

Cloning and over-expression of recombinant human insulin –like growth factor-I in *E. coli*

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Background and Aims: Human Insulin-like growth factor (hIGF-I), is a 70-residue, single-chain therapeutic protein that has three disulfide bonds in its structure. IGF-I has a long-term impact on cell proliferation, differentiation, and apoptosis. *E. coli* facilitates protein expression by its relative simplicity, its inexpensive and fast high-density cultivation. Rapidly synthesized heterologous proteins in *E. coli* often cause the formation of inclusion bodies. Producing protein in the form of inclusion bodies is desirable because proteolysis decreases, and purification might be facilitated. The present study was designed to clone and over-express IGF-I in *E. coli*

Methods: In the present study, IGF-I synthetic gene in PET 15b expressed as inclusion body in *E. coli*. The expression of hIGF-1 protein was rationally compared in two different *E. coli* hosts (Origami B, BL21 (DE3)). Furthermore, the accumulation of inclusion bodies in two strains was compared after sonication. In this research, the effect of different expression conditions such as induction temperature (28, 33, 37°C), medium (M9, LB, TB) and the IPTG concentration (0.1mM, 0.3mM, 0.5mM) on quantity and quality of recombinant IGF-I production were studied too. The results of experiments were analyzed by SDS-PAGE, western blotting and cell growth and recombinant protein production kinetics.

Results: In this research, it was shown that expression of IGF-I in Origami B host strain was more noticeable than BL21 (DE3). Moreover, the accumulation of inclusion bodies in Origami strain was higher than BL21 (DE3). Also induction temperature affect on quantity and quality of recombinant IGF-I production especially on purification yield. It was found that IPTG concentration can influence on productivity of recombinant IGF-I considerably.

Conclusions: According to these studies, host strain, induction temperature and kind of medium are considered three most effective factors on expression of IGF-I protein and under the optimized conditions the high percent of protein can be expressed.

Keywords: hIGF-I; *E. coli*; Cloning, Over-expression