SUBCLONING OF THE STREPTOKINASE GENE FROM STREPTOCOCCUS PYOGENES INTO pCold I AND pCold TF PLASMIDS

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ABSTRACT

Currently, the demand for thrombolytic drugs is continuously increasing. Pharmaceutical preparations based on the recombinant form of streptokinase are distinguished by their cost-effectiveness compared to other thrombolytic agents (Diwan et al., 2021). The nucleotide sequence of the streptokinase (Ska) gene, isolated from the bacterium Streptococcus pyogenes, is registered in the international GenBank database under the accession number PX644861.1. Initially, this gene was cloned into the pET-28a(+) plasmid (Vokhidov et al., 2025), and its expression characteristics were studied in E. coli host cells. This study presents the results of subcloning the Ska gene into pCold TF and pCold I vectors to enhance the solubility and yield of the recombinant protein in subsequent expression stages (Bhatwa et al., 2021; Qing et al., 2004)..

Introduction

Currently, the demand for thrombolytic drugs is continuously increasing. Pharmaceutical preparations based on the recombinant form of streptokinase are distinguished by their cost-effectiveness compared to other thrombolytic agents (Diwan et al., 2021). The nucleotide sequence of the streptokinase (*Ska*) gene, isolated from the bacterium *Streptococcus pyogenes*, is registered in the international GenBank database under the accession number PX644861.1. Initially, this gene was cloned into the pET-28a(+) plasmid (Vokhidov et al., 2025), and its expression characteristics were studied in *E. coli* host cells. This study presents the results of subcloning the *Ska* gene into pCold TF and pCold I vectors to enhance the solubility and yield of the recombinant protein in subsequent expression stages (Bhatwa et al., 2021; Qing et al., 2004).

Methods

The previously cloned pET-28a(+)-Ska plasmid was propagated in competent *Escherichia coli* XL1 cells and subsequently purified. The streptokinase gene within the vector was then subjected to restriction digestion using *Nde*I and *Bam*HI endonucleases. The digested products were separated by 1% agarose gel electrophoresis, and the target gene was

purified from the gel using an Agarose Gel Extraction Kit (Vazyme, China). The purified *Ska* gene was initially subcloned into the pCold TF vector, which had been linearized by digestion with *Nde*I and *Bam*HI restriction enzymes. For subsequent subcloning into the pCold I plasmid, the *Ska* gene was excised from the recombinant pCold TF plasmid using *Nde*I and *Xba*I endonucleases. Correspondingly, the pCold I vector was prepared by digestion with *Nde*I and *Xba*I restriction enzymes and subsequently purified using the Agarose Gel Extraction Kit (Vazyme, China).

The restriction enzyme-digested *Ska* gene and the linearized vectors were ligated using T4 DNA ligase (Thermo Fisher Scientific, Lithuania) at 16 °C for 18 hours.

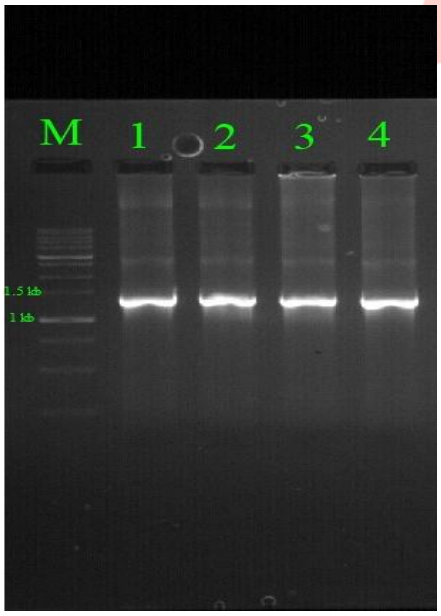
The resulting vector constructs were transformed into *E. coli* XL1 cells using the heat-shock method. The transformed cells were cultured on LB agar medium supplemented with 100 µg/mL ampicillin. Selected colonies were inoculated into liquid LB medium containing ampicillin, and the recombinant plasmids were subsequently purified. To verify the success of the subcloning procedure, the plasmids were digested with ***Nde*I** and ***Bam*HI** restriction enzymes and analyzed by PCR using specific primers (*Primer F1* and *Primer R*) designed for the ***Ska*** gene **(Table 1)**.

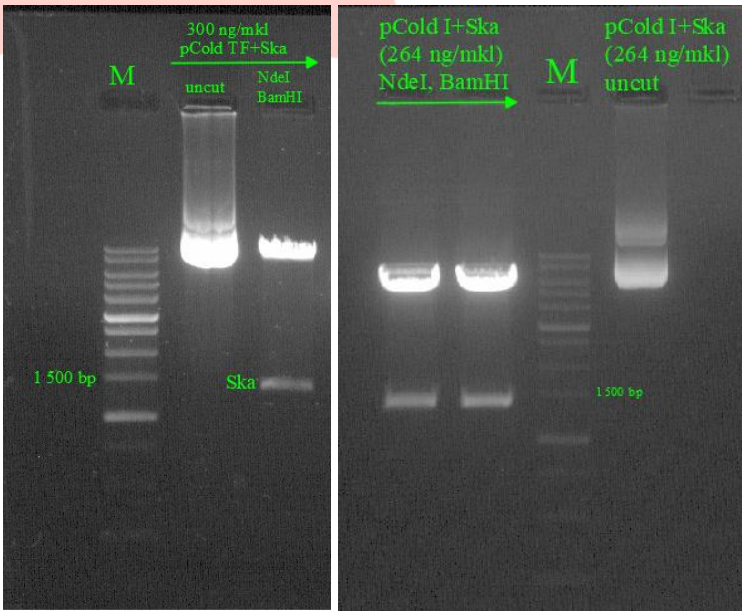
Table 1

Primer F1	5'-CGAGGCATATGATGAAAAATTACTTATCTTTTGGGAT-3'
Primer R	5'-AATGGATCCGCTTGCTTTTTTTGTCAGT-3'

Results

Following the subcloning process, recombinant plasmids were purified from the obtained colonies. The success of the cloning procedure was verified by PCR analysis using specific primers designed for the streptokinase gene. Consequently, the amplification of a PCR product with an expected size of approximately 1.3 kb was observed (Figure 1).





A	B

Figure 1. Analysis of the subcloning process: (A) 1% agarose gel electrophoresis of PCR products amplified from recombinant plasmids. M: 1 kb DNA ladder; Lanes 1, 2: *pCold I+Ska*; Lanes 3, 4: *pCold TF+Ska* vectors. (B) Results of the restriction digestion analysis.

Conclusion

The *Ska* gene isolated from *S. pyogenes* was successfully subcloned into the pCold I and pCold TF plasmids. The resulting new vector constructs enable the high-yield expression of the streptokinase enzyme in a soluble form at low temperatures in subsequent studies.

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