CLONING OF HUMAN ERYTHROPOIETIN GENE IN pVAX1 VECTOR FOR PRODUCTION OF r-DNA epo

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ABSTRACT: The epo.hu gene is already cloned in pTarget vector. In the present study we have released the epo.hu gene insert from the pTarget.epo.hu using plasmid isolation, RE digestion and ligated with pVAX1 vector. Prepared the competent cell to transformation of ligated product in E.coli DH5α. Make the LB Agar plate and the large number of colonies (approximately 25) were picked from the overnight grown transformants. The individual colonies were inoculated in fresh ampicillin (50 μg/ml) containing LB broth and allowed to grow for large scale production for further experiments. This can be used for expression and immunological studies.

Key words: Erythropoietin gene, pVAX1 vector, replicase vector, gene cloning

INTRODUCTION
Erythropoietin is a glycoprotein hormone that controls erythropoiesis or red blood cell production. It is a cytokine for erythrocyte (red blood cell) precursors in the bone marrow also called hematopoietin or hemopoietin, it is produced by the capillary endothelial cells in the kidney and liver, it is the hormone that regulates red blood cell production. It also has other known biological functions. For example, erythropoietin plays an important role in the brain's response to neuronal injury. Erythropoietin is also involved in the wound healing process [4]. Erythropoietin expression increases in five-sixths nephrectomized rats, after muscle targeted gene transfer by in vivo electroporation, using plasmid DNA expressing rat epo (pCAGGS-epo) [1]. Myelodysplastic syndrome (MDS) may be induced by certain mutagenic environmental or chemotherapeutic toxins; however, the role of susceptibility genes remains unclear. The G/G genotype of the single- nucleotide polymorphism (SNP) rs1617640 in the erythropoietin (epo) promoter has been shown to be associated with decreased epo expression [5]. Keeping the above facts in view, the present work was undertaken to clone the human erythropoietin gene in replicase based eukaryotic pVAX1 vector.

MATERIALS AND METHODS
Vector: pVAX1 vector was used.
epo.hu gene: The epo gene already cloned in pTarget vector was used as the candidate gene, the sequence accession no. #AM933611
Host Bacterial strains: Escherichia coli (E.coli) DH5α (Proteges, Madison, USA) host strain was used for transformation experiments.

Preparation for epo.hu gene:

The plasmid DNA expressing rat epo (pCAGGS-epo) [1]. Myelodysplastic syndrome (MDS) may be induced by certain mutagenic environmental or chemotherapeutic toxins; however, the role of susceptibility genes remains unclear. The G/G genotype of the single- nucleotide polymorphism (SNP) rs1617640 in the erythropoietin (epo) promoter has been shown to be associated with decreased epo expression [5]. Keeping the above facts in view, the present work was undertaken to clone the human erythropoietin gene in replicase based eukaryotic pVAX1 vector.

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pVAX1 vector preparation and ligation:

Pmel was used to create blunt end using 50 µl reaction mix. A 10 µl reaction mixture was for blunt end ligation containing T4 DNA ligase (Fermentas) 1 µl, pVAX1 Vector 2 µl, epo.hu gene 5 µl, ligation buffer (10X) 1µl, nuclease free water 1 µl. The reaction mixture was incubated overnight at 14°C. The linearised plasmid was checked and quantitated on 1% agarose gel electrophoresis.

Preparation of competent cells and Transformation

Took the 500 µl and equal volume of ice-cold 2xTSS is added in tube and the cell suspension mixed gently. Then 2 µl Ligated DNA and mixed the 100 µl cell suspension (competent cell), mixture incubate for 5-6 min at 4°C, add the 0.9 ml of SOC, incubate 37°C with shaking for 1 hr to allow expression of the antibiotic-resistance gene. Transformants are selected by standard methods [2].

Screening of recombinant clones:

A large number of colonies (approximately 25) were picked from the overnight grown transformants. The individual colonies were inoculated in fresh ampicillin (50 µg/ml) containing LB broth and allowed to grow for 18 to 24 hours.

RESULTS

Sub cloning of epo.hu gene in pVAX1.epo.hu

The epo.hu gene cloned in pTarget vector was released by digesting with EcoRI enzyme then makes the 2 fragments one is vector’s size 5.6 kb and second is 0.58 kb of epo.hu gene (Fig. 1). This Eco RI enzyme create staggered end. The pVAX1 vector was linearized by digesting with Pmel enzyme for used to create blunt end size 3.0 kb (Fig. 2), gel eluted, purified by phenol chloroform precipitation method. Blunt end ligation was done and ligated vector was transformed in E.Coli (DH5α) cells. Transformed product was spread in L.B agar plate, after incubated over night at 37°C colony seen.

DISCUSSION

epo.hu gene has become the main candidate for the development of recombinant DNA against anemia. We have tried to transform the epo.hu gene in replicase based eukaryotic vector namely pVAX1. The epo.hu gene was cloned in pVAX1 vector which is a mammalian expression vector. This vector is approved by US FDA for use the recombinant plasmid containing gene in right orientation was selected and characterized. This recombinant plasmid if injected in human, will lead to production of epo in the body which in turn will enhance the erythropoiesis. It will be of great value in India since anemia is a major problem. The recombinant plasmid can be stored and transported at room temperature hence no refrigeration is needed. Erythropoietin (epo) genomic gene was also cloned and its expression vector pOP13/epo was constructed [3]. In another study, A 600 bp synthetic erythropoietin gene encoding all 166 amino acids of the epo protein and 27 amino acids of the signal peptide had been constructed. The results indicated that the nucleotide sequence of the synthetic epo gene was identical to that of the original [7].
In the transformation colony showed the epo.hu gene is transformed in pVAX1 vector on the plate. Further we can use of pVAX1.epo.hu for large scale production in L.B broth and after confirmation by PCR and sequencing can be use in expression and immunological study.

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REFERENCES


