EXPERIMENTS IN MOLECULAR BOLOGY. BIOCHEMICAL APPLICATIONS

Experiments in Molecular Biology: Biochemical Applications

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Preface

This laboratory manual is designed for upper-level undergraduates majoring in the biological sciences who want practical experience in molecular biology and nucleic acid biochemistry methods. One of its attractive features is that the text not only provides a detailed description of the steps in an experimental procedure but also presents the theoretical concepts on which the experiments are based. This serves to reinforce and complement concepts and models taught in a formal lecture course on biochemistry. This manual is a practical companion to any of several undergraduate biochemistry texts. A second strength is that the experiments are sequentially linked: The results of one experiment serve as a starting point for the next to provide students with a feeling of what it is like to work on a research project. A third distinction is the topic of the experiments, the *Escherichia coli topA* gene and its protein product, DNA topoisomerase I.

Experiments begin with the *topA* gene cloned into a lambda vector. The *cysB* gene, genetically linked to the *topA* gene, is used as a genetic marker in biological complementation tests in subcloning the DNA fragment into a plasmid expression vector for overproduction of topoisomerase I. Production of topoisomerase I is followed by enzyme activity using a DNA relaxation assay in an agarose gel, protein analysis using gel electrophoresis in the presence of sodium dodecyl sulfate, and Western blot detection using anti-topoisomerase I antibody. This set of experiments demonstrates the importance of molecular methods for overproduction of proteins and illustrates basic biochemical methods and concepts for enzyme analysis. More specifically, it reinforces concepts of DNA structure and topology. The last set of experiments involves subcloning a DNA fragment from the *topA–cysB* region into an M13 cloning vector for preparation of single-stranded DNA.

In summary, three of the most important prokaryotic cloning systems (lambda, plasmids, and M13) involving molecular, biochemical, and genetic approaches for analysis of cloned DNA are presented. Appendices list recipes, convenient sources for supplies and equipment, as well as DNA sequence information for the topoisomerase I gene. One especially helpful section describes recommendations for new instructors on potential pitfalls of specific experiments. This section reflects over 7 years of experience gained while offering this course to junior and senior biochemistry majors at Michigan State University. These students have enjoyed performing the experiments and especially their successful outcome. They also valued the content and organization of the experiments in this laboratory manual.

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