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Evaluation of a novel fusion system for soluble protein overexpression in *Escherichia coli*

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Abstract

Proteins production requires a successful correlation between expression, solubility and purification steps. As an expression system, *Escherichia coli* combines its low cost and ease of use with rapid expression, being widely used for heterologous protein production. This host cell has however several drawbacks, namely at the expression of insoluble proteins aggregated into inclusion bodies. Many efforts have been made to overcome such problems, including the optimization of expression conditions and the use of solubility fusion tags. The use of fusion tags for protein production remains challenging since none of the available fusion systems work universally with every partner protein. A novel fusion system had been recently discovered and submitted to a patenting process by Hitag Biotechnology, Lda. This fusion system consists of low molecular weight peptides/proteins from recombinant antigens, which have demonstrated to increase soluble protein expression levels in *E. coli*.

This work aims at the evaluation of the effects of two novel fusion tags on soluble protein expression in *E. coli*. Specific primers were designed to amplify and sub-clone gene sequences that encodes for frutalin, *Cryptosporidium parvum* 12kDa protein and *Giardia lamblia* cyst wall protein. These target proteins present therapeutic and diagnostic interests and had shown to be difficult-to-express in *E. coli*. Proteins were first fused to novel tags and than expressed in *E. coli*. Proteins purification was carried out by affinity chromatography, using nickel-NTA columns. Pooled fractions were dialysed against phosphate buffer pH 7.4 and latter analysed by SDS-PAGE. Protein expression levels were determined by Bradford assay.

When fused to novel tags, all target proteins were successfully expressed in *E. coli*. Comparing to the respective non-fused proteins, both novel tags used in this work promoted an increase from three to nine folds on soluble proteins expression levels. The SDS-PAGE analysis confirmed the purity of Ni-NTA pooled fractions, corroborating also these results. Tag1-fusions achieved higher production yields than fusions with Tag2.

In this work, three different target proteins were used to evaluate two novel fusion tags. The soluble overexpression effect offered by this novel fusion system may provide an important advance in recombinant protein expression processes in *E. coli*.