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Short communication

Highly efficient protein expression and purification using bacterial hemoglobin fusion vector

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Abstract

Recently developed bacterial hemoglobin (VHb) fusion expression vector has been widely used for the production of many target proteins due to its distinctive properties of expressing fusion protein with red color which facilitates visualization of the steps in purification, and increasing solubility of the target proteins. However, after intensive use of the vector, several defects have been found. In this report, we present a modified VHb fusion vector (pPosKJ) with higher efficiency, in which most of the defects were eliminated. First, it was found that thrombin protease often digests target protein as well as inserted thrombin cleavage site, so it was replaced by a TEV cleavage site for more specific cleavage of VHb from target protein. Second, a glycine-rich linker sequence was inserted between 6× his-tag and VHb to improve the affinity of 6× his-tag to Ni–NTA resin, resulting in higher purity of eluted fusion protein. Third, *Eco*RI and *Xho*I restriction sites located elsewhere in the vector were removed to make these restriction sites available for the cloning of target protein coding genes. A pPosKJ vector was fully examined with an anti-apoptotic BCL-2 family member of *Caenorhabditis elegans*, CED-9. A C-terminal VHb fusion expression vector (pPosKJC) was also constructed for stable expression of target proteins that may be difficult to express with an N-terminal fusion. Vaccinia-related kinase 1 (VRK1) was also successfully expressed and purified using the vector with high yield. Taken together, we suggest that the VHb fusion vector may be well suited for high-throughput protein expression and purification.

Keywords: *Vitreoscilla*; Bacterial hemoglobin; Fusion expression vector; CED-9; Vaccinia-related kinase 1

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1. Introduction

Several fusion protein expression vectors have been developed for the expression of recombinant proteins in *Escherichia coli*. It has been known that fusion expression vectors enhance the productivity, solubility, and uniformity of the target proteins compared with nonfusion proteins, and bacterial N-terminus often stabilize eukaryotic proteins in a bacterial ce ll (Bachmair et al., 1986; Putkey et al., 1985; Straus and Gilbert, 1985). For example, maltose-binding protein and glutathione *S*-transferase-binding protein fusion expression vectors are designed to produce fusion proteins that can be purified from cell lysates by substrate-affinity chromatography (di Guan et al., 1988; Guan and Dixon, 1991; Maina et al., 1988; Smith and Johnson, 1988). Other vectors are designed to produce target proteins fused with a his-tag which can be used for the purification by metal-affinity chromatography (Bujard et al., 1987; Studier et al., 1990).

Recently, a bacterial hemoglobin fusion expression vector (pKW32) has been developed to produce fusion proteins with red color which facilitates visualization of the steps in purification (Park et al., 2003). The vector was designed to produce target proteins fused with VHb (*Vitreoscilla* hemoglobin coded by *vgb*) which is highly desirable as a fusion polypeptide because of high productivity and solubility of the protein in *Escherichia coli* cell, and its red color. Moreover, the expression of VHb in heterologous bacteria or yeast enhances cell growth rate and yields of recombinant proteins under oxygen limiting conditions, because VHb functions as an oxygen carrier to the terminal oxidases (Dikshit et al., 1992; Park et al., 2002; Ramandeep et al., 2001; Tsai et al., 1995a,b). This physiological property of VHb enables the fusion expression vector to produce larger amount of fusion protein with higher solubility. In fact, several target proteins that were previously difficult to express in soluble form in *E. coli*, were successfully expressed and purified using the vector, and the target proteins include HIV integrase (Park et al., 2003), several microorganisms' soluble domain of cyoA and cybd, mouse BCL-W, mouse DREAM, *Drosophilla melanogaster* GUS, *Caenorhabditis elegans* CED-9, and so on.

However, after intensive use of the vector, several defects have been found. Some of the target proteins showed internal cleavage with thrombin due to its low specificity, and expressed VHb fusion proteins tend to have lower affinity to Ni–NTA resin than his-tagged proteins. In this paper, we report the construction of pPosKJ vector, which is modified from the pKW32 by eliminating most of the defects of the vector. We also report the construction of a C-terminal VHb fusion expression vector for the stable expression of the proteins which were difficult to be expressed with N-terminal fusion tag.

2. Materials and methods

2.1. Bacterial strains and materials

Escherichia coli strain DH5α and BL21(DE3) were used for the cloning and expression of recombinant genes, respectively. The pKW32 and pPosKJ vectors used in this study contain T5/lacO promoter which can be recognized by the *E. coli* RNAP (Bujard et al., 1987) and lacI gene is located inside of the vectors. Restriction enzymes, *pfu* DNA polymerase, HiTrap ion exchange columns, Ni–NTA chromatographic resin, and TEV protease were purchased from New England Biolabs, Bioneer (South Korea), Amersham Biosciences, Qiagen, and Invitrogen, respectively. Plasmid preparation, enzymatic manipulation of DNA, site-directed mutagenesis, bacterial transformation, and PCR amplification were performed according to standard protocols.

2.2. Modifications of bacterial hemoglobin fusion vector

Replacement of thrombin cleavage site with TEV cleavage site was performed by PCR amplification. PCR fragment coding for TEV protease cleavage site proceed by VHb and linker coding gene was amplified from pKW32 using upstream primer 5′-CCATCACGGATCCATGTTAGACCAG-3′ and downstream primer 5′-GCGCGCATATGGCCTT