Preface

Microbial strain engineering is used to improve production of bioproducts. Classical strain engineering is performed by repeated cycles of random mutation and selection. These methods have greatly contributed to strain improvement, but have serious drawbacks. Uncharacterized "non-specific" secondary deleterious mutations will be introduced into the genome during each mutagenesis cycle, and accumulate in the selected strain. Classical methods also do not allow the introduction of new genetic material and are not suitable for complex strain development applications such as metabolic engineering of organisms to enable cell-based conversion of biomass into biofuels.

For complex strain engineering projects such as metabolic engineering for biofuels production, a starting point "chassis" organism must be selected. This may be a commonly used industrial organism such as *Escherichia coli* or *Sacharromyces cerevisiae*. While these industrial organisms are not inherently adapted for production of biofuels, new genes and functions can be rapidly imported using existing comprehensive strain engineering toolkits. Many of these methods draw upon the fully annotated genome sequences of *E. coli* and *S. cerevisiae* that ushered in a new age of rationale design-based strain engineering.

Alternatively, a native organism with existing biochemical pathways and production potential for biofuels is selected as the chassis. However, native strains often are not adapted for industrial fermentation and lack existing molecular biology tools necessary for efficient strain engineering.

Recently, fully annotated genome sequences of many important native microbial organisms have become publically available as a resource for researchers. The availability of these genomic resources will enable adaptation of *E. coli* or *S. cerevisiae*-based rationale design strain engineering methods to native organisms.

In this book, powerful new genetic engineering-based strain engineering methods are presented for rational modification of a variety of model organisms. These methods are particularly powerful when utilized to manipulate microbes for which sequenced and annotated genomes are available. Collectively, these methods systematically introduce genome alterations in a precise manner, allowing creation of novel strains carrying only desired genome alterations.

In *Section 1*, *E. coli*-based bacterial strain engineering strategies are reviewed. State-of-the-art methods for targeted gene knockout are presented, as well as their sequential application for scarless genome modification. Methods for random gene knockout by transposon mutagenesis are also described.

Cutting edge methods for identification of adaptation-selected genes are presented in chapters describing genome engineering using oligonucleotide-mediated targeted gene replacement and microarray-based genetic footprinting of random transposon libraries.

Methods to optimize synthetic operons for metabolic engineering applications are described. Methods for introduction of genes and operons into the bacterial chromosome are presented in a chapter on integration plasmid-based chromosomal expression of native and foreign genes.

Strategies to assemble combinations of tagged integration plasmids, gene knockouts, or knockout collections (e.g., Keio collection) are discussed in a chapter on high-through-

put double mutant assembly via conjugation. Protocols to assemble multiply modified strains are provided in a chapter on P1 transduction.

In *Section 2*, analogous microbial engineering strategies for eukaryotic cells are presented, using the yeast *S. cerevisiae* as a model. This section also includes chapters describing creation and phenotypic trait selection with signature-tagged barcoded mutant collections and libraries of mutant transcription factors; these methodologies have application in a wide range of microorganisms.

In *Section 3*, examples of the proliferative adaptations of these base technologies to strain engineer industrially important prokaryotic or eukaryotic microbial systems are presented. Introductory chapters on transformation and broad host range plasmid vectors provide design guidance to develop robust methods for the critical first step of efficiently introducing functional DNA into new microbes. This effort is guided by identification in the annotated genome of genes whose products are detrimental to efficient transformation, for example, restriction endonucleases and secreted nonspecific nucleases. Targeted elimination or neutralization of these genes improves broad host range plasmid transformation. In the case of fungi, nonhomologous recombination genes are also identified and eliminated, to facilitate development of targeted homologous recombination-based methods. This subsection then describes methods for applied strain engineering of microbial organisms (prokaryotic and eukaryotic) with bioenergy potential for which sequenced and annotated genomes are available. Once basic DNA transformation, replicating plasmids, and homologous recombination-based chromosome integration methods in new organisms are available, other techniques described in Sections 1 and 2 can be adapted. For example, to facilitate application of the *E. coli* integration plasmid technology described in Chapter 8, phage integration sites can be integrated into the genome at a permissive site by homologous recombination, and the corresponding phage integrase supplied on a broad host range plasmid.

Written for: Molecular and cellular biologists, molecular geneticists, bioengineers, and microbiologists working in academia, pharmaceutical and biotechnology that perform microbial strain engineering.

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