

SHORT PROTOCOLS IN MOLECULAR BIOLOGY

Fourth Edition



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A Compendium of Methods from
CURRENT PROTOCOLS IN MOLECULAR BIOLOGY

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A Compendium of Methods from Current Protocols in Molecular Biology

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Preface

This volume 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 the principal methods covered in CPMB. Designed for use at the lab bench, it is intended for graduate students and postdoctoral fellows who are familiar with the detailed explanations found in CPMB. However, sufficient detail is provided to allow experienced investigators to use it as a stand-alone bench guide.

Although mastery of the techniques herein will enable the reader to pursue research in molecular biology and related disciplines, 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, we recommend cross-referencing the commentaries and detailed annotations in *Current Protocols in 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 alongside more experienced investigators.

HOW TO USE THIS MANUAL

Organization

This manual is organized by chapters, with individual protocols contained in units. Each unit includes listings of materials, the protocol steps, and references for each technique. Full references for the entire manual can be found in *APPENDIX 5*. The sequence and organization of material in this manual generally follows that of *Current Protocols in Molecular Biology*. Although the unit numbers may not correspond in all cases, the unit titles are identical in both versions. Thus, users who own both manuals will find it easy and convenient to cross-reference CPMB when more explanatory details are 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 *APPENDIX 3A* to *3E*) describe commonly used techniques such as basic microbiology and basic manipulation of enzymes, DNA, and RNA, while later chapters describe more advanced techniques. 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, *UNIT 2.1A* for phenol extraction/alcohol precipitation, *UNIT 2.5A* for agarose gel electrophoresis, and so on. 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.

The appendixes provide recipes for reagents and solutions (*APPENDIX 1*), a list of useful measurements and data (*APPENDIX 2*), commonly used biochemical techniques (*APPENDIX 3*), the names and addresses of suppliers (*APPENDIX 4*), and complete listings for all cited references (*APPENDIX 5*).

Protocols

Many units 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, corresponding recipes are listed in *APPENDIX 1* except for medium recipes and buffers for restriction endonucleases—the locations of these recipes are cross-referenced parenthetically in the Materials list. 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, lysis buffer, 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 reagent in *APPENDIX 1*, except in the case of commonly used buffers and solutions—e.g., TE buffer, PBS, and 1 M CaCl₂.

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

EQUIPMENT

Standard pieces of equipment in the modern molecular biology laboratory are listed in the accompa-

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 analytical and preparative

Bench protectors plastic-backed (including “blue pads”)

Centrifuges a low-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 for spinning down large bacterial cultures 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 or membrane.

Fraction collector

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

Fume hood

Geiger counter

Gel dryer

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.

Liquid nitrogen

Magnetic stirrers (with heater is useful).

Microcentrifuge Eppendorf-type, maximum speed 12,000 to 14,000 rpm

Microcentrifuge tubes 1.5-ml

Microwave oven to melt agar and agarose.

Mortar and pestle

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

pH meter

pH paper

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.

Policemen rubber or plastic

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

Radiation shield (Lucite or Plexiglas)

Radioactive ink

Radioactive waste container for liquid and solid waste

Refrigerator 4°C

Safety glasses

Scalpels and blades

Scintillation counter

Seal-A-Meal bag sealer or equivalent

Shakers orbital and platform, room temperature or 37°C

Spectrophotometer UV and visible

Speedvac evaporator

Thermal cycler

Tissue culture equipment CO₂ humidified incubator, phase-contrast microscope, liquid nitrogen storage container, and laminar flow hood.

UV cross-linker

UV light sources long- and short-wave

UV transilluminator

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.

nying box. These items are used extensively in this manual. The Materials list that precedes each protocol includes only “specialized” items—i.e., items that might not be readily available in the laboratory or that require special preparation.

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. *APPENDIX 4* lists the names, locations, and phone numbers of 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 materials: (1) radioactive

substances, (2) toxic chemicals and carcinogenic or teratogenic reagents, (3) pathogens and infectious biological agents, and (4) certain recombinant DNA construct. It is essential that these materials 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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