

Promoter-Trap Plasmid for Identifying Promoters

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The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing a promoter-trap vector for use in Gram-positive bacteria.

Overview

Promoters are genetic regulatory elements that drive gene expression in cells under certain conditions. One way to identify promoters is to use a promoter-trap vector, which is a plasmid containing a multiple cloning site at the 5' end of a promoter-less marker gene. An unidentified DNA fragment is cloned into the multiple cloning site, and expression of the marker gene is monitored to identify active promoter elements in the unidentified DNA.

The Invention

UW-Madison researchers have developed a promoter-trap vector for use in Gram-positive bacteria such as Bacillus cereus. The promotertrap vector was constructed to contain genes for ampicillin and chloramphenicol resistance and can replicate in E. coli and B. cereus. A multiple cloning site containing EcoRl, Sac-I, Kpn-I, Smal, BamHI, and Xbal restriction sites was inserted at the 5' end of a promoter-less, green fluorescent protein (GFP) marker gene. Expression of this modified GFP can be quantified by measuring fluorescence intensity and is amenable to flow cytometry and cell sorting.

Applications

- · Identifying promoters
- · Assessing gene expression under various environmental conditions
- · Monitoring pathogen-host interactions

Key Benefits

- · Very few promoter-traps have been developed for Gram-positive bacteria
- GFP requires no exogenous substrate for detection of gene expression.

Stage of Development

Successfully used to identify several promoters from a library of B. cereus genomic DNA.

Additional Information

For More Information About the Inventors

Jo Handelsman

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