Microbial Cell Factories

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Research

Use of pIVEX plasmids for protein overproduction in *Escherichia coli*

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Published: 02 June 2005

Microbial Cell Factories 2005, **4**:18 doi:10.1186/1475-2859-4-18

Received: 21 April 2005
Accepted: 02 June 2005

This article is available from: http://www.microbialcellfactories.com/content/4/1/18

Abstract

Background: The pIVEX plasmids are vectors optimized for expression in the Rapid Translation System (RTS) cell-free system under control of bacteriophage T7 transcription elements. Even if these plasmids are intended for use *in vitro*, it is usually worthwhile to compare both cell-free and bacterial expression from the same genetic construct. However, some RTS users encountered problems when they introcuded these plasmids into *Escherichia coli* host strains producing the T7 RNA polymerase.

Results: We verified that difficulties in transforming the commonly used BL21(λDE3) strain with pIVEX arose from the presence of a strong T7 promoter combined with a high-copy number plasmid, independent of gene expression. When these vectors were introduced into this strain harboring a compatible plasmid carrying the lactose repressor (*lacI*), we improved the transformation efficiency by 4 orders of magnitude. Moreover, we designed a transformation protocol that allows, after induction, the overproduction of pIVEX-encoded proteins in the BL21(λDE3) strain.

Conclusion: Using the correct plasmid/host combination and transformation-expression protocol, we could directly compare overproduction of the same pIVEX-encoded proteins from both *in vivo* and *in vitro* expression systems.

Background

Recent developments in cell-free systems offer new and promising possibilities for producing recombinant proteins [1]. The RTS, commercialized by Roche, is an exchange cell-free system with improved productivity [2]. The continuous supply of consumable substrates and removal of reaction products provide a yield of several milligrams of protein. This system uses bacteriophage T7 RNA polymerase to perform transcription, while an enriched *E. coli* S30 extract provides the translational machinery. Thus, protein production in RTS requires a preliminary cloning step of the target gene into a vector, downstream of the T7 promoter. For this purpose, the pIVEX family of expression plasmids has been optimized for *in vitro* use. They include a T7 promoter comprising the T7 gene 10 translation enhancer [3], an efficient prokaryotic Shine-Dalgarno sequence with an optimum distance to the start codon, a multiple cloning site, and a T7 terminator which prevents 3'-terminal exonucleolytic degradation of the mRNA. These vectors are very convenient since multiple cloning sites were designed to allow gene fusions with several tags either at the N- or C-terminal of target proteins by conserving the same restriction strategy and reading frame compatibility.