Methods in Molecular Biology™

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Microbial Metabolic Engineering

Methods and Protocols

Edited by

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Preface

Classic strain improvement is a long process that relies on screening of naturally occurring or chemically induced mutations for improved strain performance. Rapid technology advancement has accelerated strain improvement by opening the field of metabolic engineering. Metabolic engineering is the practice of genetically optimizing metabolic and regulatory networks within cells to increase production and/or recovery of certain substance from cells. The approaches may be as simple as manipulation of a single gene, and it could also be as complex as reconstruction of the cell's networks.

The objective of *Microbial Metabolic Engineering* is to provide an overview of strategies and techniques of metabolic engineering within the scope of microbial applications mainly focused on bacteria and yeasts. The first part of the book describes methods to engineer genes, pathways, or the whole genome in the production host. The complementary approaches of rational design and random screening to tap into natural diversity and engineered diversity are both illustrated. The second part of the book describes the use of modern biotechnology tools in microbial metabolic engineering. It includes use of genetic tools, omics tools, FACS analysis, and flux balance analysis for identification of targets of genes/pathways for metabolic engineering. It also includes use of microfermentors and fermentation control techniques for rapid evaluation of engineered strains. The third part of the book describes several successful examples of metabolic engineering for real-world applications such as whole-cell biosensors and acetate control in large-scale fermentation. It also addresses several challenges in commercial production such as using biomass hydrolysates as feedstock and minimizing phage contamination.

Microbial Metabolic Engineering is intended both for researchers (molecular biologists, biochemists, microbiologists, physiologists, and bioinformaticians) in academia who are interested in understanding the gene functions and cellular network in microbes, and for those in industry who are interested in developing commercial products from microbial fermentations. It also provides fermentation engineers and process engineers an illustration of what could be achieved by metabolic engineering of the microbe that may have a significant impact on fermentation and downstream processing. The book provides step-by-step instructions, and could also be used as a text book in teaching undergraduate labs. In fact, some strains described in the book have been used in undergraduate labs in Cambridge University, UK. The chapters were written by renowned investigators in the field who practice the method on a regular basis. Procedures are described with enough details so that users can carry out the method without further reference to other sources. Advice and suggestions from the experienced investigators and troubleshooting of possible roadblocks are captured in the Notes section at the end of the chapters. In all, this book is intended to provide an overview of the key topics in microbial metabolic engineering and to be used as the guidebook for researchers who practice metabolic engineering for microbial applications.

Wilmington, DE, USA ***Qiong Cheng***

Preamble

Metabolic engineering is, at its simplest, the purposeful alteration of metabolism to produce molecules of interest. The science and technology in this field continue to advance at a remarkable pace, and improvements in DNA sequencing, DNA synthesis, enzyme engineering, analytical chemistry, and protocols for understanding and utilizing large and increasingly accessible databases of enzymes and metabolic pathways are all contributing to making the practice of metabolic engineering increasingly robust. Researchers have been emboldened by their new, hard-won capabilities, and are tackling ever more difficult problems, developing microbial catalysts for everything from high value pharmaceutical products through to nutritional compounds, high volume chemicals, all the way to very high volume fuel molecules. A number of commercially successful outcomes of detailed metabolic engineering programs have started to appear, and industrial companies old and new are looking to metabolic engineering as a tool to help deliver an increasingly broad range of molecules from renewable resources.

Putting it all together requires working across multiple scales with a broad range of tools and disciplines. In practical terms, creating microbes that produce desired products at commercially relevant rates, titers, and yields—usually under conditions that put severe stress on the producing organism—can challenge the most sophisticated of experimental approaches and often requires working at the very limits of our understanding of cellular and metabolic processes. Molecular tools and classical and accelerated strain selection and improvement protocols are all critically important elements in successful programs. With engineering scale-up, many previously "solved" problems can appear in a new light, and require iterative engineering/metabolic engineering revisions. Doing all this in timeframes consistent with commercial practice adds another significant challenge in designing optimum experimental protocols. As a result, metabolic engineers require access to numerous tools, and often need to pursue multiple, simultaneous approaches to a single end—iteratively combining deterministic, theory-based approaches with empirical, evolutionary-based approaches to optimize productivity.

While this can be daunting, the tools and approaches are being streamlined and simplified, and are becoming more accessible. It was not long ago, for example, that the use of metabolic flux analysis and related mathematical analyses to guide metabolic engineering approaches was exclusively reserved to a small handful of expert practitioners, and underlying data sets were difficult to access and use. As a sign of the times, I was amazed to find as I was writing this preamble that my son—a third year chemical engineering student—had a holiday problem set for one of his courses that required him to perform a constraint-based analysis of a genome-scale metabolic network. To proceed, he used databases and software tools that he downloaded from the internet—and set about solving his problem of finding a triple mutant in *E coli* that would maximize the anaerobic production of lactic acid from glucose. It was quite remarkable to see the next generation pursuing this level of sophistication and inquiry in so facile a manner. As more and more scientists and engineers are able to access ever more powerful methodologies, the field of metabolic engineering will become increasingly able to deliver timely and relevant outcomes.

This volume brings together contributions from a wide-ranging group of expert academic, institute, and industrial practitioners. The range of methodological approaches and tools that are exemplified give a good sense of the creativity and multidisciplinarity that are required to proceed from an initial idea to the large-scale production of a commercial product. Using the types of approaches and protocols outlined in this volume, we can look forward to being able to produce—with increasing speed and confidence—an increasingly large number of products from renewable resources through the use of microbial metabolic engineering.

London, UK ***John Pierce***

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