

The *Francisella novicida* transposon mutant collection

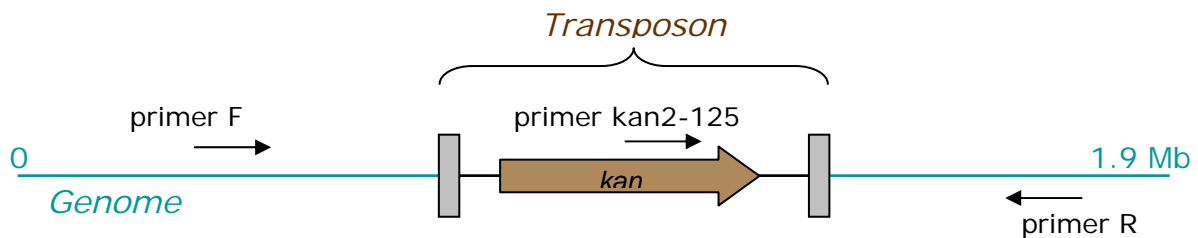
(Gallagher, et al. 2007. PNAS 104(3):1009-14)

CONFIRMING INSERTIONS

(updated Jan 20, 2007)

To confirm the insertion locations of the *F. novicida* transposon mutants using PCR:

1. Design primer pairs corresponding to the genomic DNA sequence flanking the reported transposon insertion location.* The primers should correspond to locations that are on either side and at least 50 bases away from the insertion location. Additionally, the primer locations should be far enough apart from each other to produce a PCR product of detectable size when wild-type genomic DNA is used as template (i.e., no transposon present). Primers F and R in the following figure represent the designed primers. (In this example, the direction of the transposon relative to the genome is “F”.)



* To retrieve flanking nucleotide sequence data for the transposon insertions, return to the transposon insertion viewing page at www.francisella.org and view the gene containing the insertion(s). Click the “[view surrounding nucleotides]” link above the list of transposon insertions. Enter the number of upstream and downstream nucleotides to retrieve. The returned data displays the genomic sequence data. The nucleotide in red represents the insertion location (which may be approximate). **Note that for insertions that are in the “R” direction in the genome, the sequence data returned is the reverse complement sequence of the forward genome strand.**

2. Carry out PCR with primers F and R using wild-type genomic DNA as template and verify the production of the appropriate-sized band. This step is a positive control for the efficacy of the designed primers.
3. Carry out the same PCR reaction using the mutant genomic DNA as template. The reaction should produce either no amplification product or a product that is larger than the previously-produced product by the length of the transposon. The transposon lengths are as follows:

| Transposon | Length (nt) | Tn-specific primer |
|------------|-------------|--|
| <KAN-2> | 1,221 | kan2-125 (5'- AACGCAGACCGTTCCGTGGC-3') |
| T15 | 1,284 | erm-106 (5'- GTTTTATATTTTTCTCGTTCATTATAACCCTCC-3') |
| T17 | 1,446 | kan2-125 (5'- AACGCAGACCGTTCCGTGGC-3') |
| T18 | 1,299 | kan2-125 (5'- AACGCAGACCGTTCCGTGGC-3') |
| T20 | 1,401 | kan2-125 (5'- AACGCAGACCGTTCCGTGGC-3') |

4. Confirm the presence of the insertion by carrying out PCR using the transposon-specific primer and the appropriate flanking primer (primer R for insertions that are in the “F” direction relative to the genome (as in the example above) and primer F for insertions that are in the “R” direction). An amplification product should be observed for mutant DNA but not wild-type DNA. The product size from the mutant DNA should correspond to the distance from the transposon-specific primer to the junction (for kan2-125, this is 125 bases, for erm-106, 106 bases, etc.) plus the distance from the junction to the flanking primer.