
Preface

Advances in our understanding of the biology of the cell rely on our increasing knowledge of protein behaviour and the complex interplay of proteins with other biomolecules. Proteins are essential components of living organisms and have a role in virtually every cellular process: they are enzymes, form cellular scaffolds, and are central to signalling, transport, and regulatory functions. To study these diverse roles, it is necessary to be able to work with sufficient quantities of suitably stable and functional protein samples. While some proteins can be isolated from native sources for this purpose, many cannot as they are either intrinsically unstable or are present in impractically low quantities. Moreover, the study of mutant forms of a given protein is often central to understanding its structure and activity, and such mutants must be synthesized *in vitro*.

Since the 1970s, recombinant DNA technologies have provided a solution to producing proteins in non-native host cells. However, the attainment of high yields of recombinant proteins, particularly recombinant membrane proteins, is an enduring bottleneck in the post-genomic sciences that is only now being addressed in a truly rational manner. Several host systems have been developed for the production of recombinant proteins, ranging from prokaryotes, such as *Escherichia coli*, to higher eukaryotes, such as mammalian cell-lines. This book describes strategies and protocols for the use of yeast as a production host. With its extensive literature and sequenced genome, the well-known yeast species, *Saccharomyces cerevisiae*, provides many opportunities to optimize the functional yields of a target protein of interest. *Pichia pastoris*, for which there is now a freely available genome sequence, is a popular alternative. Both have been used for the industrial production of pharmaceutical proteins and are described here.

In the last few years, significant advances have been made in understanding how a yeast cell responds to the stress of producing a recombinant protein, and how this information can be used to engineer improved host strains. The molecular biology of the expression vector, through the choice of promoter, tag, and codon optimisation of the target gene, is also a key determinant of a high-yielding protein production experiment. The use of statistical approaches to examine how parameters such as medium composition, growth variables (e.g., temperature, oxygen availability), and the precise details of the induction regime influence recombinant protein yield has also been implemented more widely by researchers in the field.

This book examines the process of preparation of expression vectors, transformation to generate high-yielding clones, optimisation of experimental conditions to maximise yields, scale-up to bioreactor formats, and disruption of yeast cells to enable the isolation of the recombinant protein prior to purification. I hope the chapters describing these steps enable you to adopt yeast as a protein production host for your research.

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