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A Method for Inserting Genetic Material into Genomic DNA

AzTE Case # M10-168

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Invention Description

Homologous recombination is the process by which similar DNA sequences exchange information with one another. Since homologous recombination is a rare event, various selection markers are used for isolation of recombinants that have undergone homologous recombination. Though a variety of positive selection markers exists, very few negative selection markers are available, such as are necessary to select against random integrations and/or eliminate marker genes. Currently, for example, the herpes simplex-thymidine kinase (HSV-TK) gene is the primary negative selection marker used. This system, however, uses a nucleoside analog for the selection, thereby making it potentially able to produce undesirable mutations elsewhere in the genomic DNA.

Researchers at the Biodesign Institute of Arizona State University have developed a novel negative selection marker for the rapid and efficient isolation of genetic recombinants. This negative selection scheme consists of using a fusion protein, GyrB-PKR, in conjunction with the antibiotic, coumermycin A1. Under selective conditions, this marker regulates eukaryotic translation machinery leading to the shut off of protein synthesis. This is further unique compared to other negative selection markers that act on replicating DNA.

In this system, genetic recombinants are enriched in the selective media through the loss of the negative selection marker. This system therefore provides a superior method of isolating clones in a safe and effective manner, without increasing the chances of generating alterations in the DNA elsewhere in the genome.

Potential Applications

- Vaccine development
- Cellular system analysis
- Development of genetically modified mammals

Benefits and Advantages

- No extraneous marker sequence is left in the genetic locus of interest.
- Universal to all eukaryotic organisms
- Avoids the use of special cell lines or noxious mutagenic chemicals that can be potentially damaging to genomic DNA
- Can be used with any system, both DNA and RNA, that undergoes homologous recombination

Intellectual Property Status:

Patent Pending

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