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(54) Title: EXPRESSION VECTOR FOR RECOMBINANT U-CONOTOXIN THIA OR THIAALAMUT EXPRESSION IN E.COLI

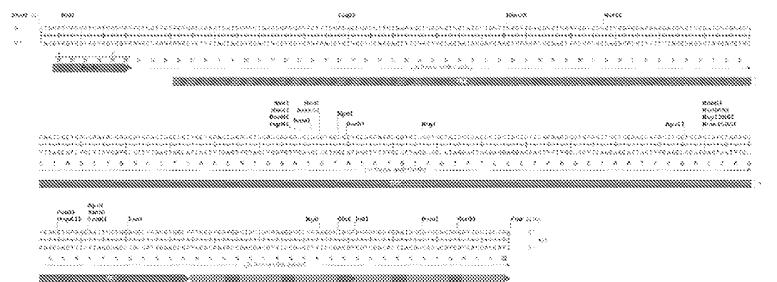


Fig. 1

(57) Abstract: Subject matter of the invention is a construct for an expression vector for recombinant μ -conotoxin THIA or THIAAlaMut expression, characterized in that it contains either the nucleotide sequence of μ -conotoxin THIA SEQ ID NO: 1 or the nucleotide sequence of μ -conotoxin THIAAlaMut SEQ ID NO: 4, both of which at the 5' end contain a sequence encoding six histidines (6His) linked by a serine-glycine-serine linker (SGS) to a construct encoding a TRX::THIA fusion protein containing the μ -conotoxin THIA gene and the gene encoding a leader protein, or a TRX::THIAAlaMut fusion protein containing the μ -conotoxin THIAAlaMut gene and a leader protein, where the said leader protein is thioredoxin (TRX) modified by point mutagenesis in which the amino acid methionine at position 37 has been replaced by lysine. Another subject matter of the invention is an expression vector containing the construct according to the invention under the control of a constitutive promoter. Another subject matter of the invention is an isolated E. coli cell containing the expression vector according to the invention. Another subject matter of the invention is a method for producing μ -conotoxin THIA or THIAAlaMut in E. coli with the expression vector containing the construct according to the invention, characterized in that it includes the following steps: a) Transformation of an E. coli cell with the expression vector containing the construct according to the invention, under the control of a constitutive promoter, encoding either a TRX::THIA fusion protein with amino acid sequence SEQ ID NO: 2 or a TRX::THIAAlaMut fusion protein with SEQ ID NO: 5; b) Expression of the TRX::THIA or TRX::THIAAlaMut fusion protein; c) Isolation and purification of the TRX::THIA or TRX::THIAAlaMut fusion protein; d) Disulfide bridges forming by subjecting the purified TRX::THIA or TRX::THIAAlaMut fusion protein to glutathione treatment in GSH/GSSG and dialysis in buffer; e) Cyanogen bromide cleavage of the TRX::THIA or TRX::THIAAlaMut fusion protein with disulfide bridges formed; f) Purification of the cleaved THIA or THIAAlaMut peptide.

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