

Protocol for inserting transgene using Tn7 (6/1/06)

This system uses Tn7 to insert transgenes at a defined neutral site in the chromosome (*attTn7*). The site is highly conserved and is known to work as a Tn7 attachment site in *E. coli* and its relatives. The *attTn7* sequence is conserved in most (all) bacteria and whether or not Tn7 can insert in them is simply a matter of testing.

The RP4 *oriT* sequence that I have recently added to these vectors allows for easy conjugation of plasmids into various bacteria using the *E. coli* strain SM10 that supplies all required proteins for RP4-dependent conjugation *in trans*. This protocol describes introduction of transgene DNA into bacteria by standard bacterial transformation.

1. Clone your favourite gene into multiple cloning site of pGRG25 or pGRG36. These plasmids are identical, except that pGRG25 does not have the *Sma*I site. Unique sites in pGRG36 are *Pac*I, *Sma*I, *Not*I, *Xho*I, *Avr*II and *Asc*I sites in the multiple cloning site. Sequences of both of these plasmids can be found using the GenBank accession # DQ460223.
2. Prepare DNA for transformation. Note these plasmids all carry the pSC101 temperature sensitive origin, so they must be grown at 30-32°C to allow replication. They are also low-copy plasmids, and will produce much lower amounts of DNA than standard *ColE1* origins (for example pUC19). I use the Qiagen kits with the very low-copy plasmid modifications to obtain a good yield of DNA.
3. Transform plasmid into your strain, recover as usual, and select for plasmid on LB+ampicillin (100ug/ml) at 32°C (though many people use 30°C for this replicon, it will take 2 days to see a colony; at 32°C very small colonies will be visible after just one day).
4. Pick 3 colonies (the process works in approximately 50-95% of cells, so picking 3 or more will ensure that you get one with a chromosomal insert) and streak out once on LB+ampicillin, 32°C.
5. Start cultures of each in LB at 32°C (no ampicillin – this step allows for some loss of plasmid) and grow overnight. Dilute cultures appropriately to recover single cells (I dilute 10^{-7} and plate 50 uL of culture) and plate on LB plates. Grow at 42°C to block replication of the plasmid.
6. Streak colonies once on LB at 42°C to ensure complete loss of the plasmid. Do PCR on colonies (I suspend a small colony in 50 uL of water and use 2 uL as a template for PCR) using the primers: 5'GATGCTGGTGGCGAAGCTGT and 5'GATGACGGTTTGTCACATGGA. These flank the Tn7 attachment site in the *glmS* gene. In the absence of a Tn7 insertion, they will yield a 678 bp product. In the presence of Tn7, this product will increase in size by 904 bp + the size of your insert (i.e. it will be 1582 bp + your insert). Screen all purified isolates to ensure that they are ampicillin sensitive (the plasmid is retained in less than 1 in 10,000

cells grown this way, so it should almost never be a problem). The PCR primers are to the chromosome, not to the Tn7, so they will not prime from the plasmid.

For more information, see our publication:

McKenzie, GJ & Craig, NL. 2006. Fast, easy and efficient: site-specific insertion of transgenes into Enterobacterial chromosomes using Tn7 without need for selection of the insertion event. *BMC Microbiol* **6**:39.

<http://www.biomedcentral.com/1471-2180/6/39>