SHORT PROTOCOLS IN CELL BIOLOGY

A COMPENDIUM OF METHODS FROM CURRENT PROTOCOLS IN CELL BIOLOGY

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Preface

The universe of cell biology is expanding. One way to sense this expansion is to attend a major meeting dedicated to the field, such as that of the American Society for Cell Biology. The poster sessions allow one to take a leisurely stroll through the exhibits and, even without stopping to look at any single poster, get a visceral feel for the scope of cell biology. For those who have taken such a stroll periodically over the past decade or two, the dynamic nature of cell biology and its explosive growth have been obvious—there is simply more and more about more and more. Once upon a time, poster sessions were dominated by images of cells fixed (literally) and captured in black and white by electron microscopists. While the electron microscope continues to contribute to our understanding of cell architecture, more recent poster sessions have witnessed the emergence of row upon row of computer-enhanced video images of living cells captured in the not-so-living colors of rhodamine red, fluorescein green, and the characteristic green that gives the now-famous green fluorescent protein its name. Freeze-etching has been joined by freeze-frame. Furthermore, interspersed among the video images are posters that document detailed molecular characterizations of a vast array of cellular components. Other posters record the latest functional insights obtained by knocking down or knocking out genes. Every point of the compass has its blot! Genetic studies in yeast, flies, and worms abound on the one hand, and on the other, a growing number of the presentations form bridges between basic research in cell biology and the practice of clinical medicine.

Clearly, the scientists who today identify themselves as cell biologists are a diverse community, and great value resides in that diversity. Borders that once separated research disciplines have faded, and cell biologists have come to appreciate that no single approach in isolation will open the profound mysteries of the cell. New techniques and new technologies come alongside the tried-and-true as the tools of cell biology expand along with the field. It is this changing face of cell biology and its methodologies that represented the greatest challenge in pulling together *Short Protocols in Cell Biology*. A foundational question addressed by the editors of this work involved where to draw the boundaries around the field of cell biology. Our decision has been to refuse to draw such boundaries—they are artificial at best and counterproductive at worst. Instead, we have attempted in this effort to match the diversity of our field. We include "classic" methods that remain valuable tools for the modern cell biologist and also provide protocols that are destined to become tomorrow's classics.

There is no reason to suspect that the universe of cell biology will cease expanding anytime soon. Indeed, part of the thrill of being a cell biologist is being constantly surprised by new innovations and discoveries. As a consequence, however, our community needs a reliable and user-friendly source of laboratory methods that is as expansive as the field itself. To begin to address this need, we have assembled a series of protocols that cover many aspects of cell biology. Although this set of methods is incomplete, it can be considered a "starter toolbox" that includes many of the most versatile and essential instruments of our trade.

Short Protocols in Cell Biology presents shortened versions of the methods published in *Current Protocols in Cell Biology*. Drawing from both the original "core" manual and the quarterly update service, this compendium includes step-by-step descriptions of the principal methods covered in *CPCB*. Designed for use at the lab bench, *Short Protocols* presents the protocols in a streamlined format compared with that found in *CPCB*. Sufficient detail is provided to allow investigators to use *Short Protocols* as a stand-alone bench guide. For more information and discussion, we recommend that the reader refer to the commentaries and detailed annotations in *CPCB*.

Although mastery of the techniques herein will enable the reader to pursue research in cell biology and related fields, neither this manual nor *CPCB* is intended to be a substitute for graduate-level courses in cell biology or a comprehensive textbook in the field. In addition, we strongly recommend that readers obtain first-hand experience in a laboratory alongside more experienced investigators.

HOW TO USE THIS MANUAL

Organization

Subjects in this manual are organized by chapters, and protocols are contained in units within a chapter. Units generally describe a method and include one or more protocols with materials, steps, and some annotations for each technique. The sequence and organization of material in this manual differs from that of *Current Protocols in Cell Biology*—chapters have been rearranged, renumbered, and in some cases merged, and units within chapters have been rearranged, but the chapter and unit titles remain essentially the same. Users who have access to both manuals will find it easy to identify corresponding units when more explanatory details and commentary are required.

Many reagents and procedures are employed repeatedly throughout the manual. Instead of duplicating procedures, cross-references among units are used extensively. Cross-referencing helps to ensure that lengthy and complex protocols are not overburdened with steps describing auxiliary procedures needed to prepare raw materials and analyze results. Certain units that describe commonly used techniques and recipes (e.g., gel electrophoresis, ion-exchange chromatography, immunoblotting, autoradiography) are cross-referenced in other units that describe their application. Thus, whenever it is necessary to isolate or identify a protein band in a protocol, the appropriate unit in Chapter 7—describing various procedures for gel electrophoresis—is cross-referenced (i.e., *UNIT 7.1*). For some widely used techniques (such as dialysis), readers are referred to *APPENDIX 3*. For commonly used methods in molecular and protein biology, readers are referred to *Current Protocols in Molecular Biology* and *Current Protocols in Protein Science* or their *Short Protocols* versions.

There are four appendixes to this manual. Recipes for the reagents and solutions used in each method and noted with a check mark are presented in *APPENDIX 1*. Other appendices include useful measurements and data (*APPENDIX 2*), commonly used techniques (*APPENDIX 3*), and names and addresses of suppliers (*APPENDIX 4*).

Protocols

Many units in the manual contain groups of protocols, each presented with a series of steps. The Basic Protocol is presented first in each unit and is generally the recommended or most universally applicable approach. Alternate Protocols are provided where different equipment or reagents can be employed to achieve similar ends, where the starting material requires a variation in approach, or where 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;

Standard Laboratory Equipment

Listed below are pieces of equipment that are standard in the modern cell biology laboratory—i.e., items used extensively in this manual and thus not usually included in the individual materials lists. No attempt has been made to list all items required for each procedure in the Materials list of each protocol; rather, those lists note those items that might not be readily available in the laboratory or that require special preparation. See *APPENDIX 4* for contact information for commercial vendors of laboratory equipment.

Applicator, cotton-tipped and wooden
Autoclave
Bag sealer
Balances, analytical and preparative
Beakers
Bench protectors, plastic-backed (including "blue" pads)
Biohazard disposal containers and bags
Biosafety cabinet, tissue culture or laminar flow hood; filters air and maintains air flow pattern to protect cultured cells from investigator and vice versa
Bottles, glass, plastic, and squirt
Bunsen burners
Centrifuges, low-speed (to 20,000 rpm) refrigerated, ultracentrifuge (20,000 to 80,000 rpm), large-capacity low-speed, tabletop, with appropriate rotors and adapters
Centrifuge tubes and bottles, plastic and glass, various sizes
Clamps
Conical centrifuge tubes, plastic and glass
Containers, assortment of glass and plastic, for gel and membrane washes
Coplin jars, glass, for 25–75-mm slides
Cryovials, sterile (e.g., Nunc)
Cuvettes
Desiccator and desiccant
Dry ice
Electrophoresis equipment, agarose and acrylamide, full-size and mini, with power supplies
Film developing system and darkroom
Filtration apparatus
Forceps
Fraction collector
Freezers, –20°C, –70°C, and liquid nitrogen
Fume hood
Geiger counter
Gel dryer
Gloves, disposable plastic and heat resistant
Graduated cylinders
Heating blocks, thermostatically controlled for test tubes and microcentrifuge tubes
Hemacytometer and/or electronic cell counter
Homogenizer
Humidified CO_2 incubator
Ice bucket
Ice maker
Immersion oil for microscopy
Lab coats
Laboratory glassware
Light box
Liquid nitrogen
Lyophilizer

continued

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 itemized in the materials list before the procedure begins. Many are common stock solutions, others are commonly used buffers or media, while others are solutions unique to a particular protocol. Recipes for Materials list items with a check mark are provided in *APPENDIX 1*. It is important to note that the *names* of some of these solutions might be similar for more than one unit (e.g., lysis buffer) while the *recipes* differ; it is essential to prepare reagents from the proper recipes. To avoid confusion, a parenthetical listing of the unit(s) in which each recipe is used can be found next to the name of each reagent in *APPENDIX 1*, except in the case of commonly used buffers and solutions, e.g., TE buffer and PBS.

NOTE: Unless otherwise indicated, deionized distilled water (or an equivalent) 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 cell biology laboratory are listed in the accompanying 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

Throughout the manual, we have listed commercial suppliers of chemicals, biological materials, and equipment. In some cases, the noted brand has been found to be of superior quality or it is the only suitable product available in the marketplace. In other cases, the experience of the author of that protocol is limited to that brand. In the latter situation, recommendations

Standard Laboratory Equipment, *continued*

Magnetic stirrer, with and without heater, and stir bars
Markers, including indelible markers, china-marking pens, and luminescent markers
Microcentrifuge, Eppendorf-type with 12,000 to 14,000 rpm maximum speed
Microcentrifuge tubes, 0.2-, 0.5-, 1.5-, 2-ml
Microscope slides, glass, 25–75-mm, and coverslips
Microscope with camera, upright, inverted, fluorescence, phase-contrast, dissecting
Microtiter plate reader
Mortar and pestle
Ovens, drying and microwave
Paper cutter, large
Paper towels
Parafilm
Pasteur pipets and bulbs
PCR thermal cycler and tubes
pH meter
pH paper
Pipets, graduated
Pipettors, adjustable delivery, 0.5- to 10-µl, 10- to 200-µl, and 200- to 1000-µl
Polaroid camera or video documentation system
Power supplies, 300-V for polyacrylamide gels, 2000- to 3000-V for other applications
Racks, test tube and microcentrifuge tubes
Radiation shield, Lucite or Plexiglas
Radioactive waste containers for liquid and solid wastes
Refrigerator, 4°C
Ring stand and rings
Rubber policemen or plastic scrapers
Rubber stoppers
Safety glasses
Scalpels and blades
Scintillation counter, β
Scissors
Shakers, orbital and platform, room temperature or 37°C
Spectrophotometer, visible and UV range
Speedvac evaporator
Syringes and needles
Tape, masking, electrician's black, autoclave, and Time tape
Test tubes, glass and plastic, various sizes, with and without caps
Timer
Toolbox with common tools
Trays, plastic and glass, various sizes
Tubing, rubber and Tygon
UV light sources, long- and short-wavelength
UV transilluminator
UV transparent plastic wrap (e.g., Saran Wrap)
Vacuum desiccator
Vacuum oven
Vacuum supply
Vortex mixers
Waring blender
Water bath with adjustable temperature
Water purification system
X-ray film cassettes and intensifying screens

are offered as an aid to the novice cell biology experimenter in obtaining the tools of the trade. Experienced investigators are therefore encouraged to experiment with substituting their own favorite brands. Addresses, phone numbers, facsimile numbers, and Web sites of all suppliers mentioned in this manual are provided in *APPENDIX 4*.

References

Short Protocols gives only a limited number of the most fundamental references as background for each unit. These are listed at the end of the unit in abbreviated form with full bibliographic listings in the *References* section at the end of the book. Listings for specific references cited, for example, in figures and tables can also found in the *References* section. Readers who would like a more complete entry into the literature for background and application of methods are referred to the appropriate units in *Current Protocols in Cell Biology*.

SAFETY CONSIDERATIONS

Anyone carrying out these protocols may 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) certain recombinant DNA constructs. Although some cautionary statements are included in the appropriate units, we emphasize that users must proceed with the prudence and precaution associated with good laboratory practice, and that all materials must be used in strict accordance with local and national regulations.

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RECOMMENDED BACKGROUND READING

Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. 2003. Essential Cell Biology, 2nd Ed. Garland Publishing, New York.

A basic introductory cell biology text written by the authors of Molecular Biology of the Cell.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Watson, J.D. 2002. Molecular Biology of the Cell, 4th ed. Garland Publishing, New York.

Lodish, H., Berk, A., Zipursky, L., Baltimore, D., and Darnell, J. 2000. Molecular Cell Biology. 4th ed. W.H. Freeman and Company, New York.

Two comprehensive and lucid textbooks that convey effectively the synergistic convergence of biochemistry, genetics, structural biology, and traditional cell biology to form modern molecular and cell biology.

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