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ORIGINAL ARTICLE

Cloning of Pituitary Gland Hormone in pET32a Expression Vector

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ABSTRACT

Background: Production of recombinant proteins, particularly human proteins, in bacterial hosts is of great importance in genetic engineering. The advantages of this process are fast, supplementary, and cheap production of proteins. Bacterial hosts are readily available and easy to use. In this study, we sought to determine the ability of cloning human genes in bacterial hosts using codon optimization program and pET32a vector.
Materials and Methods: One of the pituitary gland hormones was cloned in *E. coli* host with the pET32a vector and finally the cloned gene was confirmed by sequencing.
Results: Our findings showed the cloned gene matched with the optimized and designed gene according to sequence and size.
Conclusion: Human proteins can be produced in bacterial hosts after codon optimization and use of suitable vectors such as pET32a. These recombinant proteins have mutual similarities to the natural ones and can be appropriate for diagnostic procedures or even pharmaceutical purposes.

Key Words: Codon, *Escherichia coli*, Gene expression, Genetic vectors

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INTRODUCTION

In most cases, recombinant proteins have structural similarities with the natural ones. We can also increase production of recombinant forms up to the favorable rate in case of proteins produced naturally in small quantities. In addition, the process of purification for recombinant proteins is easier than natural ones [1].

In recent years, a great number of proteins have been cloned in *E. coli*. There is a wide range of hosts for recombinant protein production, but *E. coli* is the best choice [2]. The expression of human genes in *E. coli* is associated with lower expression levels because of huge differences between the dominant codons of humans and *E. coli* [3, 4].

Codon usage bias can have a profound effect on the production of heterologous proteins. A wide range of studies have been performed on codon optimization and the effects of mammalian proteins on *E. Coli* [5]. The adopted methods involve the creation of targeted mutation to remove rare codons or add-on tRNAs of rare codons in specific cell lines. Recently, low-cost production of synthetic genes (codon optimization) is an excellent alternative for these methods [6].

Codon usage is observed in many species. Use of codons in a gene is directly associated with its efficacy [7]. pET system is one of the most powerful systems for gene expression and production of recombinant proteins in *E. coli* hosts. Given the independence of target gene transcription from the host cell, mRNA and protein production in this system is not affected by cellular factors involved in protein synthesis in the host cell; therefore, production in this system is enhanced [8].

The aim of this study was to determine the cloning ability of human genes in bacterial hosts using codon optimization program and pET32a vector.

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MATERIALS AND METHODS

In this experimental study, gene characteristics, including length, nucleotide sequence, and amino acid sequence, were obtained from NCBI and other valid sites such as UniProt. For codon optimization, the Vector NTI software was used. Then, cutting site of Hind III and Xho I enzymes were placed at the beginning and end of the gene, respectively.

Then, human pituitary hormone synthetic gene was made in pBSK plasmid (BIOMATIC Co., Germany). *E. coli* DH5α strain was used for cloning and multiplication of plasmid. pET32a plasmid was applied for the cloning and expression vector.

The gene fragment was amplified by polymerase chain reaction (PCR) to pull out from the prior plasmid (pBSK), Then it was inserted into a new vector (pET32a), and its rate increased. Primers were designed by AlleleID 6 program. The forward and reverse primers were respectively 5' - AGCAAGCTTTTTTGCATTCCG - 3', and 5'- TTACTCGAGCACGCTAAAGCCCAC - 3'. Cutting sites of Hind III and Xho I enzymes were placed in these primers. PCR was performed in different concentrations of magnesium (i.e., 0.75, 1, and 1.5 µl). Annealing temperature was considered 52°C. PCR product was analyzed by horizontal gel electrophoresis.

Rapid digestion method was used to precipitate the process of enzymatic digestion of gene segments and pET32a vector.

After insertion of hormone genes into pET32a, they transformed into competent DH5α cells by using temperature shock (using at temperatures 0°C and 37°C), and the bacteria were cultured on nutrient agar containing AMP. After plasmid purification of these bacteria through minipreparation method, plasmid samples were run beside undigested pET32a in a horizontal electrophoresis. Finally, one of the purified plasmid samples that seemed to have our gene was sent with a primer to GenFanAvaran Company for sequencing.

RESULTS

In PCR reaction, we achieved the desired results. In all the three concentrations of magnesium, bands of pituitary hormone were 364 bp in size, which was better in the second column (0.75 µl concentration; Figure 1).

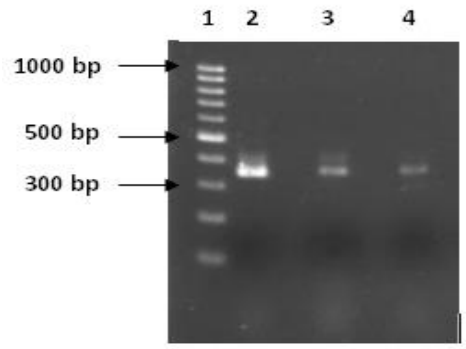

Figure 1. Polymerase chain reaction result on horizontal gel. Column 1 contains DNA ladder, and 2, 3 and 4 include PCR products with 0.75, 1, and 1.5 µl concentrations of magnesium

Transformation of plasmids containing gene to DH5α cells was favorable. After plasmid purification, these plasmids were located higher because of their lower weight compared to undigested pET32a, as a marker in electrophoresis (Figure 2).

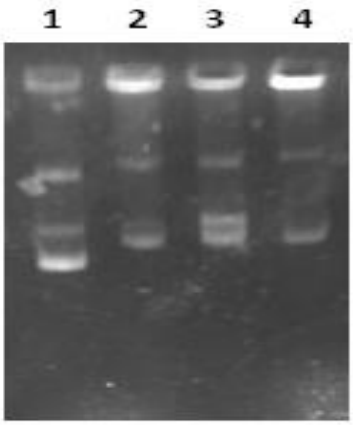

Figure 2. Result of plasmid purification on horizontal gel; columns 2 to 4 comprise of purified pET32a containing hormone gene

Gene sequencing is defined as matching of cloned gene sequence with the pre-designed sequence. The result is presented graphically, the distinct same colored peaks above baseline indicate compliance with the desired nucleotide (Figure 3).

DISCUSSION

Recombinant proteins have some advantages in comparison with other proteins. For instance, copied version of the gene can act exclusively and cause less immune system reactions than the natural ones. Moreover, recombinant proteins are efficient and cost-effective with high frequency [9].

Expression of proteins using *E. coli* is cheap, easy, and fast. Mammalian host systems have several drawbacks including poor secretion,

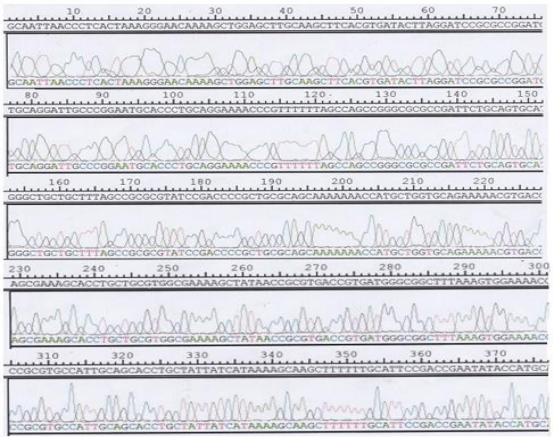

Figure 3. Gene sequencing findings

cost-intensive methods, and the possibility of product contamination with viruses [9].

Bacteria and eukaryotic cells to use different codons, which cause mammalian genes to express the bacteria poorly [10-12]. In this study, to overcome codon usage problem and enhance protein production, the respective gene was optimized and designed by Vector NTI software.

The E. coli system is used for recombinant protein production in research and industry due to advantages such as high efficiency, cost- and time-effectiveness, easy cultivation, and rapid recovery of protein [9, 13-14]. *E. coli* DH5α strain due to having protease, breaks down the recombinant protein. Thus, it cannot be used for recombinant protein expression, but since it has no plasmid, it is used for replication of plasmid [15, 16].

E. coli is a well-known microorganism for gene engineering. In this microorganism control of promoter is not difficult and the number of plasmids can be easily changed [9, 17].

E. coli BL21 is one of the most common hosts for the expression of recombinant proteins that can be cultured effectively in the simplest media. E. coli BL21 is non-pathogenic and has no membrane-bound proteins; therefore, it does not cause any degradation of the recombinant protein [16, 18-19].

In pET32a system, expression of target gene is under the control of bacteriophage T7 strong promoter, controlled by the lac operons. It has a specific amino acid sequence of 6 histidine (6His-tag) located at '5 cloning sites and is finally added to the N-terminal of protein. This sequence was applied for purification of recombinant protein using affinity chroma-tography, which has an ampicillin-resistant gene that plays an important role in screening out transformed bacteria [20].

CONCLUSION

Recombinant production of human proteins after codon optimization and by using suitable vectors such as pET32a is possible in bacterial hosts. These recombinant proteins have great similarity with natural ones and can be appropriate alternatives for use in diagnostic procedures or even pharmaceutical purposes.

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CONFLICTS OF INTEREST

None declared.

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