

An online monitoring system based on a synthetic sigma32-dependent tandem promoter for visualization of insoluble proteins in the cytoplasm of Escherichia coli.

Kraft M, Knüpfer U, Wenderoth R, Pietschmann P, Hock B, Horn U (2007) An online monitoring system based on a synthetic sigma32-dependent tandem promoter for visualization of insoluble proteins in the cytoplasm of Escherichia coli. *Appl Microbiol Biotechnol* 75(2), 397-406.

[Details](#)



Abstract

The expression of heterologous proteins in the cytoplasm of Escherichia coli is often accompanied by limitations resulting in uncontrollable fermentation processes, increased rates of cell lysis, and thus limited yields of target protein. To deal with these problems, reporter tools are required to improve the folding properties of recombinant protein. In this work, the well-known sigma(32)-dependent promoters *ibpAB* and *fxsA* were linked in a tandem promoter (*ibpfxs*), fused with the luciferase reporter gene *lucA* to allow enhanced monitoring of the formation of misfolded proteins and their aggregates in E. coli cells. Overexpression of MalE31, a folding-defective variant of the maltose-binding protein, and other partially insoluble heterologous proteins showed that the *lucA* reporter gene was activated in the presence of these misfolded proteins. Contrary to this, the absence of damaged proteins or overexpression of mostly soluble proteins led to a reduced level of luciferase induction. Through performing expression of aggregation-prone proteins, we were able to demonstrate that the *ibpfxs::lucA* reporter unit is 2.5-4.5 times stronger than the single

reporter units *ibp::lucA* and *fxs::lucA*. Data of misfolding studies showed that this reporter system provides an adequate tool for in vivo folding studies in *E. coli* from microtiter up to fermentation scales.

Beteiligte Forschungseinheiten

[Biotechnikum Miriam Agler-Rosenbaum](#) [Mehr erfahren](#)

Leibniz-HKI-Autor*innen



Uwe Horn

[Details](#)



Uwe Knüpfer

[Details](#)



Mario Kraft

[Details](#)

Identifier

PMID: 17221192