A dual expression platform to optimize the soluble production of heterologous proteins in the periplasm of Escherichia coli.

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Abstract

The functional analysis of individual proteins or of multiprotein complexes - since the completion of several genome sequencing projects - is in focus of current scientific work. Many heterologous proteins contain disulfide-bonds, required for their correct folding and activity, and therefore, need to be transported to the periplasm. The production of soluble and functional protein in the periplasm often needs target-specific regulatory genetic elements, leader peptides, and folding regimes. Usually, the optimization of periplasmic expression is a step-wise and time-consuming procedure. To overcome this problem we developed a dual expression system, containing a degP-promoter-based reporter system and a highly versatile plasmid set. This combines the differential protein expression with the selection of a target-specific expression plasmid. For the validation of this expression tool, two different molecular formats of a recombinant antibody directed to the human epidermal growth factor receptor and human 11beta-hydroxysteroid dehydrogenase type 2 (11beta-HSD2) were used. By application of this expression system we demonstrated that the

amount of functional protein is inversely proportional to the on-line luciferase signal. We showed that this technology offers a simple tool to evaluate and improve the yield of functionally expressed proteins in the periplasm, which depends on the used regulatory elements and folding strategies.

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