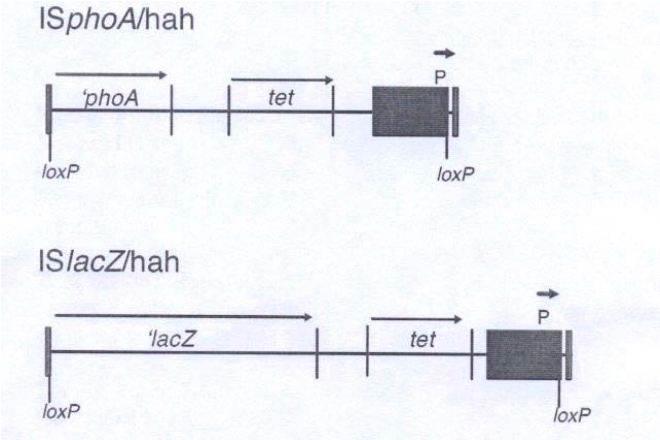
Additional information about the PA Two allele Library

Background: A comprehensive transposon mutant collection in *Pseudomonas aeruginosa* was initiated as a collaboration between Drs. Maynard Olson (UWGC) and Colin Manoil (UW Genome Sciences). The Two-allele library was created as a subset of the original library. This subset contains single colony purified isolates of, where possible, two alleles of each PAORF insertion.

Host Strain: *Pseudomonas aeruginosa* PAO1 was used as the host strain. The isolate used was obtained from Dr. Barbara Iglewski. This isolate was chosen over the sequenced one (Stover et. al. 2000) due to its wild-type twitching motility function.

Transposition: Transposition was performed according to the methods described in Bailey and Manoil, 2002. Transposons IS*phoA*/hah (4.83 Kb) and IS*lacZ*/hah (6.16 Kb) are derived from the IS50L element of transposon Tn5 and generate alkaline phosphatase (*phoA*) or beta-galactosidase (*lacZ*) translational gene fusions if inserted in a target gene in the appropriate orientation and reading frame. An outward-facing neomycin phosphotransferase promoter is expected to reduce polar effects on downstream gene expression for appropriately oriented insertions. Cre-mediated recombination excises sequences situated between the *loxP* sites in each transposon, leaving a 63 codon insertion that encodes an influenza-hemagglutinin epitope and a hexahistidine metal-affinity purification tag (see Bailey and Manoil, 2002). In below figure: *phoA*, alkaline phosphatase gene; *lacZ*, beta-galactosidase gene, *tet*, tetracycline resistance determinant; *loxP*, Cre recognition sequence; P, neomycin phosphotransferase promoter.



Searching for mutants. The Excel file "PA two-allele library" contains information about all of the available strains, which are a single colony purified subset of the original transposon library. These strains were chosen in an attempt to represent two alleles per PAORF to which insertions were found in the original library. This sheet has information about the strains, and there is a key on a second Excel sheet that describes what each heading refers to.

Receiving and confirming mutants. Mutants are shipped in agar stabs without antibiotic selection. The strains were originally selected on Tet 60 and have since been propagated

in Tet 5. You will want to save everything you are shipped, because while the strains were single colony purified this was not entirely successful for some strains. Therefore we suggest you make a frozen glycerol stock from the thick part of the streak, then test several colonies for the presence of the insertion by PCR to ensure that you have a single colony that contains the insertion in your region of interest, and freeze that isolate down as well.

Protocol for confirming your mutants by PCR…

1. Use flanking primers (you can find sequences of these on the "PA-two allele" sheet) to check that the gene is missing or you get a very long PCR product corresponding to the transposon.
2. Use a flanking primer and the transposon specific primer (lacZ-148 or Hah-138). The flanking primer you will use is based on the orientation of the transposon relative to the genome. A transposon that is labeled as "F" in the table column "Transposon Direction" will be paired with the flanking primer labeled "F", and if the transposon is "R", you will use the flanking primer "R".

Colony PCR protocol:

Cycles:

Step 1	Initial Denaturation	94° C	10 min
Step 2	Denaturing	94° C	30 sec
Step 3	Annealing	64° C	30 sec
Step 4	Extension	72° C	3 min
Step 5	go to step 2	30 cycles then to step 6	
Step 6	Final Extension	72° C	7 min
Step 7	Hold at 4° C		

Primer Hah-138 sequence:
cgggtgcagtaatatcgccct

Primer lac-148 sequence:
Gggtaacgccagggttttcc

PCR Cocktail Reagents for 10 μl rxn

H2O	3.7 μl
DMSO	0.5 μl
10X TSG buffer	1 μl
MgCl2 10 mM or 25 mM	1.5 μl
dNTPs, 10 mM mix	0.2 μl
TSG enzyme	0.1 μl
Primer 5 pmol/μl	1 μl

Primer 5 pmol/μl	1 μl
Colony boiled 10 min in 50 μl H20	1 μl

Once you have confirmed your mutant, please send an email to pamutant@u.washington.edu so that information can be incorporated into our database.

For further additional information, please reference these papers…

Stover CK, Pham XQ, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, Brinkman FS, Hufnagle WO, Kowalik DJ, Lagrou M, Garber RL, Goltry L, Tolentino E, Westbrock-Wadman S, Yuan Y, Brody LL, Coulter SN, Folger KR, Kas A, Larbig K, Lim R, Smith K, Spencer D, Wong GK, Wu Z, Paulsen IT, Reizer J, Saier MH, Hancock RE, Lory S, Olson MV. Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. Nature 406, 959-64 (2000).

Bailey J, Manoil C. Genome-wide internal tagging of bacterial exported proteins. Nat Biotechnol 20, 839-42. (2002).

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. Comprehensive transposon mutant library of Pseudomonas aeruginosa. Proc Natl Acad Sci U S A 100, 14339-44 (2003).

Held, Kiara et al. “Sequence-Verified Two-Allele Transposon Mutant Library for Pseudomonas Aeruginosa PAO1.” *Journal of Bacteriology* 194.23 (2012): 6387–6389. *PMC*. Web. 14 Sept. 2016.