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Preliminary expression of high fidelity Pfu DNA polymerase encoding fragment

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Background and Aims: In the present study the DNA fragment encoding Pfu DNA polymerase was inserted in to pET-15b expression vector and primary expression was performed using IPTG as an inducer.
Methods: Based on the DNA sequence of Pfu DNA polymerase gene two primers were synthesized. Best condition for amplification was performed. A predicted 2.3 kb fragment was agarose gel purified and cloned in to pET-15b vector. After sequencing pET-pfu construct was transformed in to E. coli BL21(DE3) expression host to test protein expression. Primary expression was induced with the addition of IPTG. Cell culture harvested and Protein expression analyzed by SDS-PAGE electrophoresis.
Results: A band of approximately 2330 bp was resulted after PCR amplification. Some colonies were produced after transforming pET-pfu in to E. coli strain XL1-Blue and then BL21(DE3). An expected 90 KD protein bond was observed after inducing by IPTG.
Conclusions: Pfu DNA polymerase encoding fragment was cloned in pET-15b expression system and primary expression was performed inBL21 (DE3) using IPTG based on T7 promoter system. SDS-PAGE analysis shown that Pfu DNA polymerase over expressed in harvested cells as well.

Keywords: DNA-dependent DNA polymerase; PCR; Cloning; pET-15b