
Preface

Heterologous gene expression in *E. coli* has been one of the most widely used methods for generating recombinant proteins for many scientific analyses and still remains the first choice for most laboratories around the world. The ease of use and low cost of production often lead researchers to initially attempt to express their proteins of interest in *E. coli* rather than opting for a eukaryotic host. Decades of development have seen the variety of methods for expressing genes in *E. coli* broaden, with improved media and optimized conditions for growth, a choice of promoter systems to regulate expression, fusion tags to aid solubility and purification, and *E. coli* host strains to accommodate more challenging or toxic proteins.

Having worked in the area of protein production for structural genomics for the past 12 years, and also having a requirement to generate human proteins, I have seen a shift from expression of many genes in *E. coli* to use of the baculovirus expression system using insect cells and more recently to mammalian cells. This revolution from prokaryotic to eukaryotic expression has been visible throughout the protein production field and is largely due to the requirement to obtain specific proteins linked to disease, for functional assays as well as structures, which may be larger, or require machinery to enable specific post-translational modifications. It is perhaps important to note, however, that the structural output from the SGC in Oxford today is still ~80% derived from *E. coli*.

This book is aimed at molecular biologists, biochemists, and structural biologists, both from the beginning of their research careers to those in their prime, to give both an historical and modern overview of the methods available to express their genes of interest in this exceptional organism. The topics are largely grouped under four parts: (I) high-throughput cloning, expression screening, and optimization of expression conditions, (II) protein production and solubility enhancement, (III) case studies to produce challenging proteins and specific protein families, and (IV) applications of *E. coli* expression. This volume provides scientists with a toolbox for designing constructs, tackling expression and solubility issues, handling membrane proteins and protein complexes, and innovative engineering of *E. coli*. It will hopefully prove valuable both in small laboratories and in higher throughput facilities. I would like to thank all the authors for their contributions and for making this a global effort.

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Methods and Protocols

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