Preface

Neuroscience is one of the most dynamic disciplines in biology. The inherent interest in the nervous system is not sufficient to explain the explosion of work in this field. The last decade has seen considerable conceptual progress, but technical advances leading to the introduction of new methods in laboratories across the world have also played a major part in driving the field forward. The techniques for investigating the nervous system that have been developed require familiarity with electrophysiology, mouse genetics, optics, brain anatomy, and many other methods. More than most areas of biology, neuroscience is a multidisciplinary activity.

The rapid transfer of technical information and experience is the partner of profound conceptual change. Although it is hard to be comprehensive in this diverse field, our goal in creating this manual is to provide a single source for core techniques in the cellular and molecular aspects of neuroscience.

The methods in *Short Protocols in Neuroscience: Cellular and Molecular Methods* are shortened versions of methods published in *Current Protocols in Neuroscience* (CPNS). This compendium includes step-by-step descriptions for a broad range of cellular and molecular neuroscience methods. The methods are complete and easy to follow and the book is easier to use at the bench than the parent volumes. *Short Protocols* is ideal for graduate students and postdoctoral fellows who are familiar with the background and theory found in CPNS, and sufficient detail is provided to allow experienced investigators to use it as a stand-alone bench guide.

Although mastery of the techniques presented in this manual, and its companion, *Short Protocols in Neuroscience: Systems and Behavioral Methods*, will allow the reader to design and complete experiments in neuroscience, this manual is not intended to be a substitute for an advanced training program in neuroscience, nor for any number of comprehensive textbooks in the field. In addition to becoming thoroughly knowledgeable of the terms and concepts of neuroscience, readers should gain first-hand experience in basic techniques and safety procedures by working in a supervised neuroscience laboratory.

HOW TO USE THIS MANUAL

Format and Organization

Subjects in this manual are organized by chapters and protocols are contained in units. Protocol units, which constitute the bulk of the book, include one or more protocols with listings of materials and steps. Recipes for unique reagents and solutions are included in *APPENDIX 1* and references are included in the *References* section at the end of the book. The book also includes several overview units which contain theoretical discussions that lay the foundation for subsequent protocol units.

Certain units that describe commonly used techniques and recipes (e.g., neural tissue sectioning and mounting, solutions for preparing coated tissue culture dishes) are cross-referenced in other units that describe their application. Thus, whenever it is necessary to prepare mounted rat brain sections for microscopy, the appropriate unit in Chapter 1—describing various procedures for brain preparation, sectioning, and mounting—is cross-referenced (i.e., *UNIT 1.1*). For some widely used molecular biological techniques, *Current Protocols in Molecular Biology* (*CPMB*) is cross-referenced throughout the manual.

Protocols

Most units in this manual contain groups of protocols, each presented with a series of steps. One or more *Basic* Protocols are presented first in each unit and generally cover the recommended or most universally applicable approaches. *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; 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. All recipes are presented in *APPENDIX 1*. It is important to note that the names of some of these special solutions might be similar from unit to unit (e.g., SDS sample buffer) while the recipes differ. To minimize confusion, the appropriate unit for a given recipe, except for those for commonly used solutions, is given parenthetically in the appendix.

NOTE: Deionized, distilled water should be used in the preparation of all reagents and solutions and for all protocol steps. When sterile solutions are required, that will be indicated in the protocol.

Equipment

When special equipment is required for an experiment, that equipment is listed in the Materials list for the protocol. The Materials list does not include every item used, rather it includes items that might not be readily available in the laboratory, items that have particular specifications, and items that require special preparation or temperature. Standard pieces of equipment used in the modern neuroscience laboratory are listed in the accompanying box. These items are used frequently in the protocols in this manual and are not necessarily listed in the Materials list.

Standard Equipment

Applicators, cotton-tipped and wooden
Autoclave
Balances, analytical and preparative
Beakers
Bench protectors, plastic-backed (including "blue pads")
Biohazard disposal containers and bags
Bottles, glass and plastic
Bunsen burners
Cell harvester, for determining radioactivity uptake in 96-well microtiter plates
Centrifuges, low-speed (6,000 rpm) and high-speed (20,000 rpm) refrigerated centrifuges and an ultracentrifuge (20,000 to 80,000 rpm) are required for many procedures. At least one microcentrifuge that holds standard 0.5- and 1.5-ml microcentrifuge tubes is essential. It is also useful to have a tabletop swinging-bucket centrifuge with adapters for spinning 96-well microtiter plates.
Clamps
Cold room or cold box, 4°C
Computer (IBM-compatible or Macintosh) and printer
Containers, assortment of plastic and glass dishes for gel and membrane washes
Coplin jars or staining dishes, glass, for 75 × 25–mm slides
Cryovials, sterile—e.g., Nunc
Cuvettes, plastic disposable, glass, and quartz
Darkroom and developing tank, or X-Omat automatic X-ray film developer (Kodak)
Desiccators (including vacuum desiccators) and desiccant
Dry ice
Filtration apparatus, for collecting acid precipitates on nitrocellulose filters or membranes
Flasks, glass (e.g., Erlenmeyer, beveled shaker)
Forceps
Fraction collector
Freezers, −20° and −80°C
Geiger counter
Gel dryer
Gel electrophoresis equipment, at least one full-size horizontal apparatus and one horizontal minigel apparatus, one vertical full-size and minigel apparatus for polyacrylamide protein gels, and specialized equipment for two-dimensional protein gels
Gloves, plastic and latex, disposable and asbestos
Graduated cylinders
Heating blocks, variable temperature up to 100°C; these thermostat-controlled metal heating blocks that hold test tubes and/or microcentrifuge tubes are very convenient for carrying out enzymatic reactions
Heat-sealable plastic bags and sealing apparatus
Hemacytometer
Hoods, chemical (fume), microbiological safety, and laminar flow (tissue culture)
Hot plates, with or without magnetic stirrer
Ice buckets
Ice maker
Incubators, 37°C for bacteria and humidified 37°C, 5% CO_2 for tissue culture
Kimwipes, or equivalent lint-free tissues
Lab coats
Light box, for viewing gels and autoradiograms
Liquid nitrogen and Dewar flask
Lyophilizer
Magnetic stirrers, (with heater is useful)
Markers, including indelible markers and china-marking pencils
Microcentrifuge, Eppendorf-type, maximum speed 12,000 to 14,000 rpm

continued

Commercial Suppliers

Throughout the manual, we have recommended 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 are offered as an aid to the novice neuroscientist in obtaining the tools of the trade. Experienced investigators are therefore encouraged to experiment with substituting their own favorite brands.

Addresses, phone numbers, and facsimile numbers of all suppliers mentioned in this manual are provided in *APPENDIX 3*.

References

Short Protocols gives only a limited number of the most fundamental references as background. These are listed at the end of the unit. Full bibliographic information for these and references cited in the unit are given in the *References* at the end of this book. Readers who would like a more extensive entry into the literature for background and

Standard Equipment, *continued*

Microcentrifuge tubes, 1.5-ml and 0.5-ml
Microscope, standard optical model (optionally with epifluorescence or phase-contrast illumination) and inverted microscope for tissue culture
Microscope slides and coverslips
Mortar and pestle
Ovens, drying, hybridization, vacuum, and microwave
Paper cutter, large size, for 46 × 57–cm Whatman paper sheets
Paper towels
Parafilm
pH meter
pH paper
Pipet bulbs, or battery-operated pipetting devices—e.g., Pipet-Aid (Drummond Scientific)
Pipets, Pasteur and graduated, glass and plastic, serological (1-ml to 25-ml)
Pipettors, adjustable delivery, volume ranges 0.5 to 10 µl, 10 to 200 µl, and 200 to 1000 µl. It is best to have one set of these three sizes for each full-time researcher and sets dedicated for radioactive and PCR experiments.
Plastic wrap, UV-transparent (e.g., Saran Wrap)
Pliers, needle nose
Polaroid camera
Power supplies, 300-V power supplies are sufficient for polyacrylamide gels; 2000- to 3000-V is needed for some applications
Racks, for test tubes and microcentrifuge tubes
Radiation shield, Lucite or Plexiglas
Radioactive waste containers, for liquid and solid waste
Razor blades
Refrigerator, 4°C
Ring stands and rings
Rotator, end-over-end
Rubber bands
Rubber policemen
Rubber stoppers
Safety glasses
Scalpels and blades
Scintillation counter
Scissors
Sectioning equipment, cryostat microtome, sliding microtome (with stage and knife), Vibratome
Shakers, orbital and platform, room temperature or 37°C. An enclosed shaker (e.g., 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.
Spectrophotometer, UV and visible
Speedvac evaporator (Savant)
Stir-bars, assorted sizes
Surgical equipment, scale for weighing animals, electric razor, syringes, hypodermic needles, dissection instruments (scissors, scalpels, forceps, hemostats, retractor, bone drill, sutures), operating microscope (optional), heating pad, sterile gauze
Tape, masking and electrician's
Thermometers
Timer
UV cross-linker (e.g., Stratalinker from Stratagene)
UV light sources, long- and short-wave, stationary or hand-held
UV transilluminator
Vacuum aspirator
Vacuum line
Vortex mixers
Wash bottles, plastic and glass
Water baths, variable temperature up to 80°C
Water purification equipment, e.g., Milli-Q system (Millipore) or equivalent
X-ray film cassettes and intensifying screens

application of methods are referred to the appropriate unit in *Current Protocols in Neuroscience.*

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) recombinant DNA constructs. Check the guidelines of your particular institution with regard to use and disposal of these hazardous materials. Only limited cautionary statements are included in this manual. Users are responsible for understanding the dangers of working with hazardous materials and for strictly following the safety guidelines established by manufacturers (e.g., MSDS) as well as local and national regulatory agencies. Radioactive substances must be used only under the supervision of licensed users, following the guidelines of the National Regularoty Commission (NRC).

Animal Handling

Many protocols call for use of live animals (usually rats or mice) for experiments. Prior to conducting any laboratory procedures with live subjects, the experimental approach must be submitted in writing to the appropriate Institutional Animal Care and Use Committee (IACUC). Written approval from this committee is absolutely required prior to undertaking any live-animal studies. Some specific animal care and handling guidelines are provided in the protocols where live subjects are used, but check with your IACUC guidelines to obtain more extensive guidelines.

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RECOMMENDED BACKGROUND READING

Bedcer, J.B., Breedlove, M.S., Crews, D., and McCarthy, M.M. (eds.) 2002. Behavioral Endocrinology, 2nd ed. MIT Press, Cambridge, Mass.

Cooper, J.R. 2002. The Biochemical Basis of Neuropharmacology, 8th ed. Oxford University Press, New York.

Charles R. Gerfen, Michael A. Rogawski, David R. Sibley, Phil Skolnick, and Susan Wray