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Modification of Escherichia coli PtolT Expression Vector via Site-Directed Mutagenesis

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Abstract : Besides having the appropriate amino acid sequence to perform the function of proteins, it is important to have correct conformation after this sequence to process. To consist of this conformation depends on the amino acid sequence at the primary structure, hydrophobic interaction, chaperones and enzymes in charge of folding etc. Misfolded proteins are not functional and tend to be aggregated. Cysteine originating disulfide cross-links make stable this conformation of functional proteins. When two of the cysteine amino acids come side by side, disulfide bond is established that forms a cystine bridge. Due to this feature cysteine plays an important role on the formation of three-dimensional structure of many proteins. There are two cysteine amino acids (C44, C69) in the Tol-A-III protein. Unlike protein disulfide bonds from within his own, any non-specific cystine bridge causes a change in the three dimensional structure of the protein. Proteins can be expressed in various host cells as directly or fusion (chimeric). As a result of overproduction of the recombinant proteins, aggregation of insoluble proteins in the host cell can occur by forming a crystal structure called inclusion body. In general fusion proteins are produced for provide affinity tags to make proteins more soluble and production of some toxic proteins via fusion protein expression system like pTolT. Proteins can be modified by using a site-directed mutagenesis. By this way, creation of non-specific disulfide crosslinks can be prevented at fusion protein expression system via the present cysteine replaced by another amino acid such as serine, glycine or etc. To do this, we need; a DNA molecule that contains the gene that encodes for the target protein, required primers for mutation to be designed according to site directed mutagenesis reaction. This study was aimed to be replaced cysteine encoding codon TGT with serine encoding codon AGT. For this sense and reverse primers designed (given below) and used site-directed mutagenesis reaction. Several new copy of the template plasmid DNA has been formed with above mentioned mutagenic primers via polymerase chain reaction (PCR). PCR product consists of both the master template DNA (wild type) and the new DNA sequences containing mutations. Dpn-l endonuclease restriction enzyme which is specific for methylated DNA and cuts them to the elimination of the master template DNA. E. coli cells obtained after transformation were incubated LB medium with antibiotic. After purification of plasmid DNA from E. coli, the presence of the mutation was determined by DNA sequence analysis. Developed this new plasmid is called PtolT-δ.

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