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(54) **MGMT-BASED METHOD FOR OBTAINING HIGH YIELDS OF RECOMBINANT PROTEIN EXPRESSION**

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See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

7,662,584 B2 * 2/2010 Penttila et al. 435/69.1
7,846,722 B2 * 12/2010 Williams et al. 435/325
2011/0034368 A1 * 2/2011 Carson et al. 514/1.1

FOREIGN PATENT DOCUMENTS

DE WO 2006/114409 A1 * 11/2006 C07D 239/49
WO 2004/031404 A1 4/2004

OTHER PUBLICATIONS

Wu et al., Expression of Human O6-Methylguanine-DNA Methyltransferase in Chinese Hamster Ovary Cells and Restoration of Cellular Resistance to Certain N-Nitroso Compounds. *Molecular Carcinogenesis* 4: 482-488. 1991.*

Barash et al. Human secretory signal peptide description by hidden Markov model and generation of a strong artificial signal peptide for secreted protein expression. *Biochemical and Biophysical Research Communications*. 294: 835-842, 2002.*

Broun et al., Catalytic plasticity of fatty acid modification enzymes underlying chemical diversity of plant lipids. *Science*, 1998, vol. 282: 1315-1317.*

Chica et al., Semi-rational approaches to engineering enzyme activity: combining the benefits of directed evolution and rational design. *Curr. Opi. Biotechnol.*, 2005, vol. 16: 378-384.*

Devos et al., Practical limits of function prediction. *Proteins: Structure, Function, and Genetics*. 2000, vol. 41: 98-107.*

Kisselev L., Polypeptide release factors in prokaryotes and eukaryotes: same function, different structure. *Structure*, 2002, vol. 10: 8-9.*

Lemos et al., Rabies virus glycoprotein expression in *Drosophila* S2 cells. I: Design of expression/selection vectors, subpopulations selection and influence of sodium butyrate and culture medium on protein expression. *J. Biotechnol.*, 2009, vol. 143: 103-110.*

Schamabach et al., Vector design for expression of O-methylguanine-DNA-methyltransferase in hematopoietic cells. *DNA Repair*, 2007, vol. 7: 1187-1196.*

Seffernick et al., Melamine deaminase and Atrazine chlorohydrolase: 98 percent identical but functionally different. *J. Bacteriol.*, 2001, vol. 183 (8): 2405-2410.*

Sen et al., Developments in directed evolution for improving enzyme functions. *Appl. Biochem. Biotechnol.*, 2007, vol. 143: 212-223.*

(Continued)

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(57) **ABSTRACT**

The present invention relates to a novel enhancer of protein production in host cells. It discloses a vector for expressing recombinant proteins in these cells, comprising a nucleotide sequence encoding a) a secretion peptidic signal, b) a 6-methylguanine-DNA-methyltransferase enzyme (MGMT, EC 2.1.1.63), a mutant or a catalytic domain thereof, and c) a recombinant protein. Said MGMT enzyme is preferably the so-called SNAP protein.