# A Tn*7*-based broad-range bacterial cloning and expression system

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**For many bacteria, cloning and expression systems are either scarce or nonexistent. We constructed several mini-Tn*7* vectors and evaluated their potential as broad-range cloning and expression systems. In bacteria with a single chromosome, including *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Yersinia pestis*, and in the presence of a helper plasmid encoding the site-specific transposition pathway, site- and orientation-specific Tn*7* insertions occurred at a single *att*Tn*7* site downstream of the *glmS* gene. *Burkholderia thailandensis* contains two chromosomes, each containing a *glmS* gene and an *att*Tn*7* site. The Tn*7* system allows engineering of diverse genetic traits into bacteria, as demonstrated by complementing a biofilm-growth defect of *P. aeruginosa*, establishing expression systems in *P. aeruginosa* and *P. putida*, and 'GFP-tagging' *Y. pestis*. This system will thus have widespread biomedical and environmental applications, especially in environments where plasmids and antibiotic selection are not feasible, namely in plant and animal models or biofilms.**

Over 150 bacterial genomes have been sequenced, but tools for genetic and expression analyses have not kept up with the 'sequencing pace'. Such tools are either scarce or nonexistent for many of these bacteria, or have a narrow host range. The result is that much of the information contained in bacterial genomes cannot be exploited to its fullest potential. Tn*7*-based vectors have the most promise in bringing us a step closer to the development of a broad-range bacterial cloning and expression system, both in terms of host-range capabilities (Tn*7* was shown to transpose in at least 20 bacterial species thus far (refs. 1 and 2, information from PubMed and unpublished data)) and ease of use. The molecular biology of Tn*7* is well understood[2,3]. Whereas most transposable elements move at low frequencies and have little target site–selectivity, Tn*7* can insert at a high frequency into bacterial chromosomes site- and orientation-specifically at Tn*7* attachment, or *att*Tn*7*, sites. These sites are located downstream of highly conserved *glmS* genes, which encode essential glucosamine-6-phosphate synthetase[2,3], but recognition by the transposase also requires critical portions of the *glmS* 3′ region[3]. Tn*7* transposition minimally requires the Tn*7* left (Tn*7*L) and Tn*7* right (Tn*7*R) ends, and the transposase complex, which comprises the products of five genes, *tnsABCDE*. TnsABC+D catalyzes high-frequency transposition into *att*Tn*7*, whereas TnsABC+E directs transposition at much lower frequencies into chromosomal non-*att*Tn*7* sites and, preferentially, conjugative plasmids[3]. Elements containing $<200$ base pairs (bp) of Tn*7*L and Tn*7*R still transpose at relatively high ($10^{-3}$ to $10^{-4}$) frequencies in the presence of a helper plasmid expressing the transposase[4]. Transposon delivery and transient expression of transposase are achieved with the help of suicide plasmids, which only persist transiently in a target bacterium because it cannot support replication of these plasmids. Here we describe a series of small, fully sequenced mini-Tn*7* vectors, which contain selection markers that, after transposition, can be excised from the bacterial chromosome with yeast Flp recombinase[5]. We demonstrate efficient mini-Tn*7* transposition into diverse target bacteria for gene complementation and expression in biofilms and an animal model, as well as for establishment of regulated gene expression systems.

## RESULTS

### Delivery vectors, mini-Tn*7* elements and helper plasmids

The fullest potential of previously devised Tn*7* systems could not be fully exploited because their individual components were not engineered for general applicability[6–9]. We therefore developed optimized delivery vectors, mini-Tn*7* elements and helper plasmids.

We constructed two suicide mini-Tn*7* delivery vectors in this study (**Fig. 1a**). The pUC18T-based vectors contain the ColE1 origin of replication and do not replicate outside of the *Enterobacteriaceae*. They thus serve as efficient suicide delivery vectors in many bacteria. By contrast, pUC18TR6K-based vectors contain the R6K origin of replication and, therefore, depend on the presence of the *pir* gene for replication. Thus, they allow suicide delivery in all bacteria, including the *Enterobacteriaceae* and closely related species[10].

We derived mini-Tn*7*T (**Fig. 1b**) from mini-Tn*7* by insertion of two strong transcriptional terminators to prevent readthrough from the *glmS* promoter after chromosomal insertion and a multiple cloning site (MCS) for cloning antibiotic selection markers, reporter genes and other DNA fragments destined for chromosomal insertion. Based on mini-Tn*7* and mini-Tn*7*T, an entire family

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of mini-Tn*7* elements was derived (**Supplementary Fig. 1** online and **Supplementary Table 1** online), ranging from those containing simple selection markers (mini-Tn*7*T-Gm, **Fig. 1b**), to those allowing construction of β-galactosidase (*lacZ*) and luciferase (*lux*) transcriptional fusions, strains for regulated expression from the *tac* promoter and self-cloning of *att*Tn*7* flanking sequences (mini-Tn*7*T-Gm with the $ori_{R6K}$ replicon (mini-Tn*7*T-Gm-REP); **Fig. 1b**). The selection markers contained in the mini-Tn*7* elements are flanked by *FRT* sites and can therefore be excised from the chromosome with Flp recombinase after mini-Tn*7* insertion. Although we only tested gentamycin (Gm) and kanamycin (Km) resistance markers ($Gm^R$ and $Km^R$, respectively) in this study, we generated several improved *FRT* cassettes with chloramphenicol (Cm), tetracycline (Tc) and trimethoprim (Tp) markers for use in other bacteria (**Supplementary Table 1**).

The most commonly used helper plasmid pUX-BF13 encodes the TnsABC+D specific, as well as the TnsABC+E nonspecific transposition pathways. To eliminate potential complications caused by the superfluous nonspecific pathway, we constructed pTNS1, a mobilizable helper plasmid encoding only the specific TnsABC+D transposition pathway. Although we mostly used pTNS1 for the studies described here, we are now also using a pTNS1 derivative, pTNS2, because its entire sequence is known and transposition efficiencies are slightly higher when compared to pTNS1.

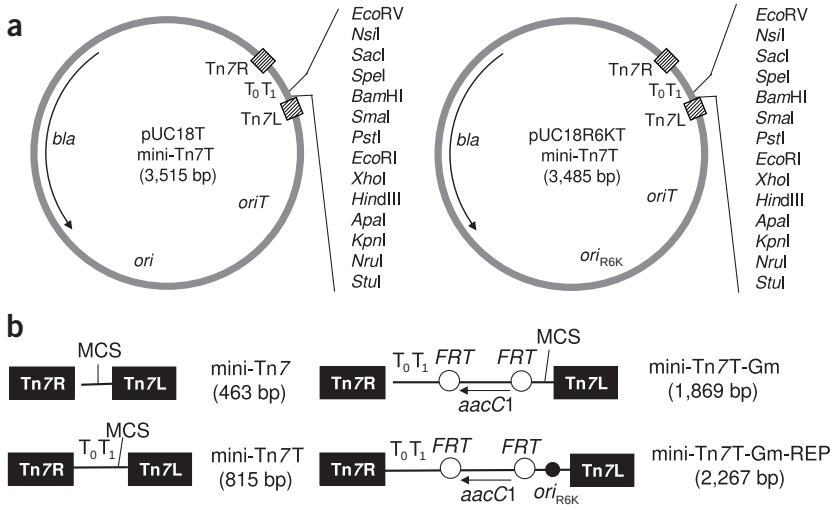


**Figure 1** | Suicide delivery vectors and mini-Tn*7* elements. (**a**) pUC18-based suicide delivery plasmids containing mini-Tn*7* base elements. These plasmids are mobilizable derivatives of pUC18-mini-Tn*7* and pUC18R6K-mini-Tn*7* (for details see **Supplementary Fig. 1**). *bla*, β-lactamase–encoding gene; *ori*, ColE1 origin of replication; $ori_{R6K}$, R6K origin of replication; *oriT*, origin of conjugative transfer; $T_0T_1$, transcriptional terminators $T_0$ and $T_1$ from bacteriophage λ and *E. coli rrnB* operon, respectively; Tn*7*L and Tn*7*R, left and right end of Tn*7*, respectively. (**b**) Maps of selected mini-Tn*7* elements. The mini-Tn*7* elements are contained on the suicide delivery vectors shown in **a**, except mini-Tn*7* which is located on pUC18. Abbreviations: *aac*C1, Gm acetyl transferase–encoding gene; *FRT*, Flp recombinase target; MCS, multiple cloning site (for mini-Tn*7* and mini-Tn*7*T the MCS are the same as in **a**; in others some sites may not be unique; for details see GenBank sequences). Maps of other transposons derived from mini-Tn*7* and mini-Tn*7*T are shown in **Supplementary Fig. 4** online.

## Mini-Tn7 transposition in *P. aeruginosa*

A previous report described the use of Tn*7* derivatives for GFP-tagging proteins in *P. aeruginosa*[8], but the vectors were very specialized: they did not allow for marker excision and the insertion site was not reported. In this study we used *P. aeruginosa* as a test strain for our newly developed vectors, but methods for Tn*7* delivery, transposition into *att*Tn*7* and verification of transposition events are similar for other bacteria.

Electroporation delivery routinely yields >500 transformants per 50 ng each of mini-Tn*7* delivery vector and helper plasmid input DNAs. Southern blot analysis of ten transformants confirmed that all Tn*7* insertions occurred in the same chromosomal DNA fragment (data not shown), and we suspected the Tn*7* insertion site to lie in the 54-bp *glmS*-PA5548 intergenic region (**Fig. 2**), as had been previously suggested[9]. PCR analysis using various primer pairs designed to the *glmS* region and Tn*7* ends verified this location and yielded the PCR fragments indicated in **Figure 2**. Sequence analysis of PCR fragments obtained from two separate transformants after Flp excision of $Gm^R$ revealed the insertions between nucleotides 24 and 25, and 25 and 26 downstream of the *glmS* stop codon (for details see GenBank accession number AY619007). These sites seem to lie in one of the inverted repeats of the putative *glmS* transcriptional terminator and most likely render it dysfunctional. We confirmed this by transposition of either mini-Tn*7*-Gm-*lacZ* (containing no transcriptional terminators) or mini-Tn*7*T-Gm-*lacZ* (containing the $T_0$ and $T_1$ terminators) into strain PAO1. Whereas substantial (267 Miller units) *lacZ* transcription could be observed in the strain containing mini-Tn*7*-*lacZ*, only background levels of transcription (10 Miller units) were measured in the strain containing mini-Tn*7*T-*lacZ*.

## Use of mini-Tn7 in other bacteria

The versatility of the various mini-Tn*7* constructs was tested by transposition into bacteria posing diverse challenges.

Because a previous report indicated that in *P. putida* strains Tn*7* derivatives did not transpose to a unique site[11], we chose this strain to address the issue of site-specific versus random transposition. Transposition of mini-Tn*7*-Gm into the *P. putida* chromosome was achieved by coelectroporation of competent KT2440 cells with pUC18-mini-Tn*7*-Gm and either pUX-BF13 or pTNS1 helper DNA. Genomic Southern blot and PCR analyses revealed that with pUX-BF13, of ten $Gm^R$ transformants tested, three (or 30%) had clean insertions at the putative *att*Tn*7* site downstream of *glmS* in the 171-bp *glmS*-PP5408 intergenic region (**Fig. 3**); five of ten transformants (or 50%) had insertions at *att*Tn*7*, but some chromosome rearrangements around *att*Tn*7* were evident. Only two (or 20%) of the insertions tested were located outside of the *glmS* region. In contrast, when we used pTNS1, all insertions occurred at *att*Tn*7* and 60% of them were clean insertions $21 \pm 1$ bp downstream of *glmS*, that is, there was no evidence of chromosome rearrangements in the *att*Tn*7* region, which were observed in the other 40% of transformants. But the 60% clean

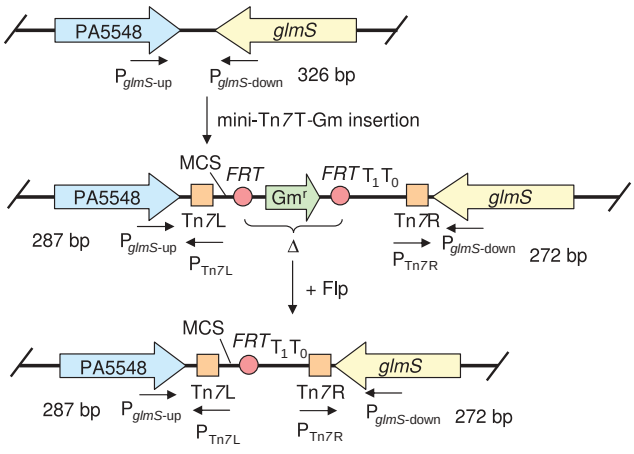


**Figure 2** | Integration of mini-Tn*7* in *P. aeruginosa*. Mini-Tn*7*T-Gm was transposed into the PA01 chromosome after coelectroporation of pUC18-mini-Tn*7*T-Gm and pTNS1, and $Gm^R$ transformants resulting from transposition of mini-Tn*7*T-Gm into the PA5548-*glmS* intergenic region were selected. In most instances, the $Gm^R$ marker was removed by Flp-mediated excision resulting in strains with unmarked mini-Tn*7* insertions. Verification of transposition events by PCR using the primer pairs shown by convergent arrows yields PCR fragments whose sizes are indicated in bp. Integration of mini-Tn*7* in other bacteria follows the same general scheme and integration sites are schematically illustrated in **Fig. 3**.

*att*Tn*7* insertion success rate achieved is more than satisfactory given the fact that no system for site-specific chromosomal gene integration now exists for this bacterium.

*Y. pestis* is on the select agent list because of bioterrorism and biowarfare concerns[12]. Although this bacterium can be genetically manipulated with relative ease, no site-specific gene integration system is now available, which would be desirable for pathogenesis studies. We coelectroporated cells of two different biovars, KIM6 (Medievalis) and AZ96-2456 (Orientalis), with pUC18R6K-mini-Tn*7*T-Km and pTNS1. Genomic Southern blot and PCR analyses of ten $Km^R$ isolates of each strain indicated that Tn*7* transposed to a unique site in the chromosome of either biovar. Sequence analysis revealed that the insertion site was identical in each strain and located 25 nucleotides downstream of *glmS* in the 482-bp *glmS-pstS* intergenic region (**Fig. 3**).

Lastly, to prove the general usefulness of the new vectors for bacteria for which the genome sequences are yet unknown, incomplete or unpublished, we transposed a mini-Tn*7*T-Gm-REP derivative containing the R6K origin of replication into the genome of *B. thailandensis* strain E264. *B. thailandensis* is closely related to *B. pseudomallei* and *B. mallei*, the causative agents of melioidosis[13] and glanders[14], respectively, and thus can serve as a surrogate for these select-agent bacteria. After self-ligation of DNA fragments containing the mini-Tn*7* element, which yielded plasmids containing the R6K origin of replication and a $Gm^R$ marker, we determined the *att*Tn*7* sites by sequencing the Tn*7*-chromosomal DNA junction sequences using $P_{Tn7L}$ and $P_{Tn7R}$ as sequencing primers. All insertions thus verified demonstrated that the *B. thailandensis* genome contains two closely-related *glmS* genes, *glmS*1 on chromosome 1 and *glmS*2 on chromosome 2. Tn*7* insertion occurred 25 bp downstream of either *glmS*1 or *glmS*2 (**Fig. 3**), but none of the four transformants tested had insertions at both loci. We then verified the insertion sites by using $P_{Tn7L}$ and a $glmS_{Bt}$-specific primer, that is, either $P_{glmS1}$-DN or $P_{glmS2}$-DN. Despite the presence of two separate insertion sites, site-specific insertions can therefore be rapidly screened using specific PCR primers that yield PCR fragments of defined sizes, depending on which *att* site is used.

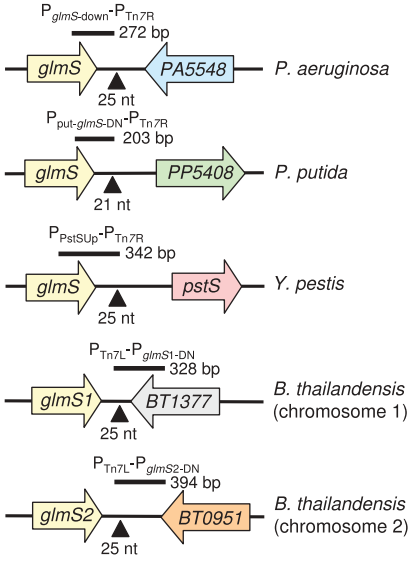


**Figure 3** | Tn*7* insertion sites in various gram-negative bacteria. Mini-Tn*7* elements containing suitable selection markers, for example, $Gm^R$ for *P. aeruginosa* PA01, *P. putida* KT2440, *B. thailandensis* E264 and $Km^R$ for *Y. pestis* strains Kim 6 (biovar *Mediaevalis*) and AZ96-2456 (biovar *Orientalis*), were transposed into the respective chromosomes. The insertion sites (indicated by triangles) for bacteria with known genome sequences (*P. aeruginosa*, *P. putida* and *Y. pestis*) were derived by PCR amplification of Tn*7*-chromosomal DNA junction sequences using species-specific primers, followed by nucleotide sequencing of the PCR products (indicated by horizontal bars labeled with the expected product sizes). The insertion sites for *B. thailandensis* were obtained by sequencing transposon-chromosomal DNA junction sequences using plasmids obtained after self-ligation of chromosomal restriction fragments containing transposons with an $ori_{R6K}$ and a $Gm^R$ marker. They were subsequently verified by sequencing the indicated PCR fragments. Boxed arrows mark genes and their transcriptional orientations.

## Applications of mini-Tn*7* elements

Two major utilities for site-specific insertion elements are the ability to perform experiments in environments where antibiotic selection is not feasible and to expand the range of well-characterized gene expression systems to bacteria other than *E. coli*[15].

To prove stable complementation in the absence of selection, we performed two experiments. First, we integrated a mini-Tn*7* containing the cloned *nfxB*$^+$-*mexC*$^+$*D*$^+$-*oprJ*$^+$ genes into the chromosome of the Δ(*mexCD-oprJ*) *P. aeruginosa* strain PAO238 (**Fig. 4a**) and showed that the cloned genes complemented its biofilm-deficient growth phenotype in the presence of subinhibitory azithromycin concentrations (**Fig. 4b** and data not shown). Second, to demonstrate the utility of mini-Tn*7* for generation of bacteria expressing GFP for *in vivo* tracking, we transposed the mini-Tn*7*T-Km-GFP into the *Y. pestis* A1122 chromosome (**Fig. 4c**) and monitored the fate of A1122-GFP cells after

intraperitoneal injection. We found that only 30 min after intraperitoneal injection, 7% of peritoneal exudate cells (PECs) were positive for A1122-GFP (**Fig. 4d**). This result shows that 'GFP-tagged' *Y. pestis* rapidly associate with mouse PECs and that the signal from single-copy, chromosomally encoded GFP is sufficiently strong for detection by fluorescence-activated cell sorting (FACS).

To obtain proof-of-principle for the utility of mini-Tn*7* vectors for establishment of expression systems, we constructed several *P. aeruginosa* expression strains. To establish a regulated expression system that is entirely chromosomally located, we cloned a promoterless *xylE* reporter gene into the MCS of mini-Tn*7*T-Gm-LAC. After integration at PAO1 *att*Tn*7* and Flp-mediated excision of $Gm^R$, we obtained a *P. aeruginosa* strain that contained a chromosomal $P_{tac}$-*xylE* expression cassette under control of the neighboring *lacI* gene with increased expression owing to the q promoter mutation (*lacI*$^q$) (**Fig. 4e**). We then measured catechol-2,3-dioxygenase (C23O) expression in isopropyl-β-D-thiogalactopyranoside (IPTG)-induced and uninduced cells of this strain. Whereas uninduced cells had little C23O activity over the entire testing period, this activity was readily inducible and measurable in cells induced for as short as 1 h (**Fig. 4f**). As previously demonstrated in *E. coli* and *P. aeruginosa*[16,17], transposable elements and site-specific gene integration systems are also useful for construction of host strains permitting establishment of a regulated α complementation and expression systems. When we transposed mini-Tn7-Gm-LacZΔM15 into the *P. aeruginosa* PAO1 chromosome (**Fig. 4g**); the resulting strain allowed IPTG-dependent blue-white screening (**Fig. 4h**) and IPTG-inducible *xylE* expression from $P_{lac}$ (data not shown) after transformation with pUCP19 (vector) or its recombinant derivative pCDO[17]. We obtained similar data after transposition of mini-Tn*7*-Gm-LAC into *P. putida* KT2440 (data not shown).

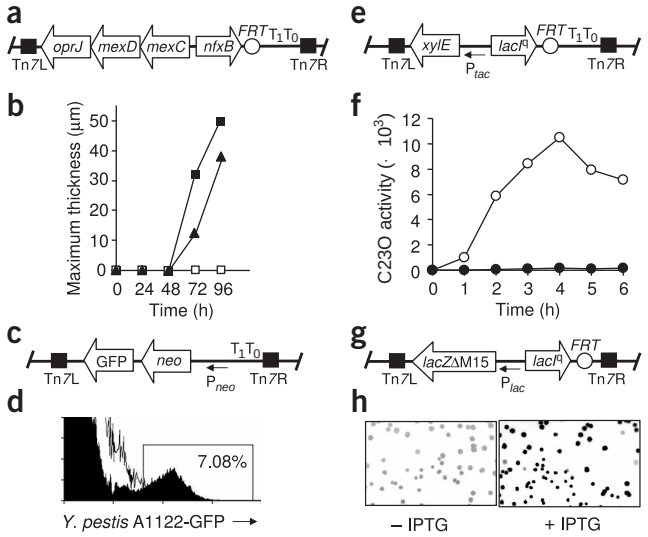


**Figure 4** | Mini-Tn*7* vector applications. (**a**,**b**) Complementation of the biofilm growth defect of *P. aeruginosa*. The PAO1 *mexCD-oprJ* operon and its *nfxB* regulator was cloned into mini-Tn*7*T-Gm and transposed into the PAO238 Δ(*mexJK-oprJ*) chromosome, followed by Flp-mediated excision of the $Gm^R$ marker. Thicknesses of biofilms formed by PAO1 (■), PAO238 (□) and complemented PAO238 (▲) in the presence of subinhibitory azithromycin concentrations were assessed by microscopy at the indicated time points after inoculation. (**c**,**d**) 'GFP tagging' of *Y. pestis*. A mini-Tn*7* construct expressing GFP from the *neo* promoter ($P_{neo}$) was integrated into the *Y. pestis* A1122 chromosome. FACS analysis was used to determine the distribution of A1122 cells expressing GFP (black area), including those within mouse peritoneal exudate cells (boxed area). Cells were harvested 30 min after intraperitoneal infection. The white area represents the signal obtained with the PBS control. (**e**,**f**) Establishment of a chromosomal $P_{tac}$ expression system. A promoterless *xylE* gene was cloned into mini-Tn*7*T-Gm-LAC and transposed into the *P. aeruginosa* PAO1 chromosome, followed by Flp-mediated excision of the $Gm^R$ marker. C23O activity in uninduced (●) and IPTG-induced (○) cells was measured at the indicated time points. (**g**,**h**) α complementation in *P. aeruginosa*. Mini-Tn7-Gm-LacZΔM15 was transposed into PAO1 and the $Gm^R$ marker was excised. The resulting strain was transformed with either pUCP19 (vector) or pCDO (vector + *xylE* insert). A ~15:1 mixture of pUCP19- or pCDO-containing cells was plated on LB medium with Cb and X-Gal in the presence or absence of IPTG.

## DISCUSSION

Most existing bacterial chromosome integration systems have a narrow host range because they are either based on phage *attB*[18–21] or species-specific[22] chromosomal integration sites. Though transposon-based systems can have a broad host range, the resulting insertions tend to be random (for example, Tn*1545*, which integrates in various Gram-positive bacteria at diverse chromosomal *att* sites[18]). Although random transposon delivery, such as that via mini-Tn*5*[23], is generally widely applicable, it has several drawbacks. First, insertions occur randomly in the genome, most often within coding sequences. Consequently, considerable effort is required to determine the transposon insertion sites and the fitness of the mutant bacteria. Second, as most bacteria lack efficient chromosomal gene transfer procedures, integrated transposons cannot easily be transferred between different mutant backgrounds for meaningful comparative analyses. Site-specific integration vectors, like mini-Tn*7*, overcome many of these hurdles. First, because they integrate at an intergenic, naturally evolved site, compromise of bacterial fitness as a consequence of gene integration is generally of little or no concern. Second, the same constructs can be rapidly integrated in a site- and orientation-specific manner into many different mutant backgrounds, thus facilitating comparative gene expression analyses. Possible drawbacks of site-specific integration systems include lack of integration sites in some bacteria or absence of host factors needed for integration.

The family of mini-Tn*7* vectors described here provides high-level versatility in terms of suicide vector delivery (pMB9 and R6K origins of replication), delivery options (electroporation or conjugation), vector capabilities (small size with carefully engineered MCS and terminators to avoid read-through from *glmS*), choice of selection markers (engineered to be devoid of restriction sites and to be excisable *in vivo*) and availability of vector sequences from GenBank. Although we have not constructed all possible combinations of suicide delivery vectors and mini-Tn*7* elements with diverse selection markers, we provide all the tools and strategies necessary to derive the appropriate vectors for the bacterium of choice.

When we used pTNS1 or pTNS2; insertions in bacteria with single chromosomes always occurred site-specifically at *att*Tn*7* sites associated with single *glmS* genes. *B. thailandensis* provided an example of a bacterium with two chromosomes, revealing two similar *glmS* genes and two *att*Tn*7* sites, highlighting the close association of *glmS* and *att*Tn*7*. The presence of two *att*Tn*7* sites

may offer the possibility of sequentially integrating two separate mini-Tn*7* constructs. This should be possible because although secondary Tn*7* transposition is subject to target immunity, Tn*7* immunity is not a global immunity, but is dependent on distance between separate insertion events[24]. But insertion of two Tn*7*s into the same genome may lead to recombination events, which could be deleterious to the cell. Furthermore, immunity and structural similarities may complicate the use of Tn*7* elements in bacteria naturally harboring transposons of the superfamily that Tn*7* is a member of, but we have not yet encountered this situation.

Mini-Tn*7* elements integrate at an intergenic site and therefore probably have little effect on bacterial fitness. But because *att*Tn*7* sites are located in the 3′ end of the *glmS* mRNA, insertions could have an adverse effect on GlmS expression, which could affect peptidoglycan synthesis. Fortunately, this does not seem to be the case because *P. aeruginosa* and *Y. pestis* wild-type and integrant strains recovered with equal efficiency from stationary phase, indicating that rates of peptidoglycan synthesis were not affected by the insertions (**Supplementary Fig. 2** online). Once integrated into the chromosome, expression or reporter gene constructs and complementing fragments are stable and do not require continued antibiotic selection. When we grew *P. aeruginosa* and *Y. pestis* integrants in the absence of selection for 100 generations, insertions were 100% stable (**Supplementary Fig. 3** online). This facilitates applications in environments where antibiotic selection is not feasible (for example, animal and plant models, biofilms, and others). Optional removal of the antibiotic resistance markers facilitates downstream applications, such as transformation with complementing plasmids or expression constructs, or allows construction of unmarked strains for applications in which the presence of antibiotic resistance markers is disallowed or is undesirable (for example, vaccine strains and strains destined for environmental release).

The new vectors allow engineering of bacteria for various environmental and biomedical applications. First, we demonstrated the use of mini-Tn*7* for single-copy, that is, as nature intended it to be, complementation in a biofilm, an environment in which plasmids are difficult to select. Second, we used the method for stable 'GFP-tagging' of *Y. pestis* for studying dissemination of this bacterium from the initial site of infection. Third, as we demonstrated with *P. aeruginosa* and *P. putida,* the mini-Tn*7* vectors allow expansion of well-characterized and familiar host and regulated expression systems to bacteria other than *E. coli*, even those for which the necessary plasmid vectors are not available because the entire expression system can be engineered into the chromosome.

In this study, we focused on Gram-negative bacteria. But as *glmS* genes are present in all sequenced bacterial chromosomes (**Supplementary Table 2** online), we predict that mini-Tn*7* elements will be applicable in many other species. Despite the universal presence of *glmS* genes in bacteria, however, Tn*7* transposition has not yet been documented in Gram-positive bacteria. This could be an indication of a possible limitation of the Tn*7* system or simply a lack of application and/or proper engineering of the system for use in these bacteria. In a few genomes, *glmS* either overlaps with or is separated by an intergenic region from the downstream gene that is shorter than the average distance of *att*Tn*7* from *glmS* (25 bp). In these cases, Tn*7* may actually insert within a coding sequence, but because *att*Tn*7* sequences do not have signature motifs and can only be experimentally determined, it is unknown whether or not this will actually occur or have any consequences in these bacteria.

## METHODS

**Bacterial strains and media.** We grew *P. aeruginosa* PAO1 (ref. 25), its Δ(*mexAB-oprM*) Δ(*mexCD-oprJ*) derivative PAO238 (ref. 26), *P. putida* KT2440 (American Type Culture Collection strain 47054) and *B. thailandensis* E264 (ref. 27) strains in LB broth (Becton Dickinson). *Y. pestis* strains Kim6+, its *pgm* derivative A1122 (biovar Medievalis) and AZ96-2456 (biovar Orientalis), all from the Centers for Disease Control and Prevention (CDC)-Fort Collins collection, were grown in heart infusion broth (Difco). We added antibiotics to media as follows: for *E. coli*, ampicillin (Ap), 100 μg/ml; Gm, 15 μg/ml; Km, 35 μg/ml; for *P. aeruginosa*, carbenicillin (Cb), 200 μg/ml; Gm 30 μg/ml; for *P. putida*, Cb, 1,000 μg/ml; Gm, 30 μg/ml; for *B. thailandensis*, Gm, 450 μg/ml; and for *Y. pestis*, Km, 35 μg/ml. β-galactosidase phenotypes were assessed on agar plates supplemented with 40 μg/ml 4-bromo-5-chloro-β-indolyl-D-galactopyranoside (X-Gal), with or without 1 mM IPTG. We grew biofilms in flow cells in FAB medium (10 mM sodium citrate, 0.5% casamino acids, 0.15 mM $(NH_4)SO_4$, 0.33 mM $Na_2HPO_4$, 0.2 mM $KH_2PO_4$, 0.5 mM NaCl, 0.1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.01 mM Fe-EDTA) in the presence of 2 μg/ml azithromycin (AZM) and analyzed them as previously described[28].

**Plasmid and transposon construction.** Standard molecular biology procedures were used[29]. Detailed procedures on construction of individual plasmids and transposons are provided in the **Supplementary Methods** online. We assembled the mini-Tn*7* elements on pUC18 or its mobilizable derivatives, which contain either the original ColE1-derived (pUC18T) or the R6K origin of replication (pUC18R6KT). The Tn*7* helper plasmid pTNS1 was derived from pUX-BF13 (ref. 7) by deletion of a 909 bp *Cla*I fragment from within the *tnsE* 5′ coding sequences. For pTNS2, we cloned a 6,698-bp *Eco*RI-*Cla*I (blunt) fragment from pTNS1 between the *Eco*RI and *Sma*I sites of pUC18R6KT, in which the *tnaA,B,C* and *D* genes are constitutively transcribed from the *E. coli lac* operon promoter.

**Tn*7* delivery and stability of recombinant traits.** Mini-Tn*7* vectors were either delivered by four parental matings, involving SM10(λ*pir*)[10]/pTNS1, HB101/pRK2013 ($Km^R$)[30], *E. coli* harboring the respective mini-Tn*7* delivery plasmid and the respective recipient strains or by coelectroporation of bacteria with equal amounts (50 ng) of mini-Tn*7* delivery vector and helper plasmid (details of these procedures are described in **Supplementary Methods**). A published procedure was used for Flp recombinase–mediated marker excision[5]. The stability of recombinant traits was monitored as detailed in **Supplementary Methods**.

**Determination of Tn*7* insertion sites.** We used colony PCR[5] to verify chromosomal Tn*7* insertions in bacteria for which the genome sequences were known. Common primers used for all bacteria were $P_{Tn7L}$ and $P_{Tn7R}$ (the locations of priming sites are shown in **Figures 2** and **3**, and primer sequences are provided in **Supplementary Methods**). Bacterium-specific primers were $P_{glmS\text{-down}}$ and $P_{glmS\text{-up}}$ for *P. aeruginosa*; $P_{put\text{-}glmSUP}$ and $P_{put\text{-}glmSDN}$ for *P. putida*; and $P_{GlmSDn2}$ and $P_{PstSUp2}$ for *Y. pestis*. We initially determined the mini-Tn*7* insertion sites in *B. thailandensis* by self-ligation of chromosomal DNA fragments with R6K origin of replication–containing transposon insertions and sequencing the

transposon-chromosomal DNA junction sequences. Subsequently, we designed two PCR primers $P_{glmS1\text{-}DN}$ and $P_{glmS2\text{-}DN}$ to determine Tn*7* insertions either downstream of *glmS1* or *glmS2* in combination with $P_{Tn7L}$ (for details see **Supplementary Methods**).

**Animal experiments and FACS analysis.** We injected mice intraperitoneal with $3 \times 10^5$ A1122-GFP or phosphate-buffered saline (PBS; control). After 30 min, we collected peritoneal exudate cells and washed them extensively in FACS buffer (PBS, 2% fetal bovine serum, 0.05% sodium azide). Cells were fixed in 1% paraformaldehyde, then washed and resuspended in FACS buffer. We analyzed viable cells, as determined by forward and side scatter, on a Cyan MLE flow cytometer using Summit software (Dako Instrumentation). Animal experiments were reviewed and approved by the Colorado State University Animal Care and Use Committee.

**Catechol-2,3-dioxygenase expression.** For measurement of C23O activity, we grew cells in LB medium with Cb to mid-logarithmic phase ($OD_{600nm} = \sim 0.5$), then measured and calculated C23O activity as previously described[17].

**Accession numbers.** GenBank accession numbers for all plasmids are available in **Supplementary Table 1**.

*Note: Supplementary information is available on the Nature Methods website.*

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**COMPETING INTERESTS STATEMENT**

The authors declare that they have no competing financial interests.

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