

IMPROVED METHOD OF ALLELIC EXCHANGE

Technology Summary

Allelic exchange is the most versatile means of genetic manipulation of bacterial strains. By this method a segment of DNA within a bacterial chromosome or episome can be replaced with a selectively modified DNA sequence of choice. Thus, gene deletions, insertions, gene fusions, single base pair changes, etc. can be introduced into a bacterial genome in a completely native context.

To simplify this frequently used method, FDA investigators have developed an improved and faster technique to introduce defined genetic alterations into the genome of *Bordetella* species such as *Bordetella pertussis*, *Bordetella parapertussis*, and *Bordetella bronchiseptica*. This method uses a single site chromosomal DNA cleavage of integrated vector to catalyze vector elimination leading to allelic replacement. It can be used as a research tool to test the importance of specific genes in virulence and to create manipulated strains with desirable properties such as mutant bacterial toxins (e.g. pertussis toxin) that no longer have toxic activity but are fully immunogenic, or increased synthesis of vaccine components.

Potential Commercial Applications

- Research tool

Competitive Advantages

- Simplified genetic manipulation without the use of selectable markers

Development Stage: Research Materials

Inventors: Earle Stibitz

Publications:

- Buboltz AM, et. al. (2009) Role of the type III secretion system in a hypervirulent lineage of *Bordetella bronchiseptica*. *Infect Immun.* 77:3969-77. PMID: [19596779](#)
- Inatsuka CS, et. al. (2010) Pertactin is required for *Bordetella* species to resist neutrophil-mediated clearance. *Infect Immun.* 78:2901-9. PMID: [20421378](#)
- Buasri W, et. al. (2012) Construction of *Bordetella pertussis* strains with enhanced production of genetically-inactivated pertussis toxin and pertactin by unmarked allelic exchange. *BMC Microbiol.* 12:61. PMID: [22524455](#)
- Shah NR, et. al. (2013) Minor modifications to the phosphate groups and the C3' acyl chain length of lipid A in two *Bordetella pertussis* strains, BP338 and 18-323, independently affect toll-like receptor 4 protein activation. *J. Biol. Chem.* 288:11751-60. PMID: [23467413](#)

Product Area: Research materials, tool, gene, manipulation

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