonies with the following protocol.

Colony PCR on bacteria to check any mini-Tn*7* insertion

Make the PCR mix according to the sceme below. Take a small fresh single colony from the selective plate (we often use ABTC plates, LB tends to inhibit PCR) and put it either directly in the PCR mix or put it in 50 ul water and boil for 5 min and from this take 5-10 ul and use as template in the PCR mix (if this last posibility is used remember to ajust the water amount in the PCR reaction). The PCR is run according the the PCR programe decribed below.

PCR-mix	Stock sol.	1 x PCR	10 x PCR	20 x PCR	x PCR	Final conc.
10x PCR-buffer*	10 x	5.0 µl	50 µl	100 µl		1 x
dNTP	10 mM	1.0 µl	10 µl	20 µl		200 µM
Primer 1: Tn7-Glms	10 pmol/µl	2.0 µl	20 µl	40 µl		0.4 pmol/µl
Primer 2: Tn7-RR 109	10 pmol/µl	2.0 µl	20 µl	40 µl		0.4 pmol/µl
Taq DNA polymerase*	5 units/ µl	0.4 µl	4 µl	8 µl		0.04 ŭ/ µl
H_2O	----	39.6 µl	396 µl	792 µl		
In total		50.0 µl	500 µl	1000 µl		

*Amershampharmacia Biotech (product number: 27-0799-62)

	PCR Program		
	96° C	5 min	
25 cycles	96° C	30 sek	denaturation
	53° C **	30 sek	annealing
	72° C	30 sek	elongation
	72° C	5 min	final elongation

**Annealing temperature used depends on which set of primers being used.
After the PCR the fragments are visualised on a 1 to 1.5 % agarose gel.

Table 2. Primers that may be used to test the mini-Tn*7* insertion

Name of primer	Sequence	Annealing site[1]	Tm	Comments
Tn7-GlmS	5'AATCTGGCCAAG TCGGTGAC[2]	Nt 310 from the start site of *glmS*	57.4	Used for every PCR to test Tn*7* insertion.
Tn7-Gm	5'ATATCGACCCAA GTACCGCC	Nt 509 from start site of *aaC1* from pAComegaGm[3]	57.4	
Gfp(478)	5´CATCATGGCAGA CAAACAAAAG	Nt 478 from the start site of *gfp*	54.4	Designed by Jens Bo Andersen
Tn7R109	5'CAGCATAACTGG ACTGATTTCAG	Nt 109 at the Tn7R sequence described in Craig, 1989[4].	58.9	Combined with Tn7-GlmS it produces a fragment of approx 150 bp. Can be used to check any Tn7 insertions.

[1] Annealing of 5'-end of the oligo.

[2] the sequence used was chosen from alignment of the *glmS* sequences from *P. syringae* pv. *tomato* (gnl|TIGR_323|psyring_3888), *P. putida* KT2440 (gnl|TIGR|pputida_10722), *P. fluorescens* DR54 (AJ276127), *P. aeruginosa* (AE004681) and *Haemophilus influenzae* Rd (U32726).

[3] Accession number U22104.

[4] Tn7R described in Craig, 1989 is identical to the Tn7L sequence described by Lightenstein (1982), having accession number: J01837.

Other useful comments concerning tagging of specific bacteria

Pseudomonas putida tagging

For selection: Minimal medium with citrate (ABTC) plates + 8-10 ug/ul Gm or 25 ug/ul Km, depending on the Tn*7*-tag. Tagging has been done both by electroporation and mobilisation, it works well with both methods, but most commonly mobilisation is used here.
For some strange reason the Km-resistance gene does not provide resistance to 25 ug/ml Km on LB plates. This phenomenon has also been observed earlier with the Tn5 transposon with the same Km resistance gene (unpublished observations).

Pseudomonas aeruginosa tagging, (this protocol was used and developed by Marie, Anders, Mikkel, Arne, and others working with *P. aeruginosa* in our lab)

Grow over night cultures of **R**ecipient (*P. aeruginosa*), **D**onor (strain with delivery plasmid) and **H**elpers (both *E. coli*) strains at 37°C in LB and the required antibiotics.

1. Next day inoculate 0.5 mL R in 10 mL LB in the morning, and incubate at 41°C no shaking. After 3 hours inoculate 0.5 mL of both of D and H in each 10 mL LB and incubate at 30°C shaking. After 4 hours the strains should be ready.
2. Take a 1:1:1:1 (1 mL each) ratio of R, D and H, mix and transfer to a filter: Poretics, polycarbonate, 25 mm, 0.22 micrometer. Make three mixtures on three filters.
3. Place the filters on each of three preheated LB plates and incubate at 30, 37 and 42°C over night.
4. Day after plate 10^0, 10^{-1} and 10^{-2} on selective plates at 37°C.

Selective plates used are e.g. PIR-plates with 60 ug/ul Gm or 500 to 1000 ug/ul Str.

Stains that have been tagged with Tn*7* in this lab by staff or visitors are:

Pseudomonas putida KT2440
Pseudomonas putida DOT
Pseudomonas aeruginosa PAO1
Pseudomonas aeruginosa PA14
Shewanella
Ralstonia
Pseudomonas putida OUS82
Pseudomonas fluorescens SBW25
Pseudomonas aeruginosa PAK
Pseudomonas syringae
Caulobacter crescentus

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