

Facile promoter deletion in *Escherichia coli* in response to leaky expression of very robust and benign proteins from common expression vectors.

Kawe M, Horn U, Plückthun A (2009) Facile promoter deletion in *Escherichia coli* in response to leaky expression of very robust and benign proteins from common expression vectors. *Microb Cell Fact* 8, 8.

[Details](#)



Abstract

Background

Overexpression of proteins in *Escherichia coli* is considered routine today, at least when the protein is soluble and not otherwise toxic for the host. We report here that the massive overproduction of even such "benign" proteins can cause surprisingly efficient promoter deletions in the expression plasmid, leading to the growth of only non-producers, when expression is not well repressed in the newly transformed bacterial cell. Because deletion is so facile, it might impact on high-throughput protein production, e.g. for structural genomics, where not every expression parameter will be monitored.

Results

We studied the high-level expression of several robust non-toxic proteins using a T5 promoter under *lac* operator control. Full induction leads to no significant growth retardation. We compared expression from almost identical plasmids with or without the *lacI* gene together in strains expressing different levels of LacI. Any combination without net overexpression of LacI led to an efficient promoter deletion in the plasmid, although the number of growing colonies and even the plasmid size – all antibiotic-resistant non-producers – was almost normal, and thus the problem not immediately recognizable. However, by assuring sufficient repression during the initial establishment phase of the plasmid, deletion was completely prevented.

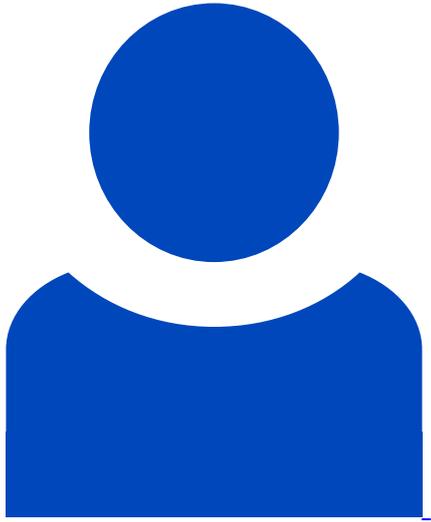
Conclusion

The deletions in the insufficiently repressed system are caused entirely by the burden of high-level translation. Since the *E. coli* Dps protein, known to protect DNA against stress in the stationary phase, is accumulated in the deletion mutants, the mutation may have taken place during a transient stationary phase. The cause of the deletion is thus distinct from the well known interference of high-level transcription with plasmid replication. The deletion can be entirely prevented by overexpressing LacI, a useful precaution even without any signs of stress caused by the protein.

Beteiligte Forschungseinheiten

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

Leibniz-HKI-Autor*innen



Uwe Horn

[Details](#)

Identifier

doi: 10.1186/1475-2859-8-8

PMID: 19171063