Markerless Gene Replacement Plasmids for *E. coli*

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The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing a simple and efficient gene replacement method that allows targeted modifications of *E. coli* DNA sequences.

Overview

Microbial genome sequencing projects uncover large numbers of new genes. Functional analyses of these genes require targeted modifications of particular DNA sequences in their chromosomal locations using mutant alleles constructed *in vitro*. For delivery of a mutant allele into a cell, the allele is typically carried on either a suicide plasmid or a linear DNA fragment.

The Invention

UW-Madison researchers have discovered a simple and efficient gene replacement method that permits targeted introduction of markerless deletions, insertions and point mutations into the *E. coli* chromosome. In this method, the mutant allele is carried on a circular plasmid that integrates into the chromosome at a homologous locus, resulting in a direct duplication. Resolution of this cointegrate via intramolecular recombination is controlled by introducing a unique double-stranded break into the chromosome by the meganuclease I-*Sce*I. The enzyme recognizes an 18-base pair sequence and generates a double-stranded break with a four-base 3` hydroxyl overhang. The method can be used in recombination-proficient *E. coli* and produces markerless replacements at high efficiency.

Applications

- Functional analyses of genes discovered via microbial genome sequencing projects

Key Benefits

- Potentially useful in numerous strains of bacteria
- Does not leave selectable marker behind
- No special growth conditions needed – method is relatively insensitive to culture medium and temperature
- Efficiency of resolution increased by two to three orders of magnitude because I-*Sce*I cleavage serves as both a selection tool and a stimulator of the resolution process

Tech Fields

- Research Tools : DNA & RNA tools
- Research Tools : Genomics & proteomics

For current licensing status, please contact Jennifer Gottwald at jennifer@warf.org or 608-960-9854