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Recent Work

Title

The Development of an In-frame Deletion System in *Desulfovibrio vulgaris* Hildenborough

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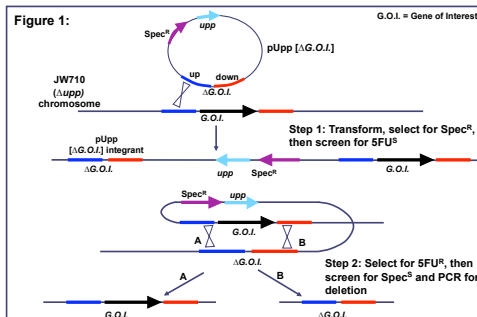
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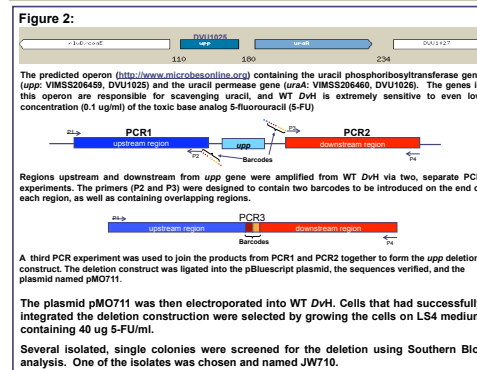
ABSTRACT

In recent years, 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 low number of selectable markers now available in *D. vulgaris*. To broaden the repertoire of genetic tools for manipulation of *D. vulgaris*, an in-frame markerless deletion system is being 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 is resistant to 5-FU. The introduction of a plasmid containing the wild-type *upp* gene expressed constitutively from the *aph*(S)-III promoter (the promoter for the kanamycin resistance gene in Tn5) into the *upp* deletion strain restored sensitivity to 5-FU. This observation is the basis for the establishment of a two-step integration and excision strategy for the deletion of genes of interest. Since this in-frame deletion does not contain an antibiotic cassette, multiple gene deletions can be generated in a single strain using this method. To construct such a markerless deletion for the R-subunit (DVU1703) of a type I restriction-modification system, Gateway Technology methods (Invitrogen) are being used. A destination vector containing the constitutively expressed wild-type *upp* gene has been constructed and is available for generating deletion vectors. Its use is reported here.

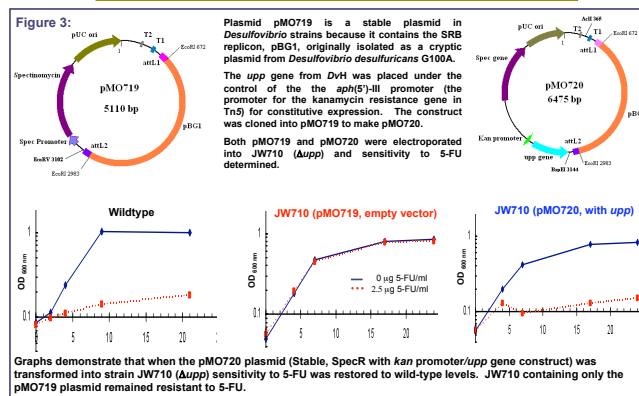
Markerless Deletion Strategy



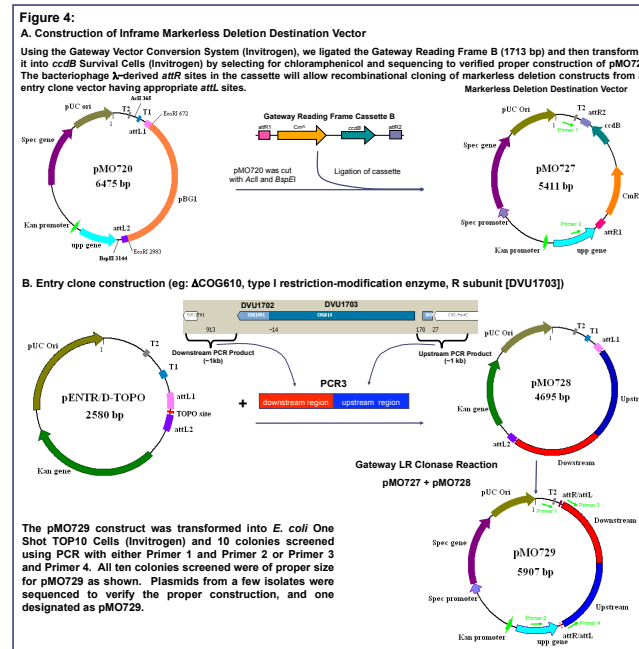
Construction of JW710 (Δupp) Strain



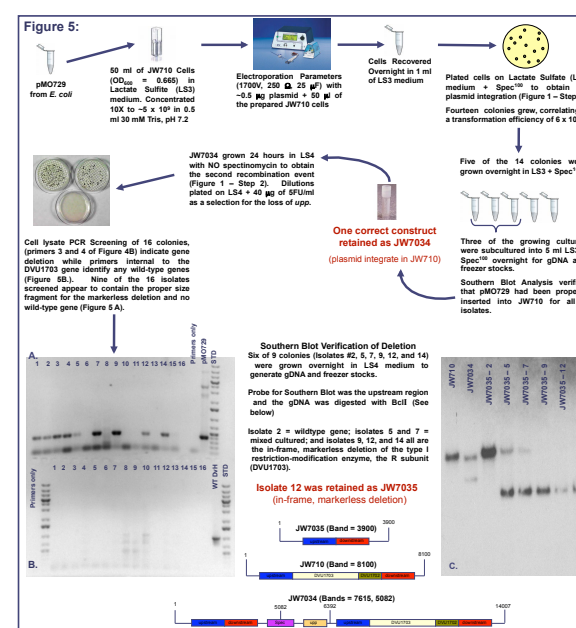
Construction of the kan promoted *upp* gene



Gateway Technology



In-Frame Deletion



CONCLUSION

- The deletion of the Type I Restriction-Modification enzyme, R subunit (DVU1703), clearly demonstrates the successful development of a two-step integration and excision strategy using *upp* as a counterselectable marker in *Desulfovibrio vulgaris* Hildenborough
- Preliminary data show wild-type growth rates for JW7035
- The Gateway construction allows for efficient generation of deletion vectors
- Because the resulting deletion mutants do not contain antibiotic resistance determinants, multiple genes can be deleted in a single strain

ACKNOWLEDGEMENT

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