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Research article

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 L.B 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:

pTarget. epo. hu recombinant plasmid containing epo.hu gene insert was revived in LB broth and plasmid DNA was isolated Sambrook and Russell [6]. Restriction endonuclease digestion of recombinant plasmid pTarget.epo.hu with *Eco*RI enzyme was done to release the epo.hu gene insert. This enzyme create staggered end. For blunting of staggered ends generated by *Eco*RI enzyme, T4 DNA polymerase was used. Reaction mixture consisted of epo gene insert 20 µl, T4 DNA polymerase buffer 10X 5 µl, T4 DNA polymerase 2 µl, dNTP mix. (10mM each) 1 µl, nuclease free water 22 µl, making total volume 50 µl. The reaction mixture was incubated at 37°C for 10 min. The blunted epo gene insert was purified using phenol chloroform, following the protocol of Sambrook and Russell [6]. The presence of purified blunted DNA was checked by running 1 µl of DNA on 1% agarose gel.

pVAX1 vector preparation and ligation:

*Pme*I was use 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 Tansformation

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. This culture can be use for immunological studies.

RESULTS

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

The epo.hu gene cloned in pTarget vector was released by digesting with *Eco*RI 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 *Pme*I 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.

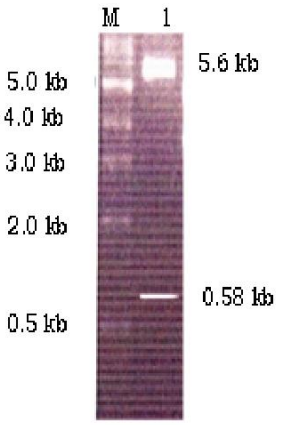

Fig. 1: pTarget.epo.hu digested with *Eco*RI enzyme

Lane M : Marker 1kb ladder

Lane 1 : pTarget.epo.hu digested with *Eco*RI releasing a gene insert .58 kb and vector 5.6 kb

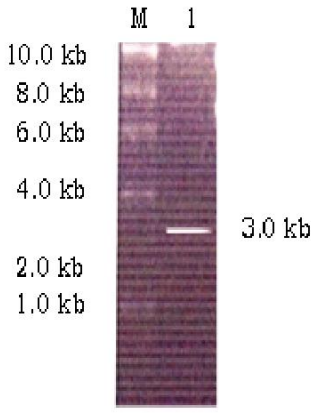

Fig.2: Preparation of pVAX1 Vector

Lane M : Marker 1kb ladder

Lane 1 : pVAX1 digested with *PmeI* enzyme

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 erythropoieisis. 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 166amino 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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