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Construction of Yeast Recombinant Expression Vector Containing Human Epidermal Growth Factor (hEGF)

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ABSTRACT

Purpose: The objective of this study was construction of recombinant hEGF-pPIC9 which may be used for expression of recombinant hEGF in following studies. ***Methods:*** EGF cDNA was purchased from Genecopoeia Company and used for PCR amplification. Prior to ligation, the PCR product and pPIC9 vector was digested with EcoRI and XhoI and ligated in pPIC9 vector and subjected to colony PCR screening and sequencing analysis. ***Results:*** PCR amplification of EGF cDNA using recombinant hEGF-pPIC9 vector as template was concluded in amplification of 197bp fragment. Construction of recombinant hEGF-pPIC9 of EGf gene was verified by PCR and sequencing. ***Conclusion:*** Construction of Recombinant hEGF-pPIC9 was the primary stage for production and expression of EFG in the future study.

Introduction

Epidermal growth factor (EGF) is a member of an extensive class of molecules, reffered to as growth factors, that intervenes in cell growth and differentiation.[1] Epidermal growth factor (EGF), a small polypeptide consist of 53 amino acids with molecular weight of about 6.3kDa, is present in copious mammalian species[2-5] There are six cysteine residues in the hEGF sequence that comprised three disulfide band.[6] Human EGF was purified from urine by Cohen & Carpenter, Starkey et al. EFG was first isolated from the parotid gland of male mice and subsequently from human urine as urogastrone.[7] Mouse EGF is derived from a 1217 amino acid precursor protein which contains 7 additional EGF-like domains. Human EGF is come apart from a 1207 amino acid precursor.[8] Studies have demonstrated that EGF can prompt abundant effect on both cells and epithelial tissue. Besides, hEGF has been noticed to have many biological actions both in vivo and in vitro. Studies have focused on the proliferative efficiency of EGF on keratinocytes, fibroblasts and epithelial cells.[9,10] The yeast Pichia pastoris is a practical system for the expression of milligram-to-gram amounts of proteins for both simple laboratory experimentation and industrialized production.[11,12] The aim of this study was construction of recombinant pPIC9/hEGF which will be used for expression of recombinant hEGF in the following studies.

Materials and Methods

Materials

All restriction endonucleases, T4 DNA ligase, Plasmid Miniprep Kit and Gel Extraction Kit were purchased from fermentas company (Vilnius, Lithuania). EGF cDNA was purchased from genecopoeia company (Accession: NM_001963.2). Plasmid pPIC9 which contains AOX1 prompter and E. coli strain DH5-α (F– Φ80lacZΔM15 Δ (lacZYA-argF) U169 recA1 endA1 hsdR17 (rK–, mK+) phoA supE44 λ– thi-1 gyrA96 relA1) were provided from Pasteur Institute of Iran (Tehran). LB medium was purchased from Sigma Aldrich (L7658-1KG).

Amplification of hEGF gene with PCR

Amplification of EFG gene was accomplished by the following primers: Forward primer 5´ATCTGGAGAAAAGAGAGGCTGAAGCTAAT AGTGACTCTCAATGTCCC3´ and reverse primer 5´AATGAATTCTTAGCGCAGTTCCCACCACTTC

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AGGTCTC3´ that contain restriction sites for XhoI and EcoRI at 5´terminal respectively (underlined).
PCR reaction contain 10 x PCR buffer, 400 µM dNTPs, 50 ng Genomic DNA, primers (EGF-F, EGF-R) 0.2 µM from each primer, 3 mM MgCl2 and 1 unit from Taq DNA polymerase (cinnagen) and following program was accomplished for amplification of desired fragment: Initial denaturation at 94 °C for 5 minute followed with 94 °C for 1 minute, 52 °C for 20 second for annealing and 72 °C for 45 second for extention that repeated for 35 cycles.

Constructing of recombinant vector and sequencing

The PCR product was purified with GeneJET PCR Purification Kit (Fermentas) and digested by EcoRI and XhoI restriction enzymes. Then this fragment with pPIC9 vector that previously digested by mentioned enzymes subjected to ligation reaction and its product was transformed to DH5-α by heat shock method.Various clone screened by colony PCR method and the positive clones analyzed by sequencing using EGF-F primer.

Results

Amplification of EGF gene

For this purpose thermal gradient PCR (50-54) was carried out to optimized temperature of primer annealing. As seen in Figure 1, PCR in annealing temperature over than 52 °C give rise to specific amplification of 197bp fragment. (Figure 1)

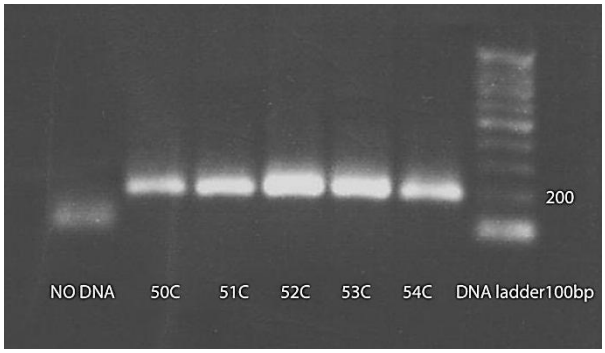

Figure 1. Optimization of PCR reaction for amplification of *EGF* gene.PCR in annealing temperature in 52 °C gives rise to specific amplification of 197bp fragment.

Cloning and sequencing of EGF gene

The double-digested PCR products were mixed with predigested pPIC9 vector (Figure 2) and introduced in ligation reaction. Then ligation product was transformed to DH5-α *E.coli* bacteria and recombinant plasmid extracted. The colony PCR was carried out on transformant using the mentioned primers and desired 197bp fragment was observed on 1% Agarose by UV transilluminator (Figure 3). In the next step, recombinant pPIC9 was analyzed by sequencing. Comparison of hEGF sequence with available sequences in Gene bank showed that obtained sequence has 100% homology with Homo sapiens epidermal growth factor (hEGF), transcript variant 3 (NM_001178131.1).

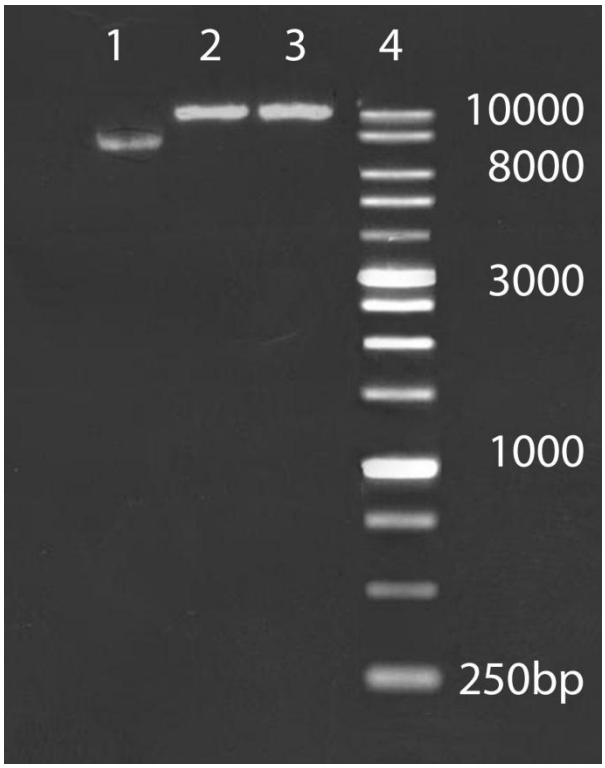

Figure 2. Digestion of pPIC9 vector: line 1; undigested pPIC9 vector line 2 and 3; digested pPIC9 vector line 4; DNA ladder (1kb).

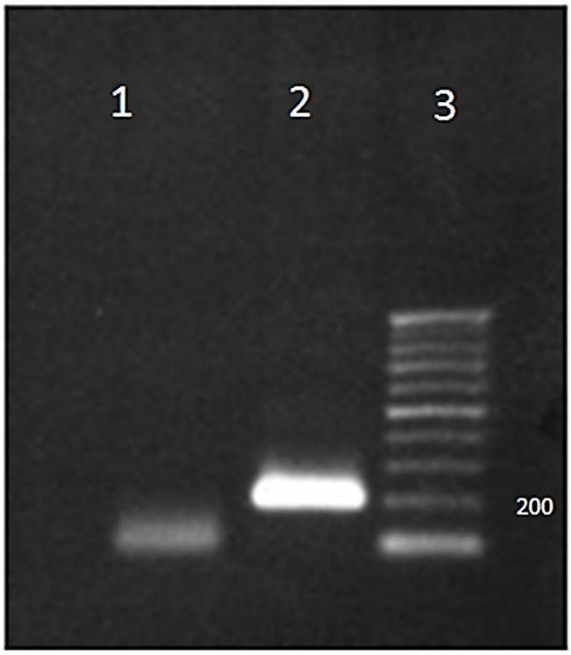

Figure 3. PCR assay for detection of desired 197bp fragment in recombinant pPIC9/hEGF vector: line 1; no DNA line 2; EGF gene (197bp) line 3; DNA ladder (100bp).

Discussion

The application of P.pastoris for the production of heterologous proteins is highly successful[13] Essential to the production of heterologous proteins in P.pastoris is construction of expression vector. This necessitates the selection of both proper expression vector and related strain.[14-16] In the present study recombinant pPIC9/hEGF was constructed. This expression vector rely on the AOX1 promoter and contains α-mating factor secretion signal for secretion of recombinant protein into extracellular environment.[17] A more superiority of Pichia is that it is remarkably suitable for fermentation and can reach high cell densities that may improve overall protein yields.[13,18,19] Also pichia has a secretory system that enable to secrete desired protein and conformation of disulfide bind was performed easily.[20] For soluble secretory proteins, undoubtedly, it is the most simple and the most trustworthy expression system with possible to yield grams of the favorite protein.[21,22]

The construction of recombinant hEGF has been conducted by many researchers in a variety of vector for many applications.[23,24] In construction of pPIC9/hEGF, the important point which should be considered is insertion of correct ORF of gene. At this stage of this research, the correction of insert ORF was confirmed by PCR and sequencing analysis. In the future study, the construction of recombinant vector pPIC9/hEGF should be more characterized for expression of recombinant protein.

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Conflict of Interest

The authors report no conflicts of interest.

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