

Bacterial surface display for the expression and purification and characterization of protein

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ABSTRACT

The enhanced green fluorescent protein (EGFP) is widely used as a marker in life science. Currently, protein purification mostly involve chromatographic methods, which are multistep, time-consuming and costly. Green fluorescent protein (GFP), a 27-kDa protein containing 238 amino acid residues, is originally isolated from the bioluminescent jellyfish *Aequorea victoria*. The most distinct property of GFP is the intense visible fluorescence without the need of cofactors, which makes GFP a good candidate of fusion protein expressed in hosts. The applications of GFP in biological and biotechnological fields are enormous, including as a biomarker, a reporter protein, as a component in biosensor, used in ecological monitoring, real-time imaging and bioprocess applications. We recently designed the INP (Ice Nucleation Protein)-TEV protease cleavage site-EGFP as a gene constructs. The analysis showed that cell surface display system successfully expressed and displayed on the surface of *E. coli* cells. In this study, after expressing the gene constructs in *E. coli* BL21 (DE3), the pellet of induced *E. coli* was collected by centrifuging and the cell was suspended in STET buffer containing lysozyme. Then, the cell debris was kept by centrifugation. After incubation with TEV protease, a 27 kD EGFP band was observed on SDS-PAGE. In this study, we introduced a simple and efficient system for protein expression and purification without any chromatographic process. The results in the study suggest that the use of lysozyme in lysis buffer is an appropriate approach for purification of surface display proteins in *E. coli*.

Keywords: Cell-surface display, EGFP, INP, lysozyme