

# Cloning and Expression of Luteinizing Hormone Subunits in Chinese Hamster Ovary Cell Line

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## Abstract

**Background:** Luteinizing hormone (LH) was secreted by the stimulating cells of the testes and ovaries in the anterior pituitary gland. The application of this hormone is in the treatment of men and women with infertility and amenorrhea respectively.

**Materials and Methods:** In the present study the alpha and beta subunits of human LH gene were cloned into the pEGFP-N1 expression vector and produced the recombinant LH hormone in Chinese hamster ovary (CHO) eukaryotic system.

**Results:** Alpha and beta subunits of LH hormone were cloned between *NheI* and *BamHI* cut sites of pEGFP\_N1 expression plasmid and confirmed by PCR. Hormone expression was evaluated in CHO cell line by Western blotting using the specific antibody.

**Conclusion:** Alpha and beta subunits of LH hormone were expressed in CHO cell line perfectly.

**Keywords:** LH, Amenorrhea, Sperm, cloning, Recombinant Hormone

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## Introduction

Luteinizing hormone (LH) is one of the gonadotropins essential for the process of reproduction. This glycoprotein hormone consists of two subunits alpha and beta. The alpha subunit is common among the hormones of this family, while the beta subunit of this type of hormone varies in amino acids and component of carbohydrate. Beta subunit is responsible for the specific function of these hormones. LH hormone was secreted from gonadotropic basophil cells of the anterior pituitary.

This hormone as well as other glycoprotein hormones consists of alpha and beta subunits connected by non-covalent bond. Its three-dimensional structure is maintained by internal disulfide bonds<sup>1</sup>. The alpha subunit consists of 92 amino acids and has two chains of N-linked oligosaccharides in ASN102 and ASN76 amino acids and five disulfide bonds. LH hormone was used to treat female infertility, male infertility in hypopituitarism cases, cryptorchidism and in vitro fertilization (IVF)<sup>2-4</sup>. Menotropins are a group of the conventional generic products. They are combinations of LH to FSH. In the present study, the expression and

production of recombinant form of LH hormone in CHO cell line was evaluated.

## Methods

CHO cell line and pEGFP/N1 plasmid were purchased from Pasteur Institute of Iran.

**LH/ pEGFP-N1 Construct design:** The cDNA sequence of alpha and beta subunits of LH hormone obtained from genebank. The IRES region was considered between two subunits and ordered for making into plasmid pGEM\_B1 (Bioneer-Korean). This plasmid was transformed into *E. coli* Top10 strain. The transformed bacterium was cultured in LB (Luria-Bertani) (Merck-Germany) Broth medium for 16 hours. The restriction enzyme sites Nhe1 and BamH1 were designed at the both ends of the  $\alpha$ -IRES- $\beta$  sequence. The fragment was sub-cloned into pEGFP-N1 plasmid (Pasteur-Iran) using the enzyme T4 DNA Ligase (Sigma-USA) and transfer to Ecoli Top 10 strain (Pasteur-Iran) cultured into plate including the Kanamycin (Sigma-USA). Finally, clones containing the expression plasmid were confirmed by PCR. The used primers in PCR reaction are shown in table 1.

**Transition of recombinant plasmid into CHO cells by Electroporation:** CHO cell line was cultured in complete culture medium (DMEM + 10% FBS + Pen/strep1X) (Gibco-Germany) at 37°C in an incubator containing 5% CO<sub>2</sub>. Then, 5×10<sup>5</sup> cells suspended in 200µl hypo-osmolar electroporation buffer (Eppendorf-USA) and 1µg pEGFP-N1-LH plasmid incubated on ice. pEGFP-N1-LH plasmid was transferred into a cell line by electroporation (Eppendorf, Germany). Also pEGFP-N1 plasmid was considered as control. After incubation they were

studied with inverted fluorescent microscopy after 4, 24 and 48 hours.

**SDS PAGE and Western blotting:** After 48 hours, the collected samples were centrifuged at 8000rpm for 5 min and protein lysis buffer (Sigma-USA) was added into deposits. After 15 min, protein loading buffer (Sigma-USA) was added into them and was heated to 80°C. The samples along with protein marker were loaded onto 12% SDS-PAGE. The protein bands were blotted to nitrocellulose membrane. Western blot analysis was carried out by using specific antibody (Abcam-UK) against LH hormone. Anti-rabbit antibody conjugated with HRP (Abcam-UK) was used as second antibody<sup>5-7</sup>. The protein band obtained from LH gene expression in the cells containing the plasmid pEGFP-N1-LH with a molecular weight about 36 KD was appeared by using DAB substrate (Sigma-USA).

**LH assay by ELISA:** The amount of hormone expressed in the supernatant of cell culture has assayed by ELISA Kit (Pishgamteb, Iran). Also, it has evaluated in one million cells extracted.

## Results

A plasmid containing LH ( $\alpha$ -IRES- $\beta$  fragment) was removed with Nhe1 and BamH1 restriction enzymes. This fragment was used for insertion into expression vector in the next step.

**The recombinant plasmid pEGFP-NI-LH confirmation:** The colonies obtained from the ligation process were evaluated using colony PCR. Finally, the PCR product was electrophoresed on agarose gel 1% that shows in figures 2 and 3.

**The transfection of CHO cell line with recombinant LH plasmid:** The introduction of the plasmid

**Table 1:** Universal and Specific primer Used in research.

Primer	Sequence	Annealing T.°C
pEGFP Universal	5'AAATGGGCGGTAGGCGTG 3'	55°C
	5'GCACCACCCCGGTGAACAG 3'	
LH	5'ATATATTTGCTAGCATGGAGATGCTCCAGGGGATGC 3'	58°C
	5'ATATATTTGGATCCAGATTTGTGATAATAACAAGTAC3'	

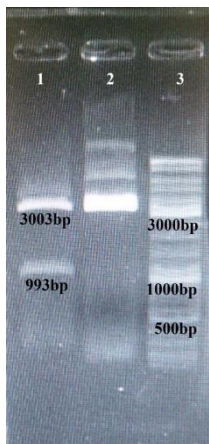


Figure 1. Electrophoresis of digested plasmid as well as control and DNA Ladder. Lane 1: Fragment removed from vector using BamHI and NheI. Lane 2: Intact vector was used as control. Lane 3: 100 bp DNA Ladder.

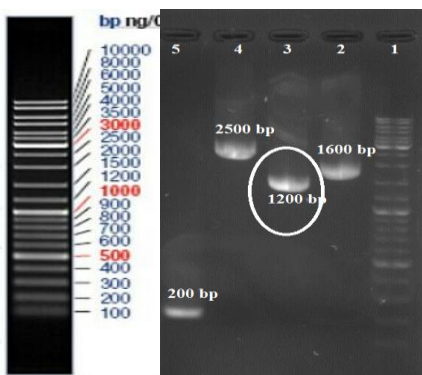


Figure 2. The colony PCR results. Lane1: 100bp DNA Ladder, Lane3: PCR product including the LH fragment, Lane2, 4 and 5: negative ones, The positive colony (lane3), the colonies was cultured on the other plate. At the next step, the new culture was cultured in liquid LB medium containing kanamycin. Then, plasmid DNA (pEGFP-N1-LH) was extracted from the bacteria and confirmed by PCR (Fig.3).

pEGFP-N1 and pEGFP-N1-LH separately in CHO cell line and GFP expression in these plasmids was accompanied by emission of green light from them (Fig. 4).

**Confirmation of recombinant LH Hormone by Western blotting:** Transfected cell line containing the pEGFP-N1-LH, pEGFP-N1 and Negative cells were prepared for analysis. Immunoblotting was carried out using specific antibody against of LH hormone. The 36 KD protein band of LH protein was shown in figure 5.

**LH amount in cell culture medium:** It was detected 0.086 miu/ml and 0.068 miu/ml LH Iin supernatant

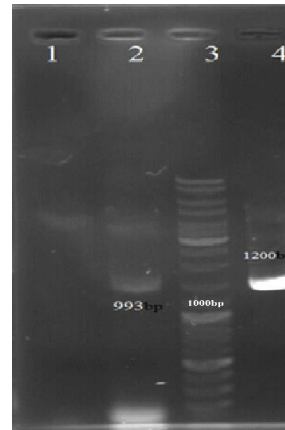


Figure 3. The PCR product of LH gene with specific LH primers and pEGFP-N1 Universal ones. Lane1: Negative control (without DNA), Lane2: PCR product of LH gene amplification using specific primers, Lane3: 100 bp DNA Ladder, Lane 4: PCR product of LH gene amplification using LH Universal primers.

medium and cell pellet extracted separately.

## Discussion

In recent years, infertility problems have affected families. In Iran, 8% of women 19-26 years, 13-14% of women 27-34 years, 18% of women 35-39 years and 18-28% men 35-40 years encountered with this problem<sup>8,9</sup>. LH hormone is one of the drugs used for treatment of infertility. Traditional forms of menotropin drugs were obtained from the urine of mothers and accompanied with different possible contaminations. The allergy along with intolerance response was one of the complications of these drugs<sup>10,11</sup>. The other used drug was the recombinant form of LH hormone. In the present project, the recombinant form of this hormone was produced *in vitro* by designing suitable eukaryotic construct. It appears that this method could be used for sufficient production of this hormone in the country<sup>12</sup>. In the present research, the human LH construct was produced using genetic engineering methods. Recombinant glycoproteins have some advantages to other proteins. One of these is the obtained protein can specifically act as well as the original one. It also showed low immune response in the body. Finally, this technology caused that the industrial production of LH hormone was accompanied with lower-cost, more accessible and lower risk of immunogenicity. Furthermore, the recombinant proteins are effectively produced. This method could be used for high level production<sup>13</sup>.

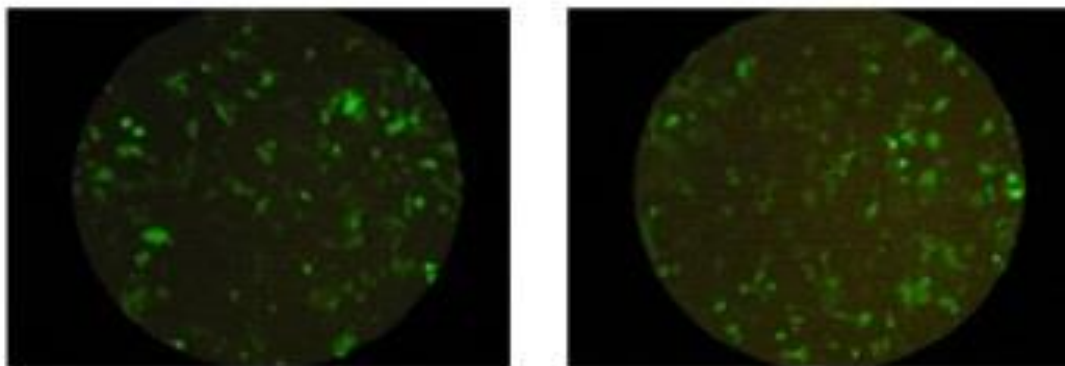


Figure 4. CHO cell line transfected with the pEGFP-N1 plasmid containing *LH* gene (Right) and CHO cell transfected with plasmid pEGFP-N1 (Left) as the control group.

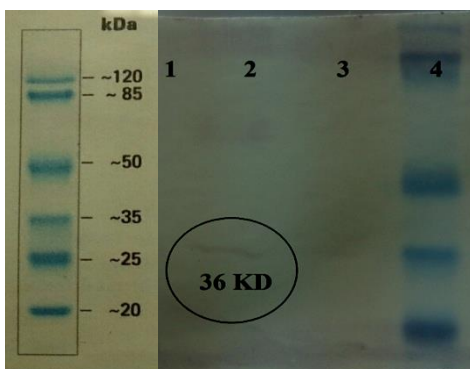


Figure 5. The confirmation of expression of recombinant LH protein in CHO cell line by Western blotting. Lane1: cells without any plasmids, Lane2: cells containing pEGFP-N1-LH plasmid and expression of 36 kDa proteins, Lane3: cells containing the pEGFP-N1 plasmid.

Here we used the pEGFP-N1 expression vector. This vector has a Green fluorescent protein (GFP) gene that makes it easier to identify and track the vector in expressing cells. GFP protein with 238 amino acids has obtained from a jellyfish for the first time. This protein emitted green light under ultraviolet radiation and used as an important tool in detecting various processes used in biology<sup>14</sup>.

Another distinguishing feature of the project is the use of CHO cells for human LH gene expression. Cultured mammalian cells had the ability to make the correct structure of the glycoprotein, the accumulation and post-translational modifications. Therefore, these cells are the optimal system for the production of recombinant proteins which could have different clinical applications<sup>15</sup>.

The researchers of the Institute of Biotechnology at

the University of Maryland in America produced 7.4 mg/L recombinant LH hormone in *Drosophila* cells<sup>16</sup>.

## Conclusion

Alpha and beta subunits of LH hormone were expressed in CHO cell line perfectly.

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## Conflict of Interest

The authors have no financial interest in the products discussed in this article.

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