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Publication Date

2009-02-08

A new counterselectable marker for *Desulfovibrio vulgaris*, the *upp* gene, allowed for the construction of a markerless deletion of a Type I restriction enzyme that exhibits increased transformation efficiency

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Acknowledgements

This work was part of the Virtual Institute for Microbial Stress and Survival (<http://VIMSS.lbl.gov>) supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics:GTL program through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.

In recent years, the genetic manipulation of the sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough has seen enormous progress; however, the current method of deletion construction via marker exchange mutagenesis does not allow for easy selection of multiple sequential gene deletions because of the need for multiple selectable markers. To broaden the repertoire of genetic tools for manipulation of *D. vulgaris*, an in-frame markerless deletion system has been developed based on the *upp*-encoded uracil phosphoribosyltransferase as an element for a counterselection strategy. In wild-type *D. vulgaris*, growth is inhibited by the toxic pyrimidine analog 5-fluorouracil (5-FU), whereas a mutant bearing a deletion of the *upp* gene, strain JW710, is resistant to 5-FU. The introduction of a plasmid containing the wild-type *upp* gene expressed constitutively from the *aph*(5')-III promoter (the promoter for the kanamycin resistance gene in Tn5) into JW710 restored sensitivity to 5-FU to wild-type levels. This observation is the basis for the establishment of a two-step integration and excision strategy for deleting genes of interest. Since this in-frame deletion does not leave behind an antibiotic cassette, multiple gene deletions can be generated in a single strain. With this method, a markerless deletion of the R-subunit (DVU1703) of a type I restriction-modification system (*hsdR*), strain JW7035, was constructed. The transformation efficiency of the JW7035 strain is greater (an approximate 2-log increase in transformants) compared to wild-type DvH when transforming stable plasmids via electroporation.