RESEARCH ARTICLE

Sequence-Defined Transposon Mutant Library of *Burkholderia thailandensis*

Larry A. Gallagher, Elizabeth Ramage, Rapatbhorn Patrapuvich, Eli Weiss, Mitch Brittnacher, Colin Manoil

Department of Genome Sciences, University of Washington, Seattle, Washington, USA

ABSTRACT **We constructed a near-saturation transposon mutant library for *Burkholderia thailandensis*, a low-virulence surrogate for the causative agent of melioidosis (*Burkholderia pseudomallei*). A primary set of nearly 42,000 unique mutants (~7.5 mutants/gene) was generated using transposon Tn*5* derivatives. The strains carry insertions in 87% of the predicted protein-coding genes of the organism, corresponding to nearly all of those nonessential for growth on nutrient agar. To achieve high genome coverage, we developed procedures for efficient sequence identification of insertions in extremely GC-rich regions of DNA. To facilitate strain distribution, we created a secondary library with two mutants per gene for which most transposon locations had been confirmed by resequencing. A map of mutations in the two-allele library and procedures for obtaining strains can be found at http://tools.nwrce.org/tn_mutants/ and http://www.gs.washington.edu/labs/manoil/. The library should facilitate comprehensive mutant screens and serve as a source of strains to test predicted genotype-phenotype associations.**

IMPORTANCE **The Gram-negative bacterium *Burkholderia pseudomallei* is a biothreat agent due to its potential for aerosol delivery and intrinsic antibiotic resistance and because exposure produces pernicious infections. Large-scale studies of *B. pseudomallei* are limited by the fact that the organism must be manipulated under biological safety level 3 conditions. A close relative of *B. pseudomallei* called *Burkholderia thailandensis*, which can be studied under less restrictive conditions, has been validated as a low-virulence surrogate in studies of virulence, antibiotic resistance and other traits. To facilitate large-scale studies of *B. thailandensis*, we created a near-saturation, sequence-defined transposon mutant library of the organism. The library facilitates genetic studies that identify genotype-phenotype associations conserved in *B. pseudomallei*.**

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Address correspondence to Larry A. Gallagher, lg@u.washington.edu.

The Gram-negative bacterium *Burkholderia thailandensis* is a close relative of *Burkholderia pseudomallei*, which is the causative agent of melioidosis and a Tier 1 select agent (1, 2). Both species are found in the soil and have large genomes with two chromosomes and high levels of gene synteny and sequence conservation (3). *B. thailandensis* is not normally virulent toward humans, but at high doses it can infect rodents, causing a melioidosis-like disease (4, 5). Like *B. pseudomallei*, *B. thailandensis* infects mammalian tissue culture cells by escaping phagocytic vacuoles, moving through the cytoplasm, inducing giant cell formation, and spreading to adjacent cells (6–9). In addition, *B. thailandensis* infects insects, nematodes, plants, and slime molds, often exceeding *B. pseudomallei* in virulence (10–15). *B. thailandensis* encodes homologues of many known *B. pseudomallei* virulence functions, including type III secretion, type VI secretion, and quorum-sensing systems (16, 17). Both species also encode a remarkable variety of functions that inhibit growth of competing microbes, including contact-dependent growth inhibition systems, type VI effectors, antibiotics, and other small molecules (17–23). Both *B. pseudomallei* and *B. thailandensis* are also intrinsically resistant to several antibiotics due to the actions of orthologous efflux pumps (24, 25). There are likely many additional similarities between the two organisms yet to be discovered. Because it may be manipulated under less restrictive laboratory conditions, *B. thailandensis* represents an attractive surrogate for *B. pseudomallei*.

Two *B. pseudomallei* auxotrophic mutants exempt from the select agent rule have recently become available (26, 27). The strains are highly attenuated in their virulence and promise to be highly useful for studying processes other than whole animal infection.

Several procedures facilitate genetic manipulation of *B. thailandensis* and *B. pseudomallei*. Natural transformation can be employed for targeted mutagenesis using PCR fragments or to transfer mutations between strains (25, 28). Procedures for transposon mutagenesis and complementation have also been developed (29–31).

Genetic analysis of several bacterial species has been accelerated by the availability of comprehensive sequence-defined mutant libraries (32–35). Such libraries provide a resource for relatively complete mutant screens as well as directed tests of the functions of specific gene products (36). In this report, we describe the construction of a comprehensive transposon mutant library for *B. thailandensis*.

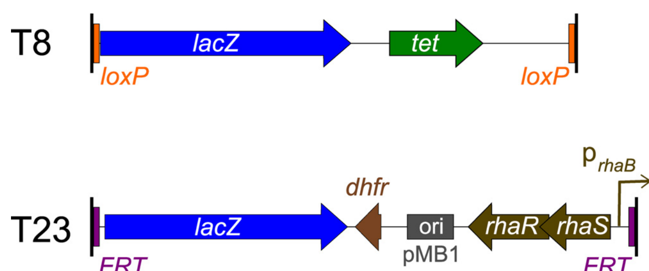

FIG 1 Transposons used for mutagenesis. Two Tn*5* derivatives were employed. Transposon T8 produces translational *lacZ* fusions when inserted in frame in a target gene, whereas transposon T23 produces transcriptional *lacZ* fusions. Both transposons carry site-specific recombination sites at their ends (*loxP* in T8 and the FRT gene in T23) enabling excision of most of the transposon and reuse of the resistance marker. T23 also carries an outward-facing, rhamnose-inducible promoter (P_{rhaB}) and genes needed for induction (*rhaR* and *rhaS*), as well as a plasmid pMB1 replication origin (ori). The rhamnose promoter functions and exhibits rhamnose control in *B. thailandensis* (see Fig. S1 in the supplemental material). Transposon T8 encodes tetracycline resistance (*tet*), while T23 encodes trimethoprim resistance (*dhfr*).

RESULTS AND DISCUSSION

Overview. We sought to create a comprehensive sequence-defined transposon mutant library of *B. thailandensis*. To achieve this goal, we first generated and sequence mapped a near-saturation collection of "random" insertion mutants. To reduce the size of the library while retaining genome coverage, a subset of strains corresponding to approximately two mutations per gene were colony purified and rearrayed. Mutants of this two-allele library were then resequenced to verify insertion assignments.

Mutant isolation and sequencing. Mutants of *B. thailandensis* E264 were generated using two transposon Tn*5* derivatives (Fig. 1). More than 64,000 such mutants were initially arrayed, and their transposon-genome junction regions were amplified using a thermostable DNA polymerase (TSG). Junction fragments were Sanger sequenced, and insertion sites were mapped to the genome (see Materials and Methods) (37). This round of sequencing identified 33,358 unique strains (~6 unique mutants per gene), corresponding to insertions in about 80% (4,492) of the organism's 5,634 predicted coding genes.

This level of genome coverage was lower than expected from previous mutant library construction projects by 5 to 10% (data not shown) (37). The apparent explanation for the low coverage was inefficient identification of transposon insertions in exceptionally high-GC regions of the genome (Fig. 2A and B). The inefficient identification of insertions in very-high-GC regions was also seen in a transposon sequencing (Tn-seq) assay of a large (530,000 members) pool of T23 insertion mutants (Fig. 2C). The pattern of reads produced by the Tn-seq assay was revealing: although insertions in very-high-GC regions were observed, the average numbers of sequence reads for these insertions were low (e.g., compare BTH_I2705 to BTH_12701 in Fig. 2C). The results suggested that although insertions in the very-high-GC regions could be generated, their identification by sequencing was inefficient.

We made two changes to improve the detection of insertions in very-high-GC regions of the genome. First, we increased the GC content of the primers used to amplify transposon-genome junction fragments for sequencing (see Text S1 in the supplemental material). Second, we carried out the amplification of junction fragments with a different polymerase (KAPA) that acts efficiently on GC-rich sequences (38). We then used the new procedure to resequence arrayed mutants that had failed to be successfully sequenced when TSG polymerase was used in the initial runs. We found that many of the insertions could now be mapped and that many were indeed in the underrepresented GC-rich regions (Fig. 2B and D). Ultimately, we resequenced 9,759 mutants that could not be mapped in the initial sequencings and identified insertions in 336 additional genes. The final primary defined mutant collection provided 86.7% coverage of predicted genes (4,886/5,634) (Table 1). Genes unrepresented in the library correspond mainly to candidate essential genes (39).

Two-allele mutant library. To create a smaller library of mutants providing genome coverage, we assembled a set of two unique mutants for genes represented in the primary mutant collection. We included two rather than one insertion per gene to reduce missed genotype-phenotype associations due to cross-contamination and other issues and to provide confirmation of associations. The two-allele library strains corresponded where possible to insertions situated between 5% and 85% of the coding sequence and at a distance from one another within each gene (Table 2). Most of the mutations (84%) were transposon T23 insertions. We confirmed the identities of 81% of the two-allele set by resequencing (Table 2).

The 12,322 mutants making up the two-allele library are listed in Table S1 in the supplemental material. The table includes sequence quality metrics for each strain and indicates which mutants were confirmed by resequencing. A map of two-allele library insertions relative to the annotated *B. thailandensis* genome is provided at http://tools.nwrce.org/tn_mutants/ (Fig. 3).

Deletion of transposon sequences by recombination. We verified the functionality of the transposon T23 FLP recombination target (FRT) sites in *B. thailandensis*. Recombination at the sites is predicted to leave a short sequence in which nearly all internal transposon sequences have been lost (Fig. 4A). The recombination was demonstrated by transiently introducing a plasmid expressing FLP recombinase into three T23 mutants to induce recombination. PCR assays showed that putative recombinant colonies had indeed lost transposon sequences and carried short insertions of DNA in their place (Fig. 4B). In addition, as expected, the corresponding recombinants had lost β-galactosidase activity in two cases in which the corresponding T28 insertions had generated active *lacZ* gene fusions and had lost trimethoprim resistance for all three strains (Fig. 4C).

TABLE 1 Makeup of the primary *B. thailandensis* mutant library

Parameter	No. for transposon(s): T8	T23	Both
Insertion mutants arrayed	9,456	54,878	64,334
Insertions successfully mapped	6,057	42,660	48,717
Duplicate, discrepant, or ambiguous insertions	373	6,662	6,735
Unique insertions:	5,684	36,298	41,982
Within genes	4,939	29,255	34,194
Intergenic	745	7,043	7,788
Genes hit internally (of 5,634)	2,371	4,729	4,886
Avg unique hits per gene in genome	1.0	6.4	7.5
Genes not hit internally	3,263	905	748

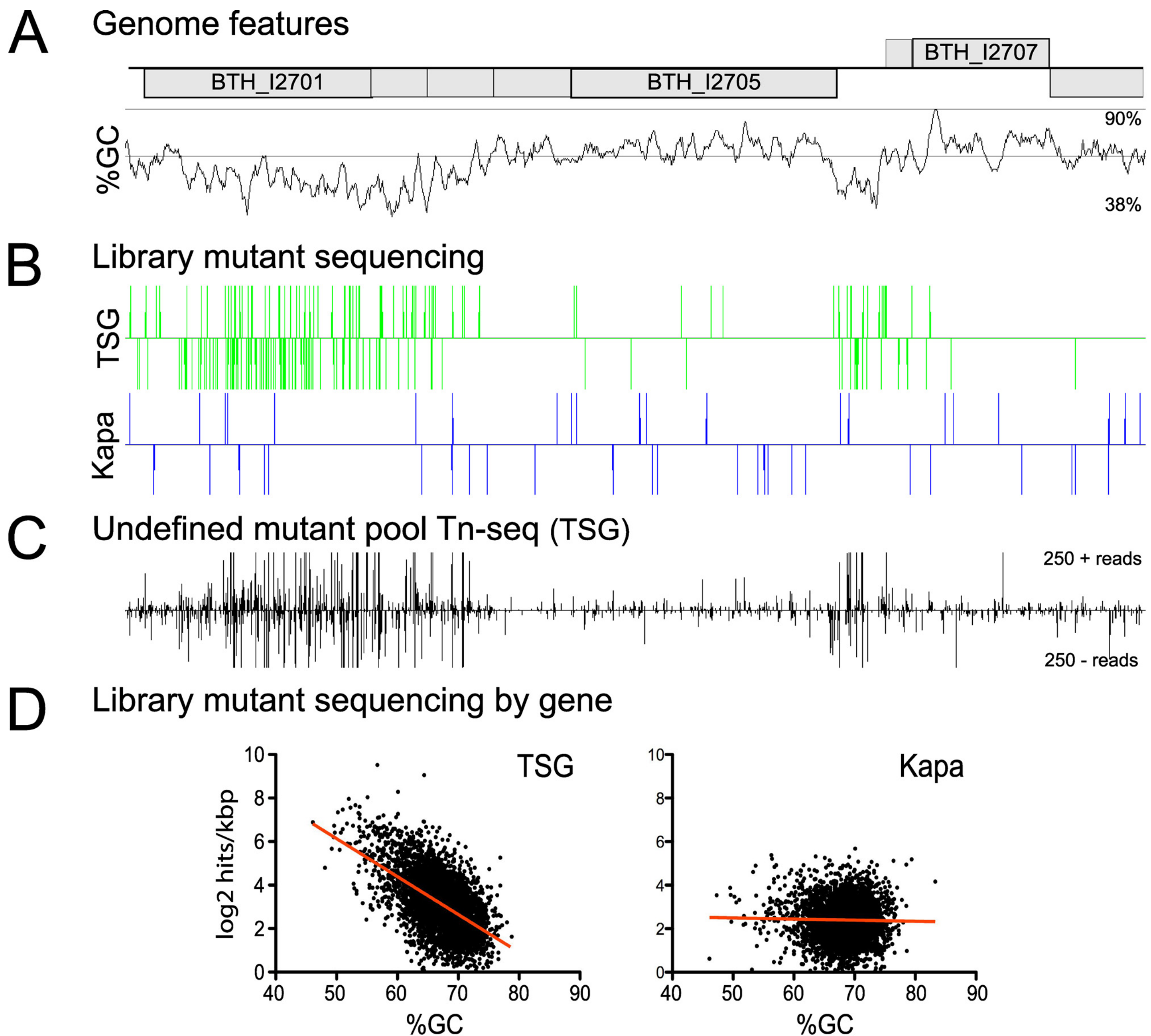

FIG 2 Transposon insertion identification efficiency as a function of GC content. The locations of transposons identified in a 10.7-kbp region of chromosome I are shown to illustrate their initial relative underrepresentation in very-high-GC-content regions. (A) Gene assignments and average percent GC content (60-bp window). Gray line, average percent GC content for the chromosome (67.3%). (B) Mutant library insertions identified using TSG polymerase amplification (top) and KAPA polymerase amplification (bottom). (C) Tn-seq insertions identified using TSG polymerase amplification. (The height of each bar reflects the average sequence read number of the corresponding transposon positions from five independent Tn-seq assays.) (D) Insertions per gene as a function of percent GC for all genes in the primary mutant library identified using TSG amplification (left) or KAPA amplification (right). Red lines correspond to least-squares fits.

Uses of the two-allele mutant library. The defined mutant library should facilitate genetic analysis of *B. thailandensis* in several ways.

- The library can be screened for strains exhibiting changes in phenotypes of interest to provide relatively complete lists of nonessential genes involved in the corresponding processes (34). Carrying out the process for multiple phenotypes can refine genome annotations and lead to discoveries (36, 40).
- The library provides a source of mutants to test hypothesized genotype-phenotype associations. For example, the *in vivo* roles of genes predicted to be involved in different processes experimentally or through bioinformatics analysis have been tested using corresponding mutants from the library (41, 42; L. A. Gallagher, unpublished results).
- Since the transposons used to generate the mutant library can generate β-galactosidase reporter gene fusions, they can be used to monitor expression of the corresponding target genes (43).
- Since the transposon used to generate most of the mutants also carries a regulated outward-facing rhamnose promoter at one end, it can be used to provide regulated expression of genes adjacent to insertion sites (44, 45).

TABLE 2 *B. thailandensis* two-allele mutant library

Parameter	No.
Total mutants	12,322
Within genes	11,510
Intergenic	812
Genes with insertions	
Total	4,667
1 unique insertion	1,033
2 unique insertions	1,480
3 unique insertions	1,449
4 unique insertions	507
>4 unique insertions	198
Genes without insertions	964
Transposon types	
T8 insertions	1,982
T23 insertions	10,340
Mutants confirmed by resequencing[a]	9,247

[a] A total of 11,407 of the 12,322 strains were successfully resequenced at least once. Mutant assignments were considered confirmed if the resequencing matched the initial assignment (8,882 strains) or if multiple resequencings confirmed a new location (365 strains). Strains for which a single resequencing indicated a new location (2,160 strains) were reassigned to the new location (1,949 strains), unless the resequencing was of poor quality, in which case the original assignment was kept (211 strains).

- Since the transposons also carry the FRT gene or *loxP* sites at their ends, resistance determinants of the transposons can be deleted by site-specific recombination. The process can facilitate the construction of multiple mutants by an iterative transformation procedure (46).

A possible concern with the library arises from the fact that the *E. coli* donor strain used for transposon mutagenesis (SM10/λpir) carries phage Mu DNA that can potentially be transferred by conjugation to recipient *B. thailandensis* strains during T8 or T23 mutagenesis (47). Such transfer could lead to Mu insertions in transposon mutant strains. While such insertions appear to be rare (a PCR assay of 66 strains chosen randomly from the two-allele mutant library detected Mu sequences in only one strain [data not shown]), it is nonetheless particularly important to verify that phenotypes found for strains of the library are due to the T8 or T23 insertions rather than secondary mutations. We would thus recommend that genotype-phenotype associations be confirmed for both insertion alleles of a gene represented in the two-allele library and/or that genetic linkage be examined by natural transformation of transposon insertion alleles into the "clean" parent background (25) followed by phenotype retesting.

We believe that the defined mutant library should facilitate a variety of genome-scale genetic studies of *B. thailandensis*. Such studies should help reveal the functional basis of important traits shared by *B. pseudomallei*, as well as provide a better understanding of how the two species differ from each other. Strains from the two-allele library may be obtained by following instructions at http://www.gs.washington.edu/labs/manoil/thailandensis_library.htm. The Transposon Mutant Library Browser (http://tools.nwrce.org/tn_mutants/) provides a simple graphical interface to facilitate identifying and requesting strains.

MATERIALS AND METHODS

Strains, media, and growth conditions. The transposon mutant library was constructed using *B. thailandensis* E264 (48). Transposon T8 (IS*lacZ*/hah-tc) and its conjugal delivery suicide plasmid (pIT2) have been described previously (37). Transposon T23 (IS*lacZ*-$p_{rhaBout}$-tp/FRT) was carried on conjugal delivery plasmid pLG99 (see Fig. S1 in the supplemental material). The growth media were LB broth (10 g tryptone, 5 g yeast extract, and 8 g NaCl per liter), tryptone-yeast extract (TYE) agar (LB broth with 1.5% agar), tryptic soy broth (TSB; Difco 211825), tryptic soy agar (TSA; Difco 236950), 2× nutrient TSA (TSA with an additional 30 g/liter TSB powder), TSB freezer medium (TSB with 10% [wt/vol] glycerol), and M9 minimal medium supplemented with 1 μg/ml vitamin B_1 and 0.2% or 0.4% pyruvate. For T23 *lacZ* reporter activity and FRT recombination tests, TYE medium was supplemented with 40 μg/ml 5-bromo-4-chloro-indolyl-β-D-galactopyranoside (X-Gal) or 50 μg/ml trimethoprim. Plasmids pIT2 and pLG69 were maintained in *Escherichia coli* using TYE containing 100 μg/ml carbenicillin.

Mutagenesis and library construction. Mutagenesis was carried out using methods similar to those used previously (37). Specifically, overnight cultures of *E. coli* donor strain SM10/λpir (49) carrying pIT2 or pLG99 and recipient strain *B. thailandensis* E264 were subcultured (1:50

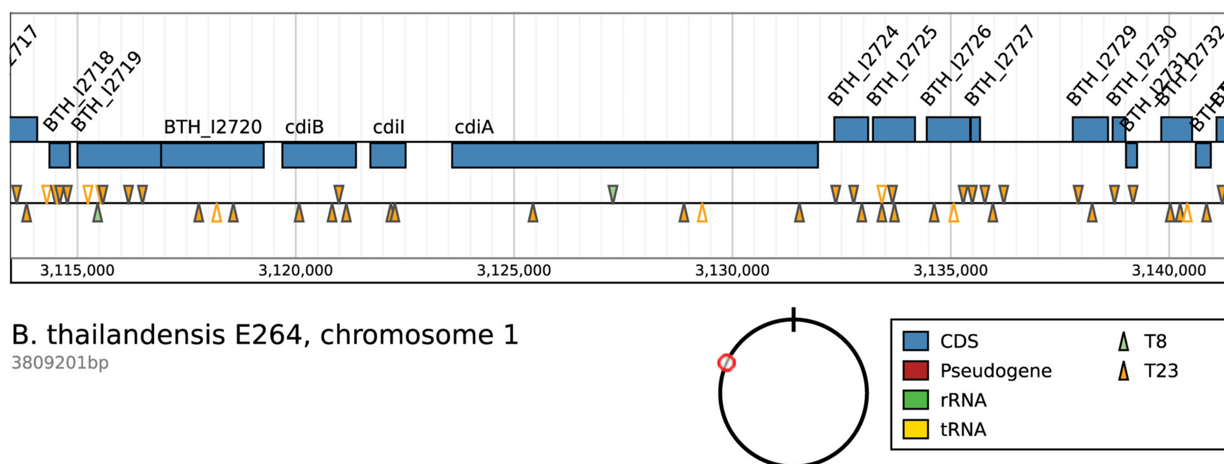

FIG 3 Web browser representation of two-allele library insertion mutations. A partial screen shot of the transposon locations in a representative region of the *B. thailandensis* genome provided at http://tools.nwrce.org/tn_mutants/ is shown. Transposons are represented as triangles, with positions above or below the line corresponding to their orientations in the genome. Filled triangles represent sequence-confirmed insertions, and open triangles represent unconfirmed insertions.

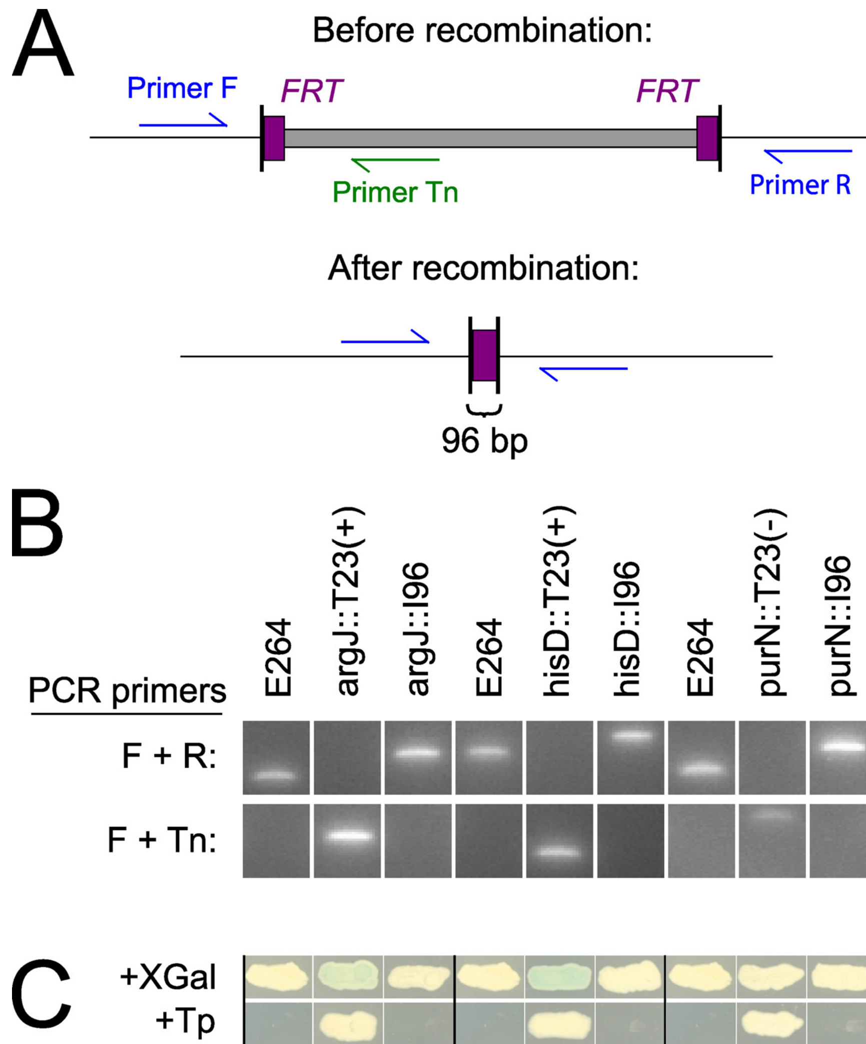

FIG 4 Transposon T23 internal sequences can be excised by FLP recombination. (A) The deletion of transposon sequences by FLP recombination at the FRT sites in T23 is illustrated, along with PCR primers used for analysis. The sequence of the 96-bp insertion left after recombination is CTGTCTCTTATACACAT CTAAGAAGTTCCTATTCCGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCCGTCGGCGAGATGTGTATAAGAGACAG (not including the 9 bp of target sequence duplicated at each insertion site). (B) Analysis of insertion sites by PCR. For three T23 insertions examined, the wild-type (E264) PCR fragment (F + R) disappeared in the corresponding transposon insertion mutant and was replaced by a slightly larger fragment in the FRT-FRT recombinant. The presence of the transposon in each case was verified by PCR using a transposon-specific primer (F + Tn). (C) Phenotypic tests of recombinants. Two of the T23 insertions analyzed carried *'lacZ* in the same orientation as the chromosomal target gene (denoted as "+") and generated LacZ$^+$ cells, which were blue on X-Gal (5-bromo-4-chloro-3-indolyl galactopyranoside) medium (top). The β-galactosidase activity was eliminated by FRT-FRT recombination, as expected. Tp, trimethoprim.

for the donor and 1:10 for the recipient) and grown for approximately 90 min on a 37°C roller. Equal volumes (0.5 to 3.0 ml) of donor and recipient subcultures were then mixed and centrifuged, the pellet resuspended in 20 μl of LB with 20 mM $MgSO_4$, spotted onto a nitrocellulose filter (0.45-μm pore size) on prewarmed TYE agar, and incubated for approximately 2 h at 37°C. Cells were washed from the filter and plated on TYE agar containing streptomycin (100 μg/ml) and either tetracycline (60 μg/ml) for pIT2 mutagenesis or trimethoprim (50 μg/ml) for pLG99 mutagenesis. After incubation for 2 days at 37°C, resistant colonies were picked and arrayed into 384-well plates containing TSB freezer medium using a QPix2 colony picking robot (Genetix). Plates were incubated for 24 h at 37°C and 16 h at room temperature and then stored at −80°C.

Mutant identification. Insertion sites were identified by semidegenerate PCR and sequencing of the transposon-genome junctions (37, 50) (see Text S1 in the supplemental material).

Tn-seq. Approximately 530,000 T23 insertion mutants were pooled (pool "BtL1"), and genomic DNA was isolated from an aliquot of the pool. The Tn-seq circle method (51) with minor modifications was used to identify the transposon insertion locations for the pooled mutants (see Text S1 in the supplemental material).

FLP recombination of T23 insertion mutants. FLP-mediated marker excision of T23 insertion mutants was carried out using plasmid pFLPe4 (29). Excision was verified by colony PCR using a transposon-specific primer and locus-specific primers flanking the site of insertion. The oligonucleotides used are listed in Text S1 in the supplemental material.

Two-allele mutant set. A Perl script and manual curation were used to choose the two mutants for each gene that best combined four criteria: (i) insertions situated between 5% and 85% of the coding sequence of the gene, (ii) insertions situated at substantial distance from one another within the 5 to 85% window (with the hits selected spaced at least 100 bp

apart for more than 85% of the genes and at least 200 bp apart for greater than 75% of the genes), (iii) sequence mapping data of high quality, and (iv) T23 insertion mutants favored over T8 insertion mutants. To assemble the set, strains from the original library were cherry-picked using the QPix2 robot into 96-well plates (Genetix X6011) containing 180 μl of TSB per well. After picking, the plates were covered with a sterile plastic lid and grown 4 h at 37°C in a shaking incubator at 200 rpm. Three serial dilutions were then made by transfer of ~5-μl aliquots of culture per well into new 96-well plates containing 60 μl of TSB per well using a grooved 96-pin replicator. The grooved pin replicator was then used to transfer ~5 μl of medium per well from each of the dilution plates onto 2× nutrient TSA medium with 100 μg/ml streptomycin and 50 μg/ml tetracycline or trimethoprim, depending on the transposon. The stamped dilution plates were grown overnight at 37°C, and an isolated single colony of each strain was picked into a 96-well, deep well block containing 1.2 ml of TSB freezer medium per well. The blocks were covered with Airpore tape sheets (Qiagen, 19571) and grown overnight at 37°C with shaking. After growth, aliquots of the culture were distributed into single-tube racks (Matrix TrakMates 3735) before freezing.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00604-13/-/DCSupplemental.

Figure S1, TIF file, 0.2 MB.
Table S1, XLSX file, 1.3 MB.
Text S1, DOCX file, 0.1 MB.

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REFERENCES

1. **Wiersinga WJ, Currie BJ, Peacock SJ.** 2012. Melioidosis. N. Engl. J. Med. **367:**1035–1044.
2. **Brett PJ, DeShazer D, Woods DE.** 1998. *Burkholderia thailandensis* sp. nov., a *Burkholderia pseudomallei*-like species. Int. J. Syst. Bacteriol. **48:** 317–320.
3. **Yu Y, Kim HS, Chua HH, Lin CH, Sim SH, Lin D, Derr A, Engels R, DeShazer D, Birren B, Nierman WC, Tan P.** 2006. Genomic patterns of pathogen evolution revealed by comparison of *Burkholderia pseudomallei*, the causative agent of melioidosis, to avirulent *Burkholderia thailandensis*. BMC Microbiol. **6:**46. doi:10.1186/1471-2180-6-46.
4. **Wiersinga WJ, de Vos AF, de Beer R, Wieland CW, Roelofs JJ, Woods DE, van der Poll T.** 2008. Inflammation patterns induced by different Burkholderia species in mice. Cell. Microbiol. **10:**81–87.
5. **West TE, Hawn TR, Skerrett SJ.** 2009. Toll-like receptor signaling in airborne *Burkholderia thailandensis* infection. Infect. Immun. **77:** 5612–5622.
6. **Galyov EE, Brett PJ, DeShazer D.** 2010. Molecular insights into *Burkholderia pseudomallei* and *Burkholderia mallei* pathogenesis. Annu. Rev. Microbiol. **64:**495–517.
7. **Stevens JM, Ulrich RL, Taylor LA, Wood MW, Deshazer D, Stevens MP, Galyov EE.** 2005. Actin-binding proteins from *Burkholderia mallei* and *Burkholderia thailandensis* can functionally compensate for the actin-based motility defect of a *Burkholderia pseudomallei bimA* mutant. J. Bacteriol. **187:**7857–7862.
8. **Harley VS, Dance DA, Drasar BS, Tovey G.** 1998. Effects of *Burkholderia pseudomallei* and other *Burkholderia* species on eukaryotic cells in tissue culture. Microbios **96:**71–93.
9. **French CT, Toesca IJ, Wu TH, Teslaa T, Beaty SM, Wong W, Liu M, Schröder I, Chiou PY, Teitell MA, Miller JF.** 2011. Dissection of the Burkholderia intracellular life cycle using a photothermal nanoblade. Proc. Natl. Acad. Sci. U. S. A. **108:**12095–12100.
10. **Fisher NA, Ribot WJ, Applefeld W, DeShazer D.** 2012. The Madagascar hissing cockroach as a novel surrogate host for *Burkholderia pseudomallei*, *B. mallei* and *B. thailandensis*. BMC Microbiol. **12:**117. doi:10.1186/1471-2180-12-117.
11. **Wand ME, Müller CM, Titball RW, Michell SL.** 2011. Macrophage and *Galleria mellonella* infection models reflect the virulence of naturally occurring isolates of *B. pseudomallei*, *B. thailandensis* and *B. oklahomensis*. BMC Microbiol. **11:**11. doi:10.1186/1471-2180-11-11.
12. **Lee YH, Chen Y, Ouyang X, Gan YH.** 2010. Identification of tomato plant as a novel host model for *Burkholderia pseudomallei*. BMC Microbiol. **10:**28. doi:10.1186/1471-2180-10-28.
13. **Hasselbring BM, Patel MK, Schell MA.** 2011. Dictyostelium discoideum as a model system for identification of *Burkholderia pseudomallei* virulence factors. Infect. Immun. **79:**2079–2088.
14. **O'Quinn AL, Wiegand EM, Jeddeloh JA.** 2001. *Burkholderia pseudomallei* kills the nematode *Caenorhabditis elegans* using an endotoxin-mediated paralysis. Cell. Microbiol. **3:**381–393.
15. **Pilátová M, Dionne MS.** 2012. *Burkholderia thailandensis* is virulent in *Drosophila melanogaster*. PLoS One **7:**e49745. doi:10.1371/journal.pone.0049745.
16. **Chandler JR, Duerkop BA, Hinz A, West TE, Herman JP, Churchill ME, Skerrett SJ, Greenberg EP.** 2009. Mutational analysis of *Burkholderia thailandensis* quorum sensing and self-aggregation. J. Bacteriol. **191:** 5901–5909.
17. **Haraga A, West TE, Brittnacher MJ, Skerrett SJ, Miller SI.** 2008. *Burkholderia thailandensis* as a model system for the study of the virulence-associated type III secretion system of *Burkholderia pseudomallei*. Infect. Immun. **76:**5402–5411.
18. **Biggins JB, Gleber CD, Brady SF.** 2011. Acyldepsipeptide HDAC inhibitor production induced in *Burkholderia thailandensis*. Org. Lett. **13:** 1536–1539.
19. **Schwarz S, West TE, Boyer F, Chiang WC, Carl MA, Hood RD, Rohmer L, Tolker-Nielsen T, Skerrett SJ, Mougous JD.** 2010. Burkholderia type VI secretion systems have distinct roles in eukaryotic and bacterial cell interactions. PLoS Pathog. **6:**e1001068. doi:10.1371/journal.ppat.1001068.
20. **Nikolakakis K, Amber S, Wilbur JS, Diner EJ, Aoki SK, Poole SJ, Tuanyok A, Keim PS, Peacock S, Hayes CS, Low DA.** 2012. The toxin/immunity network of *Burkholderia pseudomallei* contact-dependent growth inhibition (CDI) systems. Mol. Microbiol. **84:**516–529.
21. **Anderson MS, Garcia EC, Cotter PA.** 2012. The Burkholderia bcpAIOB genes define unique classes of two-partner secretion and contact dependent growth inhibition systems. PLoS Genet. **8:**e1002877. doi:10.1371/journal.pgen.1002877.
22. **Carr G, Seyedsayamdost MR, Chandler JR, Greenberg EP, Clardy J.** 2011. Sources of diversity in bactobolin biosynthesis by *Burkholderia thailandensis* E264. Org. Lett. **13:**3048–3051.
23. **Lazer Adler NR, Stevens JM, Stevens MP, Galyov EE.** 2011. Autotransporters and their role in the virulence of *Burkholderia pseudomallei* and *Burkholderia mallei*. Front. Microbiol. **2:**151. doi:10.3389/fmicb.2011.00151.
24. **Moore RA, DeShazer D, Reckseidler S, Weissman A, Woods DE.** 1999. Efflux-mediated aminoglycoside and macrolide resistance in *Burkholderia pseudomallei*. Antimicrob. Agents Chemother. **43:**465–470.
25. **Thongdee M, Gallagher LA, Schell M, Dharakul T, Songsivilai S, Manoil C.** 2008. Targeted mutagenesis of *Burkholderia thailandensis* and *Burkholderia pseudomallei* through natural transformation of PCR fragments. Appl. Environ. Microbiol. **74:**2985–2989.
26. **Propst KL, Mima T, Choi KH, Dow SW, Schweizer HP.** 2010. A *Burkholderia pseudomallei* Δ*purM* mutant is avirulent in immunocompetent and immunodeficient animals: candidate strain for exclusion from select-agent lists. Infect. Immun. **78:**3136–3143.
27. **Norris MH, Propst KL, Kang Y, Dow SW, Schweizer HP, Hoang TT.** 2011. The *Burkholderia pseudomallei* Δ*asd* mutant exhibits attenuated intracellular infectivity and imparts protection against acute inhalation melioidosis in mice. Infect. Immun. **79:**4010–4018.
28. **Kang Y, Norris MH, Wilcox BA, Tuanyok A, Keim PS, Hoang TT.** 2011. Knockout and pullout recombineering for naturally transformable *Burkholderia thailandensis* and *Burkholderia pseudomallei*. Nat. Protoc. **6:**1085–1104.
29. **Choi KH, Mima T, Casart Y, Rholl D, Kumar A, Beacham IR, Schweizer HP.** 2008. Genetic tools for select-agent-compliant manipulation of *Burkholderia pseudomallei*. Appl. Environ. Microbiol. **74:**1064–1075.
30. **Rholl DA, Trunck LA, Schweizer HP.** 2008. In vivo Himar1 transposon mutagenesis of *Burkholderia pseudomallei*. Appl. Environ. Microbiol. **74:** 7529–7535.
31. **DeShazer D, Brett PJ, Carlyon R, Woods DE.** 1997. Mutagenesis of *Burkholderia pseudomallei* with Tn*5*-OT182: isolation of motility mutants

and molecular characterization of the flagellin structural gene. J. Bacteriol. **179:**2116–2125.
32. **Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H.** 2006. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio Collection. Mol. Syst. Biol. **2:**2006.0008. doi:10.1038/msb4100050.
33. **Held K, Ramage E, Jacobs M, Gallagher L, Manoil C.** 2012. Sequence-verified two-allele transposon mutant library for *Pseudomonas aeruginosa* PAO1. J. Bacteriol. **194:**6387–6389.
34. **Salama NR, Manoil C.** 2006. Seeking completeness in bacterial mutant hunts. Curr. Opin. Microbiol. **9:**307–311.
35. **Gallagher LA, Ramage E, Jacobs MA, Kaul R, Brittnacher M, Manoil C.** 2007. A comprehensive transposon mutant library of Francisella novicida, a bioweapon surrogate. Proc. Natl. Acad. Sci. U. S. A. **104:**1009–1014.
36. **Enstrom M, Held K, Ramage B, Brittnacher M, Gallagher L, Manoil C.** 2012. Genotype-phenotype associations in a nonmodel prokaryote. mBio **3**(2):e00001-12. doi:10.1128/mBio.00001-12.
37. **Jacobs MA, Alwood A, Thaipisuttikul I, Spencer D, Haugen E, Ernst S, Will O, Kaul R, Raymond C, Levy R, Chun-Rong L, Guenthner D, Bovee D, Olson MV, Manoil C.** 2003. Comprehensive transposon mutant library of Pseudomonas aeruginosa. Proc. Natl. Acad. Sci. U. S. A. **100:**14339–14344.
38. **Quail MA, Otto TD, Gu Y, Harris SR, Skelly TF, McQuillan JA, Swerdlow HP, Oyola SO.** 2012. Optimal enzymes for amplifying sequencing libraries. Nat. Methods **9:**10–11.
39. **Baugh L, Gallagher LA, Patrapuvich R, Clifton MC, Gardberg AS, Edwards TE, Armour B, Begley DW, Dieterich SH, Dranow DM, Abendroth J, Fairman JW, Fox D III, Staker BL, Phan I, Gillespie A, Choi R, Nakazawa-Hewitt S, Nguyen MT, Napuli A, Barrett L, Buchko GW, Stacy R, Myler PJ, Stewart LJ, Manoil C, Van Voorhis WC.** 2013. Combining functional and structural genomics to sample the essential Burkholderia structome. PLoS One **8:**e53851. doi:10.1371/journal.pone.0053851.
40. **Nichols RJ, Sen S, Choo YJ, Beltrao P, Zietek M, Chaba R, Lee S, Kazmierczak KM, Lee KJ, Wong A, Shales M, Lovett S, Winkler ME, Krogan NJ, Typas A, Gross CA.** 2011. Phenotypic landscape of a bacterial cell. Cell **144:**143–156.
41. **Goo E, Majerczyk CD, An JH, Chandler JR, Seo YS, Ham H, Lim JY, Kim H, Lee B, Jang MS, Greenberg EP, Hwang I.** 2012. Bacterial quorum sensing, cooperativity, and anticipation of stationary-phase stress. Proc. Natl. Acad. Sci. U. S. A. **109:**19775–19780.
42. **Chandler JR, Truong TT, Silva PM, Seyedsayamdost MR, Carr G, Radey M, Jacobs MA, Sims EH, Clardy J, Greenberg EP.** 2012. Bactobolin resistance is conferred by mutations in the L2 ribosomal protein. mBio **3**(6):e00499-12. doi:10.1128/mBio.00499-12.
43. **Silhavy TJ, Beckwith JR.** 1985. Uses of lac fusions for the study of biological problems. Microbiol. Rev. **49:**398–418.
44. **Judson N, Mekalanos JJ.** 2000. Transposon-based approaches to identify essential bacterial genes. Trends Microbiol. **8:**521–526.
45. **Siehnel R, Traxler B, An DD, Parsek MR, Schaefer AL, Singh PK.** 2010. A unique regulator controls the activation threshold of quorum-regulated genes in Pseudomonas aeruginosa. Proc. Natl. Acad. Sci. U. S. A. **107:**7916–7921.
46. **Gallagher LA, McKevitt M, Ramage ER, Manoil C.** 2008. Genetic dissection of the *Francisella novicida* restriction barrier. J. Bacteriol. **190:**7830–7837.
47. **Ferrières L, Hémery G, Nham T, Guérout AM, Mazel D, Beloin C, Ghigo JM.** 2010. Silent mischief: bacteriophage Mu insertions contaminate products of *Escherichia coli* random mutagenesis performed using suicidal transposon delivery plasmids mobilized by broad-host-range RP4 conjugative machinery. J. Bacteriol. **192:**6418–6427.
48. **Brett PJ, Deshazer D, Woods DE.** 1997. Characterization of *Burkholderia pseudomallei* and *Burkholderia pseudomallei*-like strains. Epidemiol. Infect. **118:**137–148.
49. **Miller VL, Mekalanos JJ.** 1988. A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires *toxR*. J. Bacteriol. **170:**2575–2583.
50. **Gallagher L, Turner C, Ramage E, Manoil C.** 2007. Creating recombination-activated genes and sequence-defined mutant libraries using transposons. Methods Enzymol. **421:**126–140.
51. **Gallagher LA, Shendure J, Manoil C.** 2011. Genome-scale identification of resistance functions in *Pseudomonas aeruginosa* using Tn-seq. mBio **2**(1):e00315-10. doi:10.1128/mBio.00315-10.