PREFACE

This manual presents shortened versions of the methods published in *Current Protocols in Molecular Biology*. Drawing from both the original "core" manual as well as the quarterly update service, this compendium includes all step-by-step descriptions of methods covered in the first ten chapters of CPMB. Designed for use at the lab bench, it is intended for graduate students and postdoctorates who are familiar with the detailed explanations found in CPMB. However, sufficient detail is provided to allow the experienced investigator to use it as a stand-alone bench guide.

Although mastery of the techniques in this manual will enable the reader to pursue research in molecular genetics, the manual is not intended to be a substitute for graduate-level courses in molecular biology or a comprehensive textbook in the field. In addition to cross-referencing the commentaries and detailed annotations in *Current Protocols in Molecular Biology*, we recommend the following introductory texts: *Molecular Biology of the Gene* (4th ed., 1987), by J.D. Watson, N.H. Hopkins, J.W. Roberts, J.A. Steitz, and A.M. Weiner; *Molecular Genetics: An Introductory Narrative*, by G.S. Stent and R. Calendar; *From Genes to Clones*, by E.-L. Winnacker; and *Genetics and Molecular Biology*, by R. Schleif. In addition, *An Introduction to Genetic Analysis*, by D.T. Suzuki, A.J.F. Griffiths, J.H. Miller, and R.C. Lewontin, is a good place to learn classical genetics techniques, and *The Molecular Biology of the Cell (6th ed., 1989)*, by B. Alberts, D. Bray. J. Lewis, M. Raff, K. Roberts, and J.D. Watson contains useful information about many aspects of cellular and molecular biology. Finally, we strongly recommend that readers obtain first-hand experience in basic techniques and safety procedures by working in a molecular biology laboratory.

HOW TO USE THIS MANUAL

Organization

This manual is organized by chapters and sections, with individual protocols contained in units. Each unit includes listings of materials, the protocol steps, and key references for each technique. Full references for the entire manual can be found in APPENDIX 5. All recipes for reagents and solutions used in the protocols are provided in APPENDIX 1. Unit numbers in this manual are identical to those in *Current Protocols in Molecular Biology*; thus, users who own both manuals will find it simple to cross-reference the latter manual when explanatory detail is required.

Many reagents and procedures are employed repeatedly throughout the manual. Rather than duplicate this information, cross-references among units are used extensively. Early chapters (and appendices) describe commonly used techniques such as basic microbiology and basic manipulations of enzymes, DNA, and RNA, while later chapters describe applications including constructing libraries, DNA sequencing, mutagenesis, transfection, and protein analysis. Thus, whenever a particular enzyme is used in a protocol, the appropriate unit in Chapter 3—describing reaction conditions for that enzyme—is cross-referenced

(e.g. *UNIT 3.7* for reverse transcriptase). Similarly, throughout the book readers are referred to *UNIT 1.3* for spreading or streaking a plate, to *UNIT 2.1* for phenol extraction/alcohol precipitation, to *UNIT 2.5* for agarose gel electrophoresis, and so on. By turning to these units, the reader will find instructions for the techniques. As a result, protocols in the later chapters of the book are not overburdened with steps describing auxiliary procedures required to prepare, purify, and analyze the sample or molecule of interest.

Protocols

Many units in the manual contain groups of protocols. The *basic* protocol is presented first in each unit and is generally the recommended approach. *Alternate* protocols are provided where (1) different equipment or reagents can be employed to achieve similar ends, (2) the starting material requires a variation in approach, or (3) requirements for the end product differ from those in the basic protocol. *Support* protocols describe additional steps that are required to perform the basic or alternate protocols; these steps are separated from the core protocol because they might be applicable to other uses in the manual, or because they are performed in a time frame separate from the basic protocol steps.

Reagents and Solutions

Reagents required for a protocol are listed in the materials list before the procedure begins. As noted above, all recipes for these solutions are listed in *APPENDIX 1* (*except* for media recipes and buffers for restriction enzymes; the locations of these recipes are cross-referenced parenthetically in the materials lists). It is important to note that the *names* of some of these special solutions might be similar from unit to unit (e.g., hybridization solution, high-salt solution, etc.), while the *recipes* differ; thus, make certain that reagents are prepared from the proper recipes. To avoid confusion, parenthetical listings of the unit or units in which each recipe is used are provided next to the name of each solution in the appendix (except in the case of commonly used buffers and solutions such as TE buffer, PBS, and 1 M $CaCl_2$).

NOTE: *Deionized, distilled water should be used in all protocols in this manual, and in the preparation of all reagents and solutions.*

EQUIPMENT

Special equipment is also itemized in the materials list of each protocol. We have not attempted to list all items required for each procedure, but rather have noted those items that might not be readily available in the laboratory or that require special preparation. Listed below are standard pieces of equipment in the modern molecular biology laboratory, i.e., items used extensively in this manual and thus not included in the individual materials lists.

Autoclave

Balances

Centrifuges— a super-speed (20,000 rpm) refrigerated centrifuge and an ultracentrifuge (20,000 to 80,000 rpm) are required for many procedures. Vertical ultracentrifuge rotors are very convenient for preparing plasmid DNA. At least one microcentrifuge that holds standard 1.5-ml microcentrifuge tubes is essential. It is also useful to have a large-capacity, low-speed centrifuge (such as the Beckman J-6M) for spinning down large bacterial cul-

tures and a tabletop swinging-bucket centrifuge with adapters for spinning 96-well microtiter plates.

Computer and printer

Darkroom and developing tanks or X-Omat automatic X-ray film developer.

Filtration apparatus for collecting acid precipitates on nitrocellulose filters.

Fraction collector

Freezers and refrigerators for 4°, –20°, and –70°C incubation and storage.

Fume hood

Geiger counter

Gel electrophoresis equipment— at least one full-size horizontal apparatus and one horizontal minigel apparatus, two sequencing gel setups for each person engaged in large-scale sequencing projects, one vertical gel apparatus for polyacrylamide protein gels, and specialized equipment for two-dimensional protein gels as required.

Heating blocks— thermostat-controlled metal heating blocks that hold test tubes and/or microcentrifuge tubes are very convenient for carrying out enzymatic reactions.

Ice maker

Incubator (37°C) for growing bacteria. We recommend an incubator large enough to hold a "tissue culture" roller drum that can be used to grow 5-ml cultures in standard 18 × 150 mm test tubes. A convenient and durable tube roller is made by New Brunswick Scientific.

Incubator/shaker(s)— an enclosed shaker (such as the New Brunswick Controlled Environment Incubator Shaker) that can spin 4-liter flasks is essential for growing 1-liter *E. coli* cultures. A rotary shaking water bath (New Brunswick R76) is useful for growing smaller cultures in flasks.

Light box for viewing autoradiograms.

Magnetic stirrers (with heater is useful).

Microwave oven to melt agar and agarose.

Paper cutter, large size, for 46 cm × 57 cm Whatman sheets.

pH meter

Pipettors that use disposable tips and dispense 1 to 1000 µl. It is best to have a set for each full-time researcher.

Polaroid camera and UV transilluminator for taking photographs of stained gels.

Power supplies— 300-volt power supplies are sufficient for agarose gels; 2000-volt power supply required for DNA sequencing.

Radiation shield (plexiglass)

Scintillation counter

Seal-A-Meal bag sealer or equivalent

Spectrophotometer, UV and visible

Speedvac evaporator (Savant)

Tissue culture equipment— CO_2 incubator, phase contrast microscope, liquid nitrogen storage container, and laminar flow hood.

Vacuum desiccator/lyophilizer

Vacuum oven

Vortex mixers

Water baths, at least two with 80°C capacity

Water purification equipment or glass distillation apparatus to purify all water used in molecular biology experiments.

X-ray film cassettes and intensifying screens

COMMERCIAL SUPPLIERS

In some instances throughout the manual, we have recommended commercial suppliers of chemicals, biological materials, or equipment. This has been avoided wherever possible, because preference for a specific brand is subjective and is generally not based on extensive comparison testing. Our guidelines for recommending a supplier are that (1) the particular brand has actually been found to be of superior quality, or (2) the item is difficult to find in the marketplace. An appendix lists the names, locations, and phone numbers of all recommended suppliers, but these are by no means the only vendors of biological supplies. Readers may experiment with substituting their own favorite brands.

SAFETY CONSIDERATIONS

Anyone carrying out these protocols will encounter the following hazardous or potentially hazardous materials: (1) radioactive substances, (2) toxic chemicals and carcinogenic or teratogenic reagents, (3) pathogenic and infectious biological agents, and (4) recombinant DNA. Most governments regulate the use of these materials; it is essential that they be used in strict accordance with local and national regulations. Cautionary notes are included in many instances throughout the manual, but we emphasize that users must proceed with the prudence and precaution associated with good laboratory practice.

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