



Instructions for Authors

GENERAL INFORMATION

Your chapter may include a Topic Introduction and/or Protocols, as appropriate for your topic. The entire chapter should not exceed 6000 words (excluding references), and each section (i.e., each Topic Introduction and Protocol) should not exceed 1500 words (see below). Because your contribution will also be published online in *Cold Spring Harbor Protocols*, each section must ‘stand alone’ as described below. The text of the entire chapter should be in one Word file, with the sections in the following order:

- **Topic Introduction**, with its own abstract, references, figures, tables, etc. (see below)
- **Protocol(s)**, each with its own abstract, references, figures, tables, etc. (see below)

Please submit your manuscript online by logging into <http://submit.cshprotocols.org>, entering your “Author Area,” and clicking on “Accepted Commissions—Papers to be Submitted.” Once you’ve uploaded your manuscript, we will send it to an Editor, who will contact you within 4–6 weeks to request any revisions that may be needed. After you have made appropriate changes, you will upload your revised manuscript on the same site. At this stage, your manuscript will be developmentally edited by a one of our professional editors to ensure that it follows our house style and format, and you may be contacted if any clarifications/additions to your text are required. You will have a final opportunity to check over your contribution once you receive your page proofs.

TOPIC INTRODUCTION

The Topic Introduction should not exceed 1500 words and include no more than 2 figures and tables. It should provide background information on the topic. You may compare different experimental approaches, outline available resources, describe key features and considerations of the methods, discuss applications for the technique(s) presented in the detailed protocols later in the chapter, and so forth. The introductory text should be ‘stand alone’ (i.e., with its own title, list of references, etc.) and include the following:

- Title page
 - Title (this will be the chapter title as well as the title of the Topic Introduction)

- Short title (50 characters and spaces) for running head
- List of authors and their affiliations (departments, institutions, city, state/country, zip code)
- Corresponding author’s telephone number and e-mail address
- Abstract (less than 250 words)
- Main text, including subheadings
- Acknowledgments
- References (see below)
- Figure legends (see below)
- Movie legends (see below)
- Tables

PROTOCOL(S)

Each protocol should not exceed 1500 words (excluding references) and include no more than 1 figure and table. If you are submitting more than one protocol, please make sure that each protocol stands alone (i.e., has its own abstract, references, figures, tables, etc.). The text should be structured as follows:

- Title page (REQUIRED)
 - Title of protocol
 - Short title (50 characters and spaces) for running head
 - List of authors and their affiliations (departments, institutions, city, state/country, zip code)
 - Corresponding author’s telephone number and e-mail address

- Abstract (less than 250 words; REQUIRED)
- Materials (REQUIRED)

This section has two subheads, as follows:

- Reagents (list all reagents and solutions alphabetically; write out detailed recipes for solutions that contain multiple ingredients [see below] and identify these items by using the symbol <R> after the pertinent item in the Reagents list; include the vendor and catalog number only if an item is unusual or difficult to locate, or if the use of a specific item is critical to the success of the procedure)
- Equipment (list all equipment alphabetically; it is not necessary to list common items such as micropipettes or gloves)

- **Method (REQUIRED)**
Write, in linear and numbered sequence, the steps needed to perform the procedure. If desired, divide the procedure into subsections, using subheadings that correspond to major stages of the procedure. Include supplementary information and alternative/optional methods as notes. The text of each note should be italicized and inserted below the step to which it refers. Details should be provided, as follows:
 - Always include working temperatures (e.g., cold room, on ice, room temperature).
 - When the precise timing of any portion of the procedure is required, indicate this clearly. If appropriate, indicate the approximate amount of time a given step or steps should require.
 - For centrifugation steps, specify time, temperature, and speed (rpm or g). (Specify type of centrifuge if providing instructions in rpm.) Always state whether to discard/keep the supernatant/pellet.
 - For incubations, specify time, temperature, type of incubator, and shaking (if necessary) in or on what.
 - For washes, specify conditions (e.g., temperature, washing solution and volume, specific number of washes, etc.).
 - If the procedure can be interrupted at a step, say so, and include appropriate storage conditions.
 - At the end of the procedure, specify where any products are to be used (e.g., cite another procedure) and/or give long- or short-term storage conditions.
- **Troubleshooting (OPTIONAL; only include this information if available/appropriate)**
Each troubleshooting point should be divided into two parts:
 - Problem (describe the problem and include the step number at which the problem is detected)
 - Solution (describe potential causes of the problem [if known] and strategies for fixing them)
- **Discussion (OPTIONAL; include as appropriate)**
Discuss advantages, limitations, special considerations, modifications, and applications of the technique. Include examples (with figures and movies, as appropriate). Acknowledge when the procedure is derived from previous publications.
- **Acknowledgments (OPTIONAL)**
- **References (REQUIRED; see below)**
- **Figure legends (OPTIONAL; see below)**
- **Movie legends (OPTIONAL; see below)**
- **Tables (OPTIONAL)**
- **Recipes (OPTIONAL; include as appropriate)**

Write a detailed recipe for each solution or buffer in the Reagents list (see above) that contains multiple ingredients. When writing the recipes, consider the following:

- Specify whether solution must be prepared fresh. Include storage conditions (temperature and shelf life) as appropriate.
- Specify which salt (e.g., phosphates, EDTA, etc.) and hydration states should be used.
- Specify the final pH of the solution when appropriate.
- If autoclaving is necessary, specify time and psi.
- If H₂O is required, specify distilled, double distilled, sterile, etc.
- For aliquots, specify the size and number needed.
- For dilutions, specify number, degree of dilution, and diluent.
- Include the vendor and catalog number only if a reagent is unusual or difficult to locate, or if the use of a specific reagent is critical to the success of the procedure.
- Note that *Cold Spring Harbor Protocols* draws from a common database of recipe information. Because of this, the exact phrasing and names of recipes and reagents may be altered in the developmental editing process to better match items already in the database.

REFERENCES

If you are using EndNote, please select the *Genes and Development* style. Otherwise, to cite references in the text, use the last name(s) of the author(s) and the year (use first author's last name and "et al." for more than two authors). If more than one paper is cited, place in chronological order (e.g., Smith 1994; Brown 1995; Jones et al. 1996). For citations of unpublished work or personal communication, provide names and first initials—for example, (P. Jones, unpubl.) or (P. Jones, pers. comm.). These will not be listed in the reference list, which is to contain only published work. All references in the reference list should be arranged alphabetically by the last name of the first author. Do not use a numbered reference list or cite by number in the text. See Sample Reference List below. Note that all authors should be listed up to 10; if there are more than 10 authors, use "et al." after the 10th name (see Proudfoot et al. 1993b below).

Sample Reference List

- Ahnn J, Fire A. 1994. A screen for genetic loci required for body-wall muscle development during embryogenesis in *Caenorhabditis elegans*. *Genetics* **137**: 483–498.
- Ahringer J. 1991. "Post-transcriptional regulation of fem-3, a sex-determining gene of *C. elegans*." PhD thesis, University of Wisconsin, Madison.
- Barstead RJ, Waterston RH. 1991a. Vinculin is essential for muscle function in the nematode. *J Cell Biol* **114**: 715–724.
- Barstead RJ, Waterston RH. 1991b. Cloning, sequencing, and mapping of an a-actinin gene from the nematode *Caenorhabditis elegans*. *Cell Mobil Cytoskel* **20**: 69–78.
- Chalfie M Jr, Thomson JN, Sulston JE. 1983. Induction of neuronal

- branching in *C. elegans*. *Science* **221**: 61–63.
- Fischmann DA, Vaughn K, Weber F, Einheber S. 1991. Myosin binding proteins: Intracellular members of the immunoglobulin super family. In *Frontiers of muscle research: Muscle contraction and muscle dystrophy*, 2nd ed. (ed. E Ozawa et al.), pp. 211–222. Elsevier, Amsterdam.
- Gerhart J. 1980. Mechanisms regulating formation in the amphibian egg and early embryo. In *Biological regulation and development* (ed. RF Goldberger), Vol. 1, pp. 133–316. Plenum, New York.
- Johnson J. 2000. The mouse. In *Proceedings of the Tenth Annual Meeting of Mouse Genetics*, p. 83. Jackson Laboratory, Bar Harbor, ME.
- Komissarova N, Kashlev M. 2011. Arrest of transcription: *Escherichia coli* RNA polymerase translocates backward, leaving the 3' end of the RNA intact and extruded. *Proc Natl Acad Sci* (in press).
- Levesque M. 2007. A microRNA feedback circuit in midbrain dopamine neurons. *PLoS ONE* **2**: e1227. doi: 10.1371/journal.pone.0001227.
- Proudfoot L, Kusel JR, Smith HV, Kennedy MW. 1993a. External stimuli and intracellular signalling in the modification of the nematode surface during transmission to the mammalian host environment. *Parasitology* **107**: 556–559.
- Proudfoot LJ, Kusel JR III, Smith HV, Hanett W, Worms MJ, Kennedy MW, Johnson AB, Thomas E, Lin X, Corbett K, et al. 1993b. Rapid changes in the surface of parasitic nematodes during transition from pre- to post-parasitic forms. *Parasitology* **107**: 107–117.
- Sive HL, Grainger M, Harland RM. 2000. *Early development of Xenopus laevis: A laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

LEGENDS FOR FIGURES, MOVIES, AND TABLES

Legends should start with a short title that describes what is shown. The remainder of the legend should be as brief as possible and should not contain experimental detail. Parts of figures should be labeled with capital letters (e.g., A, B, and C). Please indicate clearly if the figure, table, or movie was taken from elsewhere (e.g., reprinted or modified) and obtain permission to republish (see below).

FIGURES

1. We can only accept your electronic art as an **EPS** or **TIFF** file. Virtually all common artwork programs are capable of saving files in these formats. These options can normally be found under the ‘Save As...’ or ‘Export...’ commands in the ‘File’ menu. (See *specific instructions below on saving your files in the most common programs*.)
2. All images should be saved in CMYK (color), grayscale (halftones), or bitmap (black and white line art) mode. Please note that images supplied in color will sometimes appear in black and white in print due to the additional costs involved in color printing. Please do a test to make sure your color images will also produce nicely in black and white.
3. Image resolution is **critical** to the clarity and sharpness of the final printed piece. Image resolution should be set at 300 DPI for color and grayscale artwork. All line art (bitmap images) should be 1200 DPI. When supplying images please make sure the files are at the correct resolution. Any figures that do not meet the above requirement are subject to being returned for a better quality image.
4. Use Helvetica postscript font in point sizes no smaller than 6 pt type and no larger than 10 pt type. Optimum

point size for type in artwork is 8 pt. Please make sure all fonts are embedded in the image. *CSHL Press strives to make all artwork look consistent online and in printed form. In order to achieve this consistency, it is important to use the Helvetica postscript font as well as follow the directions below for creating your artwork (see Creating Your Artwork).*

5. If your figure has multiple parts (more than one image in the figure), please make sure the part labels (A, B, C, D) are 9 pt Helvetica bold, cap.
6. Authors may mail a print of their artwork if they are concerned that elements might not convert accurately during production.
7. Please make sure that your files are properly labeled by figure number and format (e.g., Fig_1.tif or Fig_2.eps).
8. Upload only final art files that have been carefully proofread. Do not embed figure legends in the art files. All figure legends should be placed at the end of the manuscript. **DO NOT EMBED FIGURES WITHIN THE TEXT FILES.** For example, figures should not be embedded in a Word file. The figures should be separate files.

Saving Files in Illustrator and Photoshop

Adobe Illustrator

1. Under the File menu, choose save as, format (Illustrator EPS).
2. Click save and a window comes up.
3. Save as “*Illustrator EPS*.”
4. Under options, click include document fonts, click include document thumbnails.
5. Preview should be checked as 8-bit Macintosh.
6. Click okay.
7. In case we have trouble with your file, please download all fonts on the disk that were used in creating your artwork.

The preview box is very important. Make sure it is checked off as 8-bit Macintosh.

If your artwork is in 4-color, please make sure you create the artwork using CMYK (not RGB) colors.

Adobe Photoshop

1. Under the File menu, choose save as, format (TIFF).
2. Click save.
3. Under TIFF options check Macintosh; **do not check LZW compression.**
4. Click okay.

If your artwork is in 4-color, please make sure you create the artwork using CMYK colors (not RGB or Pantone colors). Please make sure the resolution is at 300 dpi.

Creating Your Artwork

1. Please submit your illustrations as Adobe Illustrator EPS files.
2. **Please create your artwork at exact size.** Artwork should not be larger than the measurements noted below.
 - 8 ½ × 10 7/8" trim size, single column layout: no larger than 5 3/4" wide and 8" long. Can go up to 7" wide if necessary but we prefer the artwork to stay in column width. Please try, whenever possible, to keep the figure size to 1/2 or 1/3 of the page size.
3. All labels should be set in Helvetica postscript font. Optimum point size for labels should be 8 pt. Type sizes can vary but should range no smaller than 6 pt type and no larger than 10 pt type unless you feel it is necessary to the meaning of the artwork. Labels should be in sentence case.
4. Part labels (A, B, C, D) should be 9 pt. Helvetica bold, cap.
5. **All fonts should be embedded in the file.**
6. Line widths (as well as dotted lines and lines with arrows) should be .5 pt to .75 as the basic line weight for drawing, with heavier or lighter line weights for emphasis.
7. If you are bringing TIFF images into Illustrator, make sure "include placed files" is checked in the options box.
8. When creating tints (shades of black or color), please make sure you do not go above 15% when type is placed over the tinted area (unless the text is knocked out to white). All color artwork should be created in cmyk mode.
9. Color choices should be harmonious throughout. There should be consistency of color choice, tone, density, and hue for each article. Avoid the use of "neon" colors. No color panels should be used for backgrounds. Shading and highlighting should be subtle.
10. Overprint black ink to aid in registration. Because black ink is opaque (and usually the last to be printed), it doesn't look much different when printed over a color as opposed to a white background. Overprinting black can prevent gaps from appearing between black and colored areas of your artwork. Under the help menu in Illustrator, directions for implementing overprinting are clearly explained.

MOVIES

Preferred movie formats are QuickTime (.mov) and Flash (.flv or f4v). Files should have a maximum width of 450 pixels and a maximum height of 400 pixels, but the appropriate dimensions depend on the proportions of the image. Movies should be cited in the text where appropriate.

TABLES

Please supply all tables in Word format (not as figures/images). Small tables that are critical to the Method section of a protocol (e.g., steps of a PCR program) should be included within the Method steps themselves (see above) and need not be numbered/cited. All other tables should be numbered and cited in the text as appropriate.

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Conflicts of Interest Upon acceptance, each author is required to declare any conflict of interest or competing financial interest that may be perceived as contributing to potential bias. Examples include employment by or stocks or shares in, one or more organizations that may be financially affected by the publication of a paper, as well as patents or patent applications whose value may be affected by the publication of the paper.

Protection of Human Subjects and Animals in Research Where data from experiments involving animals or human subjects are shown, authors must demonstrate that these were in accordance with the ethical standards of the relevant institutional and national committees for such matters.

Informed Consent Where identifying information, such as patients' names, initials, or hospital numbers, is shown, authors must demonstrate that the patient (or parent or guardian) has given written informed consent for publication and been shown the manuscript prior to publication.

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