In vitro synthesis of human insulin gene usig computational methodology

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Our study focuses on the production of Human Pro-Insulin protein, because its stability, comparatively large size molecule which enabling them to resist the degradation in the bacterial host cytoplasm, Human Pro-Insulin protein will be produced via recombinant DNA technology, and will expressed in bacterial host of E.coli , obtaining human Insulin protein after that will be easily achieved by some special proteolytic digestion , to eliminate and remove the C-peptide , after forming the inter and intra chain disulphide linkages.This study briefly depends upon designing of the suitable gene sequence for the Human pro-Insulin, genetic codons optimized using computer program to be compatible with abundant codons used by E.coli in which the protein produced, human pro-Insulin gene were assembled and amplified using PCR based techniques, then synthesized gene were inserted into cloning vector plasmid and transformed into E.coli, which were chosen carefully for the cloning step, to maximizing the number of cloned gene. Cloned gene isolated and purified then inserted into an expression vector then suffering transformation again in another bacterial E.coli strain but this strain used in the expression of the genes on plasmids and production of the their proteins relative to this genes as the following:Protein sequenceHuman pro-Insulin amino acids sequence were obtained from (Uniprot) one of the most famous protein data bank, pro-Insulin protein were -composed of 86 amino acids , consequently; DNA sequence of the human pro Insulin gene which are compatible with the amino acid sequence were obtained -using (BioEdit) software.Codon OptimizationGenetic codons of the human pro Insulin gene which found to be used rarely by the translation machinery of the E.coli, were altered by abundant codons that used extensively by E.coli, without changing in the amino acids composition, depending upon the presence of more than one codon for the same amino acid, Codon Optimization achieved by using a web based software for this step called OPTIMIZER.