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**OptSSeq: High-Throughput Sequencing Readout of Growth Enrichment Defines Optimal Gene Expression Elements**

OptSSeq is shown to be a powerful tool for synthetic biology in this proof-of-concept study.

**The Science**

OptSSeq (Optimization by Selection and Sequencing) is a newly developed approach to identifying optimally balanced enzyme levels in synthetic biofuel production pathways. This method couples selection of enzyme expression levels with high-throughput gene sequencing to track enrichment of gene expression elements from a combinatorial library.

**The Impact**

OptSSeq allowed identification of the optimal cassette for ethanol production in *E. coli* and provided answers to several key questions about ethanologenic growth rate. OptSSeq is a versatile synthetic biology tool applicable to any pathway whose function can be linked to cell growth or survival. With regard to biofuel production, it can help identify constraints *in vivo* that limit maximal product formation.

**Summary**

When designing gene cassettes for heterologous expression of biofuels in microbes it can be difficult to predict *a priori* optimal levels of gene expression needed to balance biofuel production and cellular metabolism. OptSSeq is a synthetic biology tool that identifies optimal solutions from a combinatorial library of promoter and ribosome binding site variants. OptSSeq proof-of-concept was recently demonstrated using homoethanologenesis, a two-step pathway consisting of pyruvate decarboxylase (Pdc) and alcohol dehydrogenase (AdhA and AdhB) that converts pyruvate to ethanol and is naturally optimized in the bacterium *Zymomonas mobilis*. To determine the optimal configuration in *E. coli*, both gene order and expression levels were varied, subjected to ethanologenesis-dependent growth, and enriched solutions identified by high-throughput sequencing. The analysis revealed that cassettes with promoters driving strong but not maximal rates of transcription support the fastest ethanologenic growth whereas the ribosome binding sites were of maximum strength. Between the two alcohol dehydrogenases available in *Z. mobilis*,AdhB is preferred for rapid growth in both *E. coli* and *Z. mobilis*. Lastly, by comparing predictions of growth-linked metabolic flux to enzyme synthesis costs, it was determined that optimal *E. coli* ethanologenesis was achieved by the best pdc-adhB cassette and that the remaining constraints lie in the *E. coli* metabolic network or inefficient Pdc or AdhB function in *E. coli*. OptSSeq is a general tool to tune enzyme levels in any pathway whose optimal function can be linked to cell growth or survival.

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**Publications**

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