Research Tools

Recombinant oriP Plasmid Vector for Efficient DNA Synthesis

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WARF: P08058US

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The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing a modified oriP vector that could be used to more efficiently express desirable genes for gene therapy or cell culture.

Overview

The Epstein-Barr virus (EBV) is a member of the Herpes family of viruses. The genome of this virus usually is maintained as a nuclear plasmid in the cells it infects. A replication origin (*oriP*) is included within the EBV genome.

The *oriP* vector has been a popular cell culture tool for the expression of DNA sequences of interest. It consists of plasmids with two elements from EBV. The first is the *cis* acting element (*oriP*), which initiates synthesis of DNA and helps partition DNA to dividing cells. The second is the *trans* acting element, EBNA-1, which binds to the *cis* acting element.

Although this vector is widely used, it becomes cytotoxic when its wild-type EBNA-1 moiety is overexpressed. UW-Madison researchers previously developed a non-cytotoxic *oriP* vector by removing 25 amino acids from the *trans* acting EBNA-1 element. This improved vector supports extrachromosomal replication of *oriP* without activating expression of the host genes.

The Invention

UW-Madison researchers have now improved the *oriP* vector by engineering the *cis* acting *oriP* element. The recombinant vector is significantly more efficient than the wildtype vector. It can be used to enhance stable, long term expression of a desirable gene or to express a desirable gene at higher levels.

The origin of synthesis within *oriP* is the Dyad Symmetry (DS) element, which includes two pairs of EBNA-1 binding sites flanked by half-binding sites for the human protein TRF2. The inventors discovered that the affinity of EBNA-1's binding to an origin directly correlates with the efficiency at which DNA synthesis is initiated as well as the efficiency at which extrachromosomal establishment is supported at that origin. They used these findings to construct artificial origins of DNA synthesis that are several-fold more efficient than wildtype DS in their abilities to initiate DNA synthesis and promote extrachromosomal establishment.

Applications

- Gene expression systems
- Gene transfer in gene or cell therapy
- Large vectors that carry sizable regions of genomic DNA
- Preparation of embryonic stem cells from adult somatic cells

Key Benefits

- Modified vector incorporates a significantly more efficient origin of DNA synthesis than the wildtype vector.

- Enhances transformation efficiency for cells that are difficult to transform, such as stem cells or nondividing cells like lung cells
- Provides a method of maintaining and expressing at least one heterologous DNA sequence in a cell
- Useful to deliver genes to cells *ex vivo* or *in vivo*
- The non-cytotoxic EBNA-1 derivative previously developed by the inventors may be used in the vector.
- *oriP*-based vectors are inexpensive, replicated once per cell cycle and efficiently partitioned to daughter cells; allow large fragments of DNA to be introduced to a cell and maintained extrachromosomally; and do not elicit an immune response.
- The invention also includes a less efficient recombinant vector that may be useful to transiently express a desirable gene or express a desirable gene at lower levels.

Additional Information

For More Information About the Inventors

- William Sugden

Related Technologies

- WARF reference number P04170US describes a non-cytotoxic oriP vector created by removing 25 amino acids from the trans acting EBNA-1 element.

Publications

- Lindner S.E., Zeller K., Schepers A. and Sugden B. 2008. The Affinity of EBNA1 for Its Origin of DNA Synthesis is a Determinant of the Origin's Replicative Efficiency. J. Virol. 82, 5693-5702.

Tech Fields

- Research Tools : Other research tools

For current licensing status, please contact Rafael Diaz at rdiaz@warf.org or 608-960-9847