[C2-3]

Genetic Tools for *Pseudomonas* and *Burkholderia* spp.

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The development of genetic tools for *Pseudomonas aeruginosa* and *Burkholderia* spp. has not kept up with the pace of sequencing and post-genomic high-throughput technologies. The tools developed here include a broad-host range transposon Tn*7*-based site-specific integration system, a rapid and efficient bacterial transformation method and a rapid gene replacement method. Unlike *P. aeruginosa*, select agents such as *Burkholderia pseudomallei* and *B. mallei* necessitate appropriate tools because they follow the US NIH guidelines for recombinant DNA research. This includes restrictions on use of antibiotics, which were mostly used in many other bacteria. This necessitates removal of integrated selective marker on chromosome, thus allowing us to use same selective marker for further creation of a mutant. Flp- or Cre-mediated recombination has been commonly used for excision of selective marker in many bacteria, but is not easily applicable in *Burkholderia* spp.. Therefore, appropriate system of marker excision from *Burkholderia* spp. chromosome was developed in this study. In addition, expression of Tn*7* site-specific transposase from the constitutive P1 integron promoter allowed development of an efficient site-specific chromosomal integration system for *B. pseudomallei.*

These systems will be valuable in creating mutants for various applications including gene delivery and gene deletion in *P. aeruginosa* and *Burkholderia* spp.. Therefore, development of these genetic tools will accelerate studies on bacteriology and pathogenesis of these organisms.