Research in Pharmaceutical Sciences, 2012;7(5)
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Molecular cloning and expression of reteplase in *E. coli* using tac promoter

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Background and Aims: This study was aimed to clone and express the reteplase gene, a thrombolytic agent used for the treatment of acute myocardial infarction and stroke, under controlling of tac promoter in E.coli.
Methods: Reteplase gene was amplified using polymerase chain reaction (PCR) with designed primers. The product was then cloned into pTZ57 plasmid. The cloned gene was digested out and ligated into pGEX-5x-1 expression vector. The presence of the insert was confirmed by restriction digestion and determination of the nucleotide sequence. By using 0.2, 0.5 and 1mM isopropyl beta-D thiogalactopyranoside (IPTG), reteplase was induced in E. coli TOP10 cells and analyzed by SDS-PAGE.
Results: Electrophoresis of PCR product and also double digested recombinant pTZ57 plasmid (rptz), showed a 1068bp band of reteplase. SDS-PAGE analysis showed a 60 KDa band of protein product induced with all the concentrations of IPTG.
Conclusions: in the present study reteplase gene was successfully cloned and expressed under controlling tac promoter. The induced vector can be used for future analysis of expressed protein, reteplase.

Keywords: Reteplase; t-PA; Tac promoter; Cloning