عنوان مقاله:

Molecular Cloning of DARPins G۳ in pET۲۸b Expression Vector and Optimization of the Expression of This Protein in Escherichia Coli

محل انتشار:

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خلاصه مقاله:

Background: Human epidermal growth factor receptor ۲ (HER۲) is over-expressed in breast, ovarian, gastric, and prostate cancers and is used as a tumor marker in the diagnosis of cancer. Monoclonal antibodies have been used as a diagnostic and therapeutic tool against HER۲. Because of the difficulties associated with the stability and complexity of the construct and the high cost of antibody production, we aimed to investigate, cloning, and expression of HER۲-binding DARPins genes to identify, HER۲-positive tumor markers, we aimed to investigate. Materials and Methods: After synthesis, the DARPins peptide gene was cloned into the M۱۳ vector and sub-cloned into the TOP۱۰ pet۲۸b bacterial vector. After culturing the bacteria on an agarose plate containing antibiotics, the transfected bacteria expressing the DARPins gene were selected. To ensure gene cloning, we used enzymatic digestion and recombinant plasmid delivery for sequencing. Isopropyl β-d-۱-thiogalactopyranosideIPTG was used for the induction of recombinant protein expression and the SDS-PAGE method and Western blot for expression confirmation. Results: The polymerase chain reaction (PCR) amplification product of DARPins was analyzed using agarose gel electrophoresis. Plasmid was purified from the positive clone by PCR cloning, sequenced and gene cloning was confirmed. After culturing from competent cells, protein expression was obtained from positive colonies. SDS- PAGE results showed the effect of different conditions including temperature, IPTG concentration, and time on the pET-DARPins expression. Conclusion: We were succeeded to express a new codon-optimized DARPins gene in Escherichia coli and HEK۲۹۳t system.

کلمات کلیدی: