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Publication date: 2009

Document Version
Publisher's PDF, also known as Version of record

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Citation (APA): Sohoni, S. V., Mijakovic, I., & Eliasson Lantz, A. (2009). Synthetic Promoter Library for modulation of actinorhodin production in Streptomyces coelicolorA3(2). Poster session presented at 15th International symposium on the biology of actinomycetes, Shanghai, China.
Synthetic Promoter Library for modulation of actinorhodin production in Streptomyces coelicolor A3(2)

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Introduction:
Genetic manipulation tools have been successfully used for improving properties of industrial microorganisms. Most of these approaches involve over-expression of a gene for the rate limiting enzyme or deletion of a gene situated at a branching point in case of branched pathways. These simplistic all-or-nothing approaches have been fruitful in some cases, but can fall short when careful optimization of gene expression is needed in order to tune the modified pathway with the rest of the cellular metabolism. Promoter strength plays an important role in the resulting levels of gene expression. The synthetic promoter technology, based on randomization of the promoter sequences, has been successfully employed to construct promoter libraries in order to optimize levels of gene expression.

Synthetic promoter technology is based on the fact that the spacer sequences surrounding the consensus -35 and -10 regions of bacterial promoters contribute significantly to promoter strength. Randomizing these spacer sequences results in Synthetic Promoter Libraries.

In the current study the native promoter of actII orf4 was modified by randomizing spacer sequence between -35 box and -10 box and 5 nucleotides before and 5 nucleotides after -35 box and -10 box respectively. The resulting library was screened and characterized for production of actinorhodin.

Construction of Synthetic Promoter Library

Results and Discussion

Screening of Synthetic Promoter Library

• Around 10,000 colonies were screened by visual screening
• 200 colonies having a blue actinorhodin hallow were selected for characterization
• Out of the 200 colonies, 12 colonies were subjected to detailed physiology studies

Characterization of Synthetic Promoter Library

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Acknowledgements

Authors would like to thank Prof. Gunther Muth (University of Tübingen) for providing plasmid pGM160.
Authors would also like to thank Dr. Prashant Bapat and Dr. Kiran Patil for providing algorithm for the image analysis.