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ORIGINAL ARTICLE

Cloning of Pituitary Gland Hormone in pET32a Expression Vector

Hadi Mohammadzade[1], Behzad Khansari nejad[2], Abdorrahim Sadeghi [3], Hamid Abtahi[3]

[1] Department of Biotechnology, School of Medicine, Arak University of Medical Sciences, Arak, Iran; [2] Department of Microbiology and Immunology, School of Medicine, Arak University of Medical Sciences, Arak, Iran; [3] Endocrine and Metabolism Research Center , Arak University of Medical Sciences, Arak, Iran;

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ABSTRACT

Introduction: The aim of this study was to determine the ability of the cloning of human genes in bacterial hosts using codon optimization program and pet32a vector. Production of recombinant proteins, particularly human proteins in bacterial hosts has a great importance in genetic engineering. The advantages of this process is fast, further and cheaper production of proteins. Bacterial hosts are readily available and use of them is easy.

Methods: One of the hormones of pituitary gland as a human gene was cloned in *E. coli* host with the *pET32a* vector and finally cloned gene was confirmed by sequencing.

Results: The cloned gene matched with optimized and designed gene according to sequence and size.

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 to natural forms and can be appropriate alternatives for using in diagnostic procedures or even pharmaceutical purposes. *JOURNAL OF IRANIAN CLINICAL RESEARCH* 2015;1(2):58-61

INTRODUCTION

In most cases, the recombinant proteins have structural properties of natural proteins. We can also increase production of recombinant forms to favorable rate in case of proteins that produce naturally in small quantities. In addition, purification of recombinant proteins is easier than natural proteins [1].

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

Codon bias can have a profound effect on the expression of heterologous proteins. Many works on codon optimization, is about the expression of mammalian proteins in *E. coli* [5]. Methods used to solve this problem, involve the creation of targeted mutation to remove a rare codons or addition tRNAs of rare codons in the 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 selected codons in a gene, has a direct proportion with its expression efficacy [7].

pET system, is one of the most powerful systems for gene expression and production of recombinant proteins in *E. coli* hosts. Because of 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 further [8]. The aim of this study was to determine the ability of the cloning of human genes in bacterial hosts using codon optimization program and pet32a vector.

MATERIALS AND METHODS

Gene characteristics such as length, nucleotide sequence, amino acid sequence, etc. were obtained from the NCBI and other valid sites like Uni Prot. For codon optimization, the Vector NTI

Correspondence: Hamid Abtahi, Molecular and Medicine Research Center ,Arak University of Medical Sciences, Arak, Iran. Email: abtahi@arakmu.ac.ir.

program was used. After entering the amino acid sequence of gene, selecting *E. coli* strain K12 as a host, avoiding sequences that cause identifying and unwanted cutting gene with desired restriction enzymes and observe other cases, the target sequence was obtained. Then, cutting site of Hind III and Xho I enzymes were placed, respectively, at the beginning and end of the gene.

Then human pituitary hormone synthetic gene was made in pBSK plasmid by BIOMATIC Co Germany.

E. coli DH5α strain was used for cloning and multiplication of plasmid. pET32a plasmid was applied as cloning and expression vector.

The gene fragment was amplified by PCR in order to pull out from the prior plasmid (pBSK) and being able to insert to new vector (pET32a) and its rate to be 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 reaction was performed in different concentrations of magnesium (0.75, 1 and 1.5 µl). Annealing temperature was considered 52 °C. PCR product was analyzed by horizontal gel electrophoresis.

For enzymatic digestion of gene segments and pET32a vector, rapid digestion method was used. After Ligation of hormone gene to pET32a, these were transformed into the competent DH5α cells by using temperature shocks (use of temperatures 0 °C and 37 °C), and bacteria were cultured on nutrient agar containing Amp. After doing plasmid purification on these bacteria cells by mini-preparation method, plasmid samples were run beside undigested pET32a in a horizontal electrophoresis. Then, one of the purified plasmid samples that seem to have our gene was sent with a forward primer to GenFanAvaran Company for sequencing.

RESULTS

In PCR reaction, we achieved good outputs. In all three amounts of magnesium, bands of pituitary hormone were present with size of 364 bp, which in the second column (0.75 µl concentration) band was seen better (Figure 1).

Transformation of plasmids containing gene to DH5α cells was good. After plasmid purification, these plasmids because of becoming heavier were located higher than undigested pET32a as a marker in electrophoresis (Figure 2).

Gene sequencing, was shown matching of cloned gene sequence with designed already sequence. The result is shown graphically; distinct same colored peaks above baseline indicates compliance with the desired nucleotide (Figure 3).

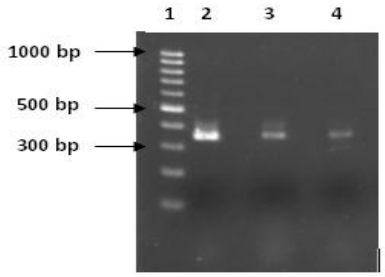

Figure 1: PCR result on horizontal gel. Column 1 contains DNA ladder, 2, 3 and 4 contain PCR products with 0.75, 1 and 1.5 concentrations of magnesium

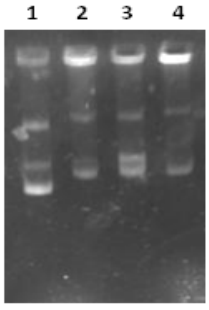

Figure 2: result of plasmid purification on horizontal gel. Column 2 to 4 contain purified *pET32a* containing hormone gene

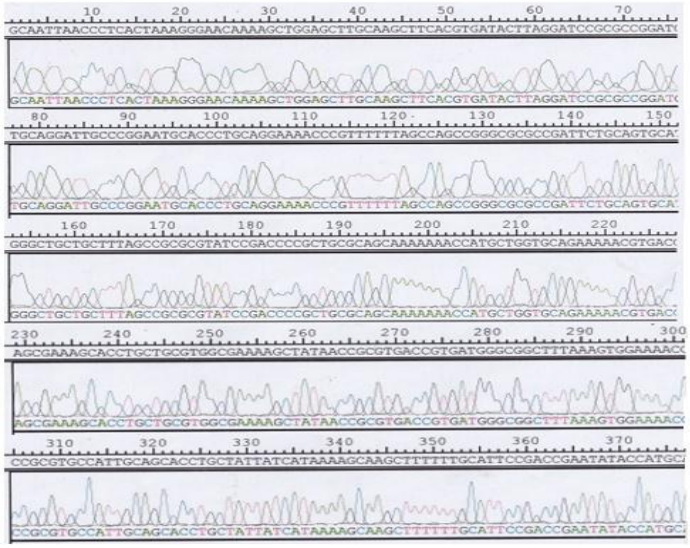

Figure 3: Result of gene sequencing

DISCUSSION

Recombinant proteins in comparison with other proteins have advantages. For instance, copied version of the gene can act specifically and cause less immune response than native form. Moreover, recombinant proteins are produced more effective, less expensive and with a higher frequency [9].

The cheapest, easiest and quickest expression of proteins can be performed by *E. coli*. Mammalian host systems have several drawbacks: poor secretion, expensive methods, and the possibility of product contamination with viruses [9].

Bacteria and mammalian prefer to use different codons. This may cause mammalian genes are expressed poorly in bacteria [10-12]. In this study, to overcome the codon usage problem and high production of protein, optimization and design of the gene was performed by Vector NTI program.

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

E. coli genome has been known better than any other microorganisms. Its genome is manipulable, control of its promoter is not difficult and the number of its plasmids is changed easily [9, 17].

Expression host *E. coli* BL21 is one of the most common hosts for the expression of recombinant proteins that can be grown effectively in the simplest culture mediums is non-pathogen and has no membrane-bound proteases, so does not cause 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. Other useful features of pET32 plasmid can be mentioned as such. It has a specific amino acid sequence of 6 histidine (6His.tag) located in '5 cloning sites and finally is added to the N-terminal of protein. This sequence was applied for purification of recombinant protein using affinity chromatography. And that has an ampicillin resistance gene that plays an important role in the screening of 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 to natural forms and can be appropriate alternatives for using in diagnostic procedures or even pharmaceutical purposes.

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