Abstract

During the last 50 years, major advances in molecular biology have been attributed to the discovery of enzymes that allow molecular cloning of important genes. One of these enzymes that has been widely noteworthy for its role in the development of biotechnology is the *T4* DNA ligase. This enzyme have applications such as; cloning fragments into either plasmid or phage vectors, Joining of double-stranded oligonucleotide linkers or adaptors to DNA, Site directed mutagenesis, Amplified fragment length polymorphism (AFLP), Nick repair in duplex DNA, RNA or DNA/RNA hybrids, Self-circularization of linear DNA and Ligase-mediated RNA detection. In this study, expression vectors *pTrcHis-T4* and *pET28-T4* separately transferred in competent Escherichia coli bacteria strains *DH5α*, *TOP10F'* and *BL21 (DE3)*, the six samples were able to grow on selective medium, and after the confirmation of transformation, colons containing *T4* DNA ligase gene was used to express recombinant protein. Final concentration of 1 mM IPTG inducer was used in step of recombinant protein expression. Then, 5 h after induction, the cells deposited and the cells lysised in the step of purification. Recombinant proteins containing histidine sequences were purified by nickel-agarose column manufacture by Qiagene Company. Finally, the purity rate of recombinant proteins was evaluated using the SDS-PAGE gel. The results showed the purity of recombinant protein is over 90% and the most expression *T4* DNA ligase recombinant protein for vector *pET28-T4* are related to the Escherichia coli strain *BL21 (DE3)* and for vector *pHis-T4* are related to E. coli strain *TOP10F'*.

Key words: Cloning; Expression; Protein Purification; Bacteriophage *T4*; *T4* DNA Ligase (*T4Dnl*) Enzyme.

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Cloning, expression *T4* DNA ligase gene (*T4Dnl*) in *E.coli* host and purification of *T4* DNA Ligase Enzyme

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