Introduction:

In order to produce antibiotics at commercial levels, various genetic tools for fine control of gene expression of biosynthetic pathways and their regulatory network are in great demands due to limited numbers of gene expression systems available for Streptomyces sp. Currently, although other systems such as kasOp and SF14p are available, ermE* promoter (ermE*P) from *Saccharopolyspora erythraeus* is the most widely used strong and constitutive promoter. Another example is a thiostrepton inducible promoter, tipA, with limited induction levels. As a result, developments of new scalable and predictable gene expression systems are in urgent needs.To this end, several constitutive promoters were recently evaluated and developed based on the RNA-seq results from *Streptomyces coelicolor* M1146 and *Streptomyces albus* J1074. It is reasonable to construct libraries of promoters with RNA-seq, because the RNA-seq is direct measurement of mRNA transcript levels, and sensitive with broader dynamic range, compared to those from microarray. However, RNA-seq data are not enough to design the gene expression system for fine control of gene expression. In this work, a novel method for screening and designing pairs of promoters and 5' UTR regions was suggested considering both transcription and translation. This study proposes a new tool, which can be usefully applied to synthetic biology and metabolic engineering for secondary metabolite productions in Streptomyces strains.

A Novel Approach for Gene Expression Optimization through Native Promoter and 5' UTR Combinations Based on RNAseq, Ribo-seq, and TSS-seq of Streptomyces coelicolor

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Abstract:

Streptomycetes are Gram-positive mycelial bacteria, which synthesize a wide range of natural products including over two-thirds of the currently available antibiotics. However, metabolic engineering in *Streptomyces* species to overproduce a vast of natural products are hampered by limited number of genetic tools. Here, two promoters and four 5' UTR sequences showing constant strengths were selected based upon multi-omics datasets from *Streptomyces coelicolor* M145, including RNA-seq, Ribo-seq, and TSS-seq, for controllable transcription and translation. Total eight sets of promoter / 5' UTR combinations, with minimal interferences of promoters on translation, were constructed using the transcription start site information, and evaluated with GusA system. Expression of GusA could be controlled to various strengths in three different media, in a range of 0.03 to 2.4 folds, compared to that of the control, ermE*P / Shine- Dalgarno sequence. This method was applied to engineer three previously reported promoters to enhance gene expressions. The expressions of ActII-ORF4 and MetK were also tuned for actinorhodin overproductions in *S. coelicolor* as examples. In summary, we provide a novel approach and tool for optimizations of gene expressions in *Streptomyces coelicolor.*