DNA Constructs for Expression of Proteins

Technology #19247

Applications

This technology is a vector system for heterologous protein expression in methylotrophic yeast that has applications in the manufacturing of biologic pharmaceuticals and commercial enzymes.

Problem Addressed

Biologic pharmaceuticals have revolutionized the drug industry, and biologics are currently one of the fastest growing areas of therapeutic development. One of the current struggles in biologic manufacturing is achieving high per-cell productivity to maximize protein titer. Methylotrophic yeast, such as *Pichia*, have recently gained popularity due to their rapid growth and post-translational modification capabilities. However, the expression vectors available for protein production in methylotrophic yeast lag far behind those for other cell systems, and there is a need for optimized vector systems to increase protein expression.

Technology

This technology is an improved vector system to drive very high expression of heterologous proteins in methylotrophic yeast. These inventors systematically identified promoter sequences, translation initiation (Kozak) sequences, secretion signal sequences, and mRNA codons that resulted in drastic increases in heterologous protein expression. The promoter sequences chosen drive very high levels of protein expression regardless of integration location, and integrations at the endogenous locus, alternate loci, or even as an episomal vector result in efficient protein production. Analysis of optimal Kozak translation initiation sequence was previously limited in methylotrophic yeast. These inventors determined the optimal Kozak sequence that increases protein production by increasing translation initiation. Biologic protein products frequently include a signal sequence that codes for extracellular secretion to facilitate isolation of the product. These inventors determined the native signal sequences in methylotrophic yeast and identified those that resulted in optimal secretion of a heterologous protein product. Finally, the inventors analyzed the effects of three-dimensional mRNA folding on protein translation, and demonstrated that codon optimization can further increase expression of heterologous protein. Taken together, these vector improvements drastically increase the per-cell yield of heterologous protein product in methylotrophic yeast.

Advantages

- Vector system for methylotrophic yeast
- Optimized promoter, signal sequence, Kozak sequence, and mRNA codon choice
- Increased per-cell yield of heterologous protein product
- Efficient expression as integrated vector or episomal plasmid

Categories For This Invention:

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Life Sciences
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Intellectual Property:

Constructs and cells for enhanced protein expression
US Patent Pending

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