Preface

As we move solidly into the 21st century, it is amazing to see that molecular biology and its related disciplines are advancing at an ever-increasing pace. Today, it is commonplace to use bioinformatic techniques to identify a gene encoding a protein of interest and then pay a company to synthesize it, codon optimized, and receive it already inserted into the expression vector of choice. This is all the more incredible when one considers that less than 100 years ago scientists were still trying to identify the substance responsible for inherited traits, a little over 50 years ago the first DNA polymerase was discovered, and only about 40 years ago the first restriction enzyme was purified.

It was the 1970s and 1980s that saw the expansive application of the prior basic research findings lead to the commonplace expression of proteins in a heterologous host. During this time recombinant DNA was first produced and shown to be stably maintained and replicated in *Escherichia coli*. The first recombinant protein, *E. coli* DNA polymerase I, was made commercially available from New England Biolabs, Inc., and a company, Genentech, was formed around the potential of recombinant DNA technology. It was also during this period that the first recombinant human protein, somatostatin, was produced.

Traditionally, *E. coli* has been the organism of choice to express proteins. Many proteins, however, were found to be insoluble or needed post-translational modifications that do not occur in *E. coli*. These issues are more likely to arise when eukaryotic proteins are expressed in *E. coli*. To address these problems, new expression hosts were developed to more effectively produce human proteins. These expression systems included insect and human cells. Although effective, these new expression systems were not as easy to manipulate or maintain as *E. coli*.

Currently, there is no perfect expression host. Membrane proteins constitute a significant percentage of the total cellular proteins but are very difficult to overexpress in a heterologous host. Furthermore, the ideal host would have the ability to express any protein, with relevant post-translational modifications, and still be as easy to work with as *E. coli*.

This volume is focused on this goal. The chapters herein describe methods, for example, to successfully express proteins in *E. coli* that would otherwise form aggregates in this host, to add post-translational modifications, to incorporate non-standard amino acid residues or moieties into *E. coli* expressed proteins, to identify binding partners, and to express membrane proteins. Although there is still no perfect expression host, and there may never be a perfect host, the work described herein moves *E. coli* closer to that ideal. In addition, a review of *E. coli* expression hosts is presented to help familiarize the researcher with the myriad of *E. coli* expression strains available. Finally, the strength of the *Methods in Molecular Biology* series is that the chapters are written in detail by scientists intimately familiar with the relevant techniques and protocols.

I hope that the reader finds the protocols described herein helpful to their research.

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