PREFACE

This is the second volume of a two-part series in *Methods in Enzymology* on tools and techniques used in synthetic biology. Synthetic biology is an engineering discipline that seeks to construct living systems that do not exist in nature. The field refers to the process by which genetic systems are designed and constructed, as opposed to any particular application. Along these lines, these volumes are organized into two areas. Volume I focuses on the assay techniques and design principles underlying the characterization of genetic parts, their combination into devices and programs, and their integration into various hosts. Volume II focuses on computational tools and biophysical models to aid in the design and organization of genetic programs and modern methods to synthesize and assemble the associated DNA.

The first set of chapters focus on computational tools to predict the function of genetic parts and to organize them into more complex systems. Biophysical methods for modeling common genetic parts are described, including promoters, codon usage, and ribosome binding sites. Computer-aided design (CAD) will become an increasingly important tool for synthetic biology, as designs become larger and more complex. Several academic simulation packages have emerged (e.g., Clotho and SynBioSS) that enable the prediction of how multiple genetic parts will behave. Several innovative grammars (e.g., EUGENE and GenoCAD) have been proposed that simplify the rules for the combination of genetic parts in a way that enables desires to organize large systems and to facilitate the sharing of designs. Ultimately, these methods will need to be able to be applied to genome-scale engineering.

The output of the CAD programs is a DNA sequence that needs to be assembled. The combination of automated DNA synthesis with modern cloning techniques has enabled a revolution in the ambition of the scale of genetic constructs. Several methods for gene synthesis are described, including error correction, which becomes critical for large constructs. The "Gibson Method" and variations thereof provide a convenient one-step method for the assembly of multiple genetic parts. This builds on restriction enzyme-based methods, such as BioBricks™ assembly. Our lab has made great use of the MEGAWHOP method for rapidly inserting large pieces of DNA into a plasmid (anybody familiar with Quickchange will get the gist of the method).

Synthetic biology ultimately needs to work at the scale of whole-genome engineering. Methods are described for the assembly, engineering, and analysis of genome-sized fragments of DNA.