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The novel Fh8 fusion technology for protein expression in *Escherichia coli*: a comparison with the traditionally used fusion systems

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The recombinant expression of natural proteins in *Escherichia coli* is limited by the lack of efficient methods for soluble production. Several efforts have been made to overcome this limitation, including the genetic fusion of highly soluble protein domains (fusion partners) to the target proteins. Among the available fusion partners, the *E. coli* maltose-binding protein (MBP), glutathione S-transferase (GST) and N-utilization substance A (NusA) are often used to enhance protein solubility. However, due to their large size, these partners can be problematic for structural and functional analyses, requiring their removal using specific proteases. The removal of the fusion partner is not always successful and the resulting cleaved target protein may also precipitate into insoluble aggregates. In recent years, new fusion partners, as the SUMO, have emerged competing directly with the traditional used ones at producing and purifying soluble proteins. This work aims at the comparison between a novel fusion partner, the small-size Fh8 tag, and the traditionally used ones for soluble protein production in *E. coli*. Six target proteins were fused to eight fusion tags including the well-known NusA, Trx, GST, MBP and SUMO. A high throughput small-scale analysis was initially performed using 10 mL cultures to select the best strain per target protein and to compare both the total expression and solubility effects promoted by the different fusion partners on the used target proteins. The NusA, Trx and MBP fusions resulted in the best *E. coli* expressions, followed by the Fh8 fusions and the SUMO fusions. In spite of the expression results in *E. coli*, the recombinant Fh8-fused proteins achieved similar solubility levels as the NusA and Trx fused ones. The Fh8 fusions presented similar or higher solubility than the GST fusions and higher solubility than the SUMO fusions. These results were also observed after performing a protein production in 500 mL cultures. The tag removal process was compared in both small-scale analysis and in the 500 mL production using the Tobacco Etch Virus (TEV) protease. After Fh8 tag removal, the cleaved target proteins remained soluble and presented similar or higher production yields than the cleaved proteins from the Trx and NusA fusions. Results from this study revealed the Fh8 as a robust fusion tag standing among the best fusion tags for protein expression and solubility in *E. coli*.