

STYLE GUIDE FOR CONTRIBUTORS

Current Protocols in Toxicology

EDITORIAL BOARD

Lucio G. Costa
University of Washington

Julio C. Davila
O'Fallon, MO

David A. Lawrence
Wadsworth Center

Donald J. Reed
Oregon State University

CURRENT PROTOCOLS

Wenshuo Zhang, Associate Developmental Editor
111 River Street, MSC 8-02
Hoboken, NJ 07030-5774
Phone: (201) 748-6286
Fax: (201) 748-6207
wezhang@wiley.com

Bill Mullen, Editorial Program Assistant
111 River Street, MSC 8-02
Hoboken, NJ 07030-5774
Phone: (201) 748-6894
Fax: (201) 748-6207
bmullen@wiley.com

**CURRENT PROTOCOLS IN TOXICOLOGY (CPTX)
EDITORIAL BOARD**

Editor

Lucio G. Costa
tel: 206-543-2831
fax: 206-685-4696
lgcosta@u.washington.edu

Address

Department of Environmental Health
Toxicology Program
4225 Roosevelt
University of Washington
Seattle, WA 98105

Julio C. Davila
julio.c.davila2010@gmail.com

O'Fallon, MO

David A. Lawrence
tel: 518-402-5684
fax: 518-474-1412
david.lawrence@wadsworth.org

Clinical Experimental Endocrinology and
Immunotoxicology
Wadsworth Center
P.O. Box 509
Albany, NY 12201-0509

Donald J. Reed
tel: 541-737-4438
fax: 541-737-4371
donald.reed@oregonstate.edu

Department of Biochemistry and Biophysics
2011 ALS Building
Oregon State University
Corvallis, OR 97331-7305

Advisory Editor

Ernest Hodgson
tel: 919-515-5295
fax: 919-513-1012
ernest_hodgson@ncsu.edu

Department of Environmental and Molecular
Toxicology
North Carolina State University
850 Main Campus Drive (P.O. Box 7633)
Raleigh, NC 27695

Quick Guide to Unit Structure

The standard elements listed below are fully described in the “Organization of the Manuscript” section of this guide

- Title Page
title, author, affiliation, phone/fax/e-mail contacts.
- Unit Title and Unit Introduction
gives context in relation to chapter; short description of individual protocols in unit
- Strategic Planning (item 3; optional)
procedural options (e.g., protocol selection, vector construction) for complex methods
- Basic Protocol(s)
 - Title
parallel with other titles in unit; more specific than unit title
 - Introduction
gives context of protocol with regard to unit as a whole; summary of procedure
 - Materials List
Solution names and special equipment; cross-references to supporting methods.
 - Steps and Annotations
steps in active voice; details for novice investigators
 - Tables and/or Figures
to illustrate setup or results; may also be included in other sections
- Alternate and/or Support Protocols
same elements as for Basic Protocol
- Reagents and Solutions
Recipe for solutions in all protocols; storage conditions (shelf life and temperature)
- Commentary
 - Background Information
theory (suitable for molecular biologists), limitations, other options for similar analyses etc..
 - Critical Parameters
points to consider before beginning
 - Troubleshooting
suggestions for commonly encountered problems
 - Anticipated Results
 - Time Considerations
- Literature Cited
follow Current Protocols style for journals/books in this guide
- Key References with Annotations
- Internet Resources with Annotations

Style Guide for Contributors

Objectives and Audience

Many subscribers to Current Protocols are trained in the subject covered, but are neither trained nor experienced in a large proportion of the procedures described. Therefore, sufficient detail must be provided to permit duplication of the protocols in any laboratory, whatever the disciplinary background or level of sophistication. For the benefit of the novice experimenter, very specific information should be included where it is important to the success of the protocol. It is preferable that you provide too much detail that can be edited at the discretion of the editorial board, rather than not enough detail.

Submission of Manuscript

The manuscript should be submitted to Current Protocols via ScholarOne Manuscripts, our electronic manuscript submission system. You will receive instructions on how to use this system in emails from our editorial office.

If you have questions, the address and phone number of the Developmental Editor are listed on the cover page of this guide. Also listed are the addresses and phone numbers of the editorial board members, whom you can contact regarding questions of scientific content or approach.

Role of Contributors

The procedure you provide should be reliable and efficient, and should provide tips and expertise based on your experience. Your name will be listed on the protocol, so the procedure will be associated directly with you.

As a contributor, you are responsible for submitting revisions or corrections to your published protocol to maintain its accuracy and timeliness. If you have improved the methods, contact your chapter editor or the Developmental Editor, and your changes will be scheduled for a future update.

Length of the Manuscript

Current Protocols does not impose strict length requirements on manuscripts. The length of the manuscript should be dictated by the topic presented. If the editors feel that the length of the unit is a concern, making coverage of the topic cumbersome, they will discuss the possibility of dividing the content into multiple units.

Organization of the Manuscript

Current Protocols uses two types of units, the overview style and the protocol style. Sample published units of the protocol style are available at <http://www.currentprotocols.com>. Contact the developmental editor for a sample overview unit.

Overview style:

An overview unit is presented as explanatory text with no protocol steps. It is not meant to be a thorough review of a subject, but rather an introduction to the major concepts; it is a useful format for summaries of key topics. You have a great deal of leeway in designing such a unit.

Protocol style:

The Quick Guide to Unit Structure outline on the previous page illustrates the required organization of the standard protocol unit. Listed below, corresponding to each element in the outline, are descriptive passages of these elements, *listed in the order in which they should appear in your manuscript*. It is important that you include all the elements described herein (except those listed as optional). Contact the Developmental Editor with any questions regarding the format or style of your submission.

1. Title Page. Include title of manuscript, all authors' names in the order in which they are to appear in the citation, all affiliations, phone and fax numbers, and an e-mail address for the corresponding author. You may also include an abstract and key words here; you will also enter them as a required field in our electronic submission form.

2. Unit Title and Unit Introduction. The unit title describes the function of the protocol(s) in your unit. Define all abbreviations and avoid the use of words such as “method,” “technique,” “procedure,” and “protocol” in the unit title.

The unit introduction should provide only a brief context for the unit (why the protocol is performed and/or how it relates to other units in the chapter). It should also describe the general approach of the methodology involved and briefly name and compare each of the protocols that are included.

3. Strategic Planning (optional). Occasionally a method is sufficiently complex that a Strategic Planning section is required. This section describes various procedural options (sometimes with flow charts), planning, experimental design, choice of reagents or conditions, etc.

4. Basic Protocol Title and Introduction. The basic protocol title is more specific than the unit title; it should describe the approach being used and differentiate it from other protocols in the unit. The introduction to the basic protocol should summarize the specific approach of that protocol.

5. Basic Protocol Materials List. The materials list should consist of two to three segments:

- *solutions and reagents*
- *special equipment* (items not readily available in the laboratory or that require special preparation). Standard lab equipment is itemized in an appendix to each Current Protocols manual.
- where applicable, a third, single run-on entry, “*Additional reagents and equipment for procedure (UNIT X.X);*” this entry is meant to avoid the listing of materials and steps for a procedure that can instead be cross-referenced to another unit. Especially for common procedures, please check whether portions of your protocols can be effectively covered by such cross-references; be sure to provide appropriate connecting information (e.g., amount of sample or cells to use).

All materials and equipment are to be listed *in order of use* in their respective categories and—if not self-descriptive (e.g., 2.5 M CaCl₂)—each listing should be accounted for by either a *recipe* (in the Reagents and Solutions section) or a *cross-reference* to a recipe elsewhere in the manual.

List suppliers only when (1) the particular brand has actually been found to be of superior quality, or (2) the item is difficult to find in the marketplace. Please provide full address, phone/fax numbers and website URLs for inclusion in the **Suppliers Appendix** (amended yearly).

6. Basic Protocol Steps and Annotations. The protocol steps should describe the actions performed, employing the **active voice** versus the passive: e.g., “Connect the outlet of the vacuum flask...” rather than “The outlet of the vacuum flask is connected to....”

When there are more than 10 steps to a protocol, provide **subheadings** to clarify the sequence of steps at each major juncture in the experiment. These, too, should be in the active tense, e.g., “Lyse the cells....”

Within steps, please provide the following parameters:

For reagent storage conditions: “Store for (shelf life) at (temperature)”

For centrifugation: “Centrifuge (duration) at (speed) x g, (temperature)”

For incubation conditions: “Incubate (time) at (temperature)”

Useful auxiliary information can be included after some protocol steps (as needed) in the form of italicized **annotations**. These may cover special tips for performing a step successfully, descriptions of *why* a step is performed,

emphasis regarding crucial parameters, descriptions of expected results (e.g., appearance of solution), alternate ways to perform the step, cautions regarding hazardous materials or other safety conditions, time considerations, storage information, and theoretical asides.

7. Alternate and/or Support Protocols. **Alternate protocols** are included when the basic protocol is inappropriate for certain important applications, or if different materials are widely used in other labs. **Support protocols** should be provided to supplement the basic protocol where necessary (e.g., to describe preparation of a complex reagent used in the basic protocol); it is preferable to list a separate protocol for preparatory techniques, than to combine everything into one extremely long protocol.

- a. **Alternate/support protocol title and introductory text (statement of purpose).** Each alternate and support protocol should have a distinguishing title (parallel in construction to the basic protocol) and an introduction describing why the particular protocol is being included in the unit (for *alternate protocol*: why it is performed instead of the basic protocol and how the steps differ; for *support protocol*: description of its relation to the protocol it is supporting).
- b. **[Additional] Materials.** Alternate and support protocols should each have their own list of materials and special equipment; however, for alternate protocols, materials and special equipment that already appear in a prior materials list(s) in the same unit should not be listed again. In such a case the heading should be "Additional Materials." For support protocols, either a full Materials list or an abbreviated Additional Materials list may be used.

8. Reagents and Solutions. This section should list recipes for all solutions or other items requiring special preparation used in all the protocols in the unit (with the exception of recipes that are cross-referenced to other units in the manual). The individual reagent names are organized in *alphabetical order*, with respective recipes usually in list format

For each ingredient listed in a recipe, provide both quantity *and* final concentration. If concentration is indicated as a percentage, indicate whether (v/v), (w/v), etc. In addition, *always provide storage conditions* (temperature and length of time) for each recipe.

9. Commentary. A complete commentary section should include each of the sections listed below.

- a. **Background Information.** A brief discussion of the theory and applications of your procedure. Some or all of the following elements could be included in this section:
 - why the procedure is performed (historical development, where pertinent);
 - the central advantages (and disadvantages) of the technique chosen (with brief description and references for alternative methods);
 - comparison of basic and alternate protocols or comparison with other methods currently in use;
 - applications of methods;
 - citation of original or useful literature and brief discussion of primary references;
 - biochemistry of reactions.
- b. **Critical Parameters.** Information that is critical to the success of the experiment, supplementing or repeating comments in the protocols or annotations.
- c. **Troubleshooting.** Discussion of problems that may be encountered in the procedure (including variations from anticipated results) with suggested remedies. Sometimes itemized in a 3-column table of Problem, Possible Cause, and Solution.

Critical Parameters and Troubleshooting are among the most popular features of Current Protocols. Remember, the commentary is being pitched to investigators who have never performed the technique.

- d. **Anticipated Results.** A discussion of the yield or other results that can be regularly achieved with this protocol, and/or the range of yields that might result from different applications, experimental conditions, or other departures from the listed protocol.

- e. Time Considerations.** Summary of the time frame for completing the full protocol (may be divided into steps for lengthy or complex procedures), again with a range for predictable departures from the technique. Discuss hands-on time as well as total time including incubation. Where appropriate, discuss number of samples that can be processed by an experienced investigator in an appropriate amount of time (e.g., "With practice, three 96-well plates can be assayed and scored in one day."). Also, if pertinent, mention convenient stopping points or steps that can be lengthened or abbreviated.

10. Literature Cited. Full references to any literature cited in the unit. References in this section should be listed alphabetically according to the following style:

a. Journal article

Baker, R.H. Jr., Suebsaeng, L., Rooney, W., Alecrim, C.C., Dourado, H.V., and Wirth, D.F. 1986. Specific DNA probe for the diagnosis of *P. falciparum* malaria. *Science* 231:1434-1436.

b. Book

Hartmann, R.K., Binderelf, A., Schon, A. and Westhof, E. 2005. Handbook of RNA Biochemistry. John Wiley & Sons, Hoboken, N.J.

c. Chapter in a book

Mathews, B. 1983. Liposome-mediated delivery of DNA to plant protoplast. *In Handbook of Plant Cell Culture, Vol. 1: Techniques for propagation and breeding* (D.A. Evans, W.R. Sharp, P.V. Ammirato, and Y. Yamada, eds.) pp. 520-540. Macmillan, New York.

All references listed in this section must be cited in the unit. Entries should include the names of all authors. Citations in the text are according to the style "(Smith, 1989; Jones and Smith, 1992)" or "as described by Ausubel et al. (1991)," where "et al." is employed for references with three or more authors.

11. Key References with Annotations (Optional). One (or more) key reference may be supplied. These may, but need not necessarily, be drawn from your literature-cited list. A key reference might be a seminal journal article, an elucidating review chapter or paper, or an important book. For each one, provide a one-sentence descriptive annotation, explaining to the reader why you consider this reference to be of particular value.

12. Internet Resources with Annotations (Optional). Listing of Web sites, FTP servers, and the like that are of particular interest or utility to the researcher. For each one, provide a one-sentence descriptive annotation signaling to the reader why you consider this resource to be of particular value.

<http://www.bbri.harvard.edu/rasmb/rasmb.html>

Web site for most recent programs and discussion group on analytical ultracentrifugation.

Figures

Appropriate figures illustrate some aspect of the protocol (equipment, flow chart of steps, appearance of gradients, etc.) or expected results. Submit electronic files as supplementary information during the manuscript submission process. See the *Guidelines for Current Protocols Illustrations and Photographs* that follow for details of acceptable image file formats.

All figures must be cited in the unit and accompanied by a detailed figure legend. Figures should be referred to as Figure 1, Figure 2, etc. If previously published, cite the original source(s) and provide a Permission Request Form (see below). Contact the Developmental Editor if you have questions.

Tables

Tables should be self-explanatory and prepared on separate pages at the end of the manuscript. Include a table number, table title, and explanatory footnotes. Cite each table in the text of your manuscript. If previously published, cite the original source(s) and provide a copyright permission form (see below).

Videos/Movies

Current Protocols is now accepting videos/movies that enhance understanding of the procedures described in the protocols. Such a video would illustrate a process involved in carrying out a protocol, particularly if that process requires special skills. For an example, see the videos available at <http://www.currentprotocols.com> on the Cell Biology Home Page.

Videos acceptable for inclusion in a unit must meet certain requirements.

- Created in QuickTime or Windows Media Player format
- No larger than 10 MB
- Run time less than 60 seconds
- Be of suitable quality for web publication

Videos will be used as submitted, if acceptable. We will do no editing. Video files should be submitted with the manuscript, but separate from it.

Each video should be cited within the manuscript at the step the video illustrates. And each video should be listed at the end of the submitted manuscript (after Figure Legends) with (1) an identifying file name, (2) a title for the video, and (3) a video legend describing the content. The title and legend will be used, with the video identification, on the website to help the reader find the appropriate video.

Abbreviations, Measurements, and Mathematical Notation

Current Protocols manuals follow the guidelines of the *American Society for Microbiology Style Manual for Journals and Books* (ASM, Washington, D.C., 1991). Please define all standard abbreviations at their first usage and clearly indicate the accepted style (bold, italics, upper- or lower-case, super- or subscript) for names of organisms, genetic elements, commercial products, etc.

Copyright Permission

If any portions of your manuscript (e.g., figures, tables) have been published previously, permission for use in this publication must be obtained from the copyright owner (*usually* the publisher but occasionally the author). Use the form provided on the last page of this style guide; when you receive signed permission from the copyright owner, forward the signed form (electronic permissions accepted) to Sarah Andrus, Editorial Program Coordinator, John Wiley & Sons, Inc., 111 River Street, Hoboken NJ 07030; sandrus@wiley.com. Photocopy the blank permission form if you need more than one. Signed email permissions from the publisher are acceptable.

Many publishers now grant permission through online services such as RightsLink. It is essential that all permission be granted for *both* print and electronic formats.

Wiley-Blackwell Policy Regarding the NIH Mandate

Current Protocols will support our authors by posting the accepted version of articles by NIH grant-holders to PubMed Central upon acceptance by Current Protocols. The accepted version is the version that incorporates all amendments made during peer review, but prior to the publisher's copy-editing and typesetting. The accepted version will be made publicly available 12 months after publication. The NIH mandate applies to all articles based upon research that has been wholly or partially funded by the NIH and that are accepted for publication on or after April 7, 2008. Please see www.wiley.com/go/nihmandate for details. The foregoing applies to NIH grantees, not NIH employees. For *NIH employees only*, we will accept the NIH Publishing Agreement.

To facilitate this process, please indicate on your Copyright Transfer Agreement whether the article you are writing for Current Protocols is based on research that has been wholly or partially funded by the NIH. You should also include funding acknowledgements in your manuscript.

Editorial License

The editorial board and John Wiley & Sons, Inc. maintain the right to rewrite, rearrange, or otherwise alter your contribution so that it will conform to the style of the manual. Should your editor desire to make changes of substance regarding content or approach, you will be consulted first or possibly asked to provide revisions. You will also be sent page proofs for approval.

Please do not hesitate to contact the Developmental Editor or our offices at any time. We would appreciate any suggestions you might offer.

Current Protocols Art Guidelines for Authors

GENERAL REQUIREMENTS

- ❑ Publication quality digital files must be provided for all figures.
- ❑ The font used for all labeling in figures should be Helvetica (or a similar sans serif font). All graphs should have axis labels.
- ❑ If the figure requires a key (e.g., “◆ morphine, □ dexamethasone, ● nimesulide”), the key should be part of the figure (not the figure caption).
- ❑ Panel identifiers should be bold Helvetica capital letters (**A, B, C**, etc.) and should appear in the upper left-hand corner of each panel.

DIGITAL FILES

- ❑ **Preferred formats:** Digital files should be in EPS or TIF format. TIF format with a resolution of 266-300 dpi produces the best results for halftone images; EPS format produces the best results for line art and graphs.
- ❑ **Other acceptable formats:** If you are unable to supply files in a preferred format, we may be able to use files in other formats (e.g., JPG, PhotoShop, Illustrator, and ChemDraw) . Please be sure that the files are of print publication quality and to provide us with information about the file format and software version used to create the image.
- ❑ **Screenshots** should be JPG, GIF, or TIF files saved at screen resolution (i.e., 72-96 dpi).
- ❑ **PowerPoint:** If you have created a graph or flowchart in PowerPoint, submit the images as PowerPoint files. However, images created with other software (e.g., Illustrator) should be submitted as TIF, EPS, or the original application format. Importing those images into PowerPoint will significantly reduce their print quality.
- ❑ **PDF and Microsoft Word:** Figures converted to PDF or imported to Microsoft Word will usually produce very poor results and sometimes be unusable by production. These formats can be useful during manuscript review, but for final submission figures should be in one of the formats listed above.

COLOR FIGURES

- ❑ All Current Protocols titles are printed in black-and-white; however, figures will appear in color on CP Online.
- ❑ Wherever possible, use shades of gray or patterns, not color, to distinguish features of your figures for readers using the print version of CP.
- ❑ If color is essential to the meaning of the figure, we will (at the editors' discretion) host a color version of the figure on *www.currentprotocols.com* and include a note advising print readers to view the color image online.

PERMISSION REQUEST FORM

To:

Date:

I am preparing a manuscript to be published in the following Current Protocols publication, which is published by John Wiley & Sons, Inc.:

Author/Title: _____

Chapter, Unit, Figure/Table No. _____

Publication Date _____ Approximate number of pages: _____

I request the non-exclusive right to include the following material in this and all subsequent editions of the publication, in all languages, and in all media of expression known or later developed, and in any derivative works published or prepared by John Wiley & Sons, Inc., or its licensees, and in versions made by non-profit organizations for use by blind or physically handicapped persons, for distribution throughout the world.

Authors(s) and/or editor(s) _____

Title of publication _____

Title of selection _____ Copyright date _____

From page _____, line _____, beginning with the words _____

To page _____, line _____, ending with the words _____

Figure No. _____ on page _____ Table No. _____ on page _____

(If necessary, attach continuation sheets)

Please indicate agreement by signing and returning the enclosed copy of this letter. In signing, you warrant that you are the sole owner of the rights granted and that your material does not infringe upon the copyright or other rights of anyone. If you do not control these rights, I would appreciate your letting me know to whom I should apply.

Thank you,

Requester's name

Requester's return address

AGREED TO AND ACCEPTED BY: _____

Name of copyright owner

Authorized signature

Title

Date

Credit and/or copyright notice: _____