nature protocols
Recipes for Researchers

Guide to authors

Protocols for publication in *Nature Protocols* are predominantly commissioned by the *Nature Protocols* Editors. They are peer reviewed, fully edited and styled prior to publication. These protocols contain followed by separate sections comprising a brief introduction, materials, procedure (with critical steps highlighted), timing information, troubleshooting, anticipated results and references. Figures and diagrams are included, where appropriate. Our detailed 'Guide to authors' is below. If you would like to propose a protocol for consideration by the Editors please submit a presubmission enquiry using our Manuscript Tracking System. If you have any problems using this system please contact us. Please do contact the Editorial Office (protocols@nature.com) if anything is unclear.

Manuscript Tracking System

Please use our Manuscript Tracking System to submit your protocol. If you have been invited to submit a protocol, specific links for submitting your protocol are supplied in e-mails from the Editors.

General submission guidelines

Nature Protocols is a forum for the publication of proven protocols. Thus we do not publish novel primary research and the authors of the protocol must have previously used their method to produce results reported in a peer-reviewed primary journal. Nevertheless, we appreciate that protocols are constantly evolving, thus if the methodology has been improved on since the original publication, we invite our authors to notify us of the modifications that they have made and provide us with the latest version of the protocol, as this will be most useful to our readers.

Unless we are notified otherwise in a cover letter on submission, submission to *Nature Protocols* is taken to imply that there is no significant overlap between the submitted protocol and any other protocols from the same authors under consideration or in press elsewhere. We require that authors have previously published their protocols, however please ensure that there is no direct repetition of text between this protocol and previous publications of the method, as this would constitute self-plagiarism. Further information about NPGs policy regarding plagiarism can be found at http://www.nature.com/authors/editorial_policies/plagiarism.html.

Submission is also taken to imply that all coauthors have approved the contents of the protocol and its submission by the corresponding author, and that the corresponding author is authorized to represent all coauthors in pre-publication discussions with the journal. (The corresponding author for editorial purposes need not be the senior author, nor the person to whom correspondence is addressed after publication.) The primary affiliation for each author should be the institution where the majority of their work was done. If an author has subsequently moved, the current address may also be stated.

A high priority of *Nature Protocols* is that all protocols be accessible to nonspecialists. The commissioned protocols are subject to substantial editing to achieve this goal. After acceptance, a copy editor will make further changes to ensure that the text and figures are readable and clear to those outside the field and that papers conform to our style. Contributors are sent proofs and are welcome to discuss proposed changes with the editors, but *Nature Protocols* reserves the right to make the final decision about matters of style and the size of figures. Commissioned protocols will undergo peer review, thus commissioning does not guarantee publication. The editors also reserve the right to retract a protocol, even after it has been accepted, if it becomes apparent that there are serious problems with the scientific content or with violations of our publishing policies.

The Nature journals, including *Nature Protocols*, share a number of common policies. Further details of the standard policies can be found here.

Protocol format

We recommend that the authors of commissioned protocols follow these guidelines wherever possible to facilitate the refereeing and editorial process. The Editorial Office welcomes feedback regarding the practicality of these guidelines and the format in general, as we are keen for *Nature Protocols* to be as useful as possible to researchers.

As we use Microsoft Word in the Editorial office, this is our preferred format for text; however, we can also accept plain ASCII text (.txt) and RTF format (.rtf).

Title. The title should succinctly describe the method and, if appropriate, its application. It should be no longer than 30 words.

Authors. Include those who have contributed intellectually and practically to the development of the technique. For all authors please include first name, middle initial (if appropriate), last names, postal address (Department, Institution, City and Country) and e-mail address. Also include the telephone number and fax number for the corresponding author. A link to a lab home page or staff information page may also be included for appropriate authors.

Abstract/summary. Include a summary of the protocol (maximum 150 words), briefly describing the protocol and its applications. The suggested format is to include one or two sentences summarizing the protocol; then to explain more specifically the stages of the protocol, and if appropriate, how these compare with other protocols; and then to include one or two sentences describing the results that can be expected. If possible, include a final sentence indicating how long the whole protocol takes. We would prefer that the abstract contained information about the procedure rather than a summary of the results that have been obtained previously using the method.

Introduction. (use heading 'INTRODUCTION') The purpose of the introduction is to enable readers to make a decision as to the suitability of the protocol to their experimental problem. Initially you should introduce the technique under discussion. Include references to key papers where the protocol has been used previously, including those published by your own group or to reviews that discuss applications of the protocol (see below for how to cite references). You are actively encouraged, where appropriate, to reference other protocols in *Nature Protocols*. Use subheadings where appropriate; these could include:

- Development of the protocol: Include references to your own peer-reviewed primary research publications.
- Applications of the method: If you think that the protocol could be adapted for use in a wider range of applications than presented you should discuss the full diversity of the applications of the method.
- Comparison with other methods: Where applicable, reference should be made to alternative methods that are commonly used to achieve the same result as the protocol. The advantages and disadvantages of your protocol compared to other alternatives should be discussed.
- Experimental design: Because our style does not allow for additional information outside of a numbered step in the procedure, it is often useful to include a subsection entitled 'Experimental design' where procedure-specific information can be placed. In this section, please provide all information about the design of the experiments that readers would need to be able to adapt the protocol to their own experimental situation. This section should also include a discussion of the controls necessary for the protocol. For protocols describing the preparation of organic molecules, a scheme showing the sequence of reactions should be included (all schemes should be labeled as figures).
- Limitations. The possible limitations of the protocol should be discussed. It should be made clear in which situations the Protocol has been successfully employed, in which situations it is reasonable to expect the Protocol to function and in which situations the Protocol would be unreliable or otherwise unsuccessful.

Materials. (use heading 'MATERIALS') REAGENTS, EQUIPMENT, REAGENT SETUP and EQUIPMENT SETUP are the only subheadings that are allowed. You must include at least one of these headings. Within REAGENT SETUP and EQUIPMENT SETUP the title of the reagent or equipment being discussed forms a second level of subheading.

Include a list of the essential materials, split into reagents (use heading 'REAGENTS') and equipment (use heading 'EQUIPMENT'). This should include information about the suppliers used for reagents (e.g., Company, web address and catalog number). If you have found that deviations from a particular reagent, or its source, have adverse effects on the outcome of the protocol this should be made clear by the word 'CRITICAL' followed by a brief explanation. Toxic or harmful agents should be made clear by the word 'CAUTION' followed by a brief explanation of the hazard and the precautions that should be taken when handling the agent. Please also highlight any specialist equipment required. The information you provide about the sources of reagents and equipment will enable us to link your protocol to Nature Product Finder to help users obtain the equipment and materials they need. Please provide as much information as you can to help us successfully identify the product, e.g., company name, company website, product code. If you need to include detailed information about specific reagents or equipment, please list in additional optional sections, called 'REAGENT SETUP' and 'EQUIPMENT SETUP'. These sections are suitable for details of composition of buffers or the setup of equipment.

REAGENT SETUP is the appropriate section to include details regarding, e.g., the required sample specification (in terms of minimum protein quantity and allowed buffer components) for a mass spectrometry experiment; a way to prepare a complicated buffer; and the pre-treatment of solvents and/or reagents to make sure they are moisture-free and/or air-free. For each item listed in the REAGENT SETUP section please indicate whether it should be made up fresh or can be stored and if so under which conditions (e.g., temperature) and for how long. In addition please state whether % solutions are wt/vol or vol/vol. EQUIPMENT SETUP is the appropriate section to include details regarding, e.g., the setup of HPLC separation methods.

For protocols that use live vertebrates or higher invertebrates, authors must state that all experiments should be performed in accordance with relevant guidelines and regulations. For manuscripts reporting experiments on human subjects, authors must also include a statement confirming that informed consent must be obtained from all subjects. These statements should appear as CAUTIONS. These must be placed in the reagents section, procedure and in the legend of any tables and figures that show data collected using human or animal subjects. Referees may be asked to comment specifically on any cases in which concerns arise.

Step by step methodology. (use heading 'PROCEDURE') This is the major part of the protocol and must be a numbered list, ideally with numbers in **bold**; do not follow the numbers with a period (full stop). Use the active tense rather than the passive tense, for example, "Pipette 20 ml of buffer A into the flask", instead of "20 ml of buffer A are/were pipetted into the flask". If the protocol naturally breaks into separate stages, then include subheadings and resume the numbered list. Include a TIMING callout with each subheading and state how long the section will take to complete. Subheadings are particularly appropriate after steps in the protocol where the procedure can be stopped (pause point), i.e., when the experiment can be stopped and resumed at a later date. Any pause points should be indicated with the heading 'PAUSE POINT', followed by a brief description of the options available, for example "Can be left overnight at 4 °C or frozen for up to a month at -20 °C".

Highlight critical steps in the protocol that must be performed in a very precise manner e.g., where the time and temperature of a step is crucial or the use of RNase free solutions is required; thus providing the user with hints to maximize the likelihood of success. Make these clear with the heading 'CRITICAL STEP', followed by a brief explanation.

Highlight any toxic or harmful chemicals that are used. Make these clear by preceding them with the wording 'CAUTION' prior to their first mention and include brief details of the hazard and the appropriate handling information.

Include diagrams and/or photographs of equipment set-up, where appropriate. If the protocol is complicated you should consider including a flow diagram to demonstrate how the stages fit together. We welcome movies of particularly complicated procedures.

Where there are alternative routes to reach the next stage of the protocol, please give enough background so that the reader will be able to make an informed decision on the route to choose. Letters of the Latin alphabet (A), B), C)...) should be used to identify the different options, and Roman numerals (i), ii), etc.) should be used to break down the appropriate steps. For example:

This step can be performed using option A or option B depending on whether...

1. First option
   (i) First part
   (ii)Second part, etc.
2. Second option
   (i) First part
   (ii) Second part, etc.

Please note that these options cannot have subheadings.

Please state all centrifugation speeds in *g* and include the length of time and temperature of the centrifugation e.g., centrifuge at 14,000*g* for 5 mins, 4 °C.

Please include TROUBLESHOOTING callouts after steps where problems are encountered, that are subsequently mentioned in the Troubleshooting section.

Timing. (use heading 'TIMING') If possible, please include a timeline indicating the approximate time a step, or set of steps, will take, e.g., Steps 1–3, 30 min.; Steps 6+7, 2 h. Provide this information as a summary at the end of the procedure, as a list. If you think it would be more user friendly you could refer to time needed for each section or detail what needs to be performed on each day of the protocol.

Troubleshooting. (use heading 'TROUBLESHOOTING') Include information on how to troubleshoot the most likely problems users will encounter with the protocol. Please provide this information in the form of a table with the columns 'Step', 'problem', 'possible reason', 'solution'. The step number should be given where the problem is first observed (not where it occurred). The appropriate steps should also be flagged in the main text by adding 'TROUBLESHOOTING' callouts. If troubleshooting text refers to only one or two steps, it can also be formatted as normal text with subheadings referring to the steps or sections that the information pertains to.

Anticipated results. (use heading 'ANTICIPATED RESULTS') Include information about, or examples of, the likely outcome to users, for example, likely yield of protein, typical microscopy images, etc.

If possible, please include one set of data from an experiment that worked very well and a second for an experiment that required troubleshooting to obtain meaningful results. If not described in detail in the introduction, this is a good place to include directions on how to interpret and analyze the raw data including equations if necessary.

This is the appropriate section to include any analytical data for chemical compounds synthesized as part of the procedure. An example of how this should be formatted is shown below:

Fmoc-L-Serine-OAll (1). White solid, mp 82.5–84.0 °C
$[\alpha]_D$ = + 0.3 (c 7.5 in EtOAc at 20 °C)
TLC (CH_2Cl_2:CH_3OH 15:1 v/v) R_f = 0.14
^{1}H NMR (500 MHz, CDCl) δ 2.33 (br s, 1H), 3.94 (br d, *J* = 10.3 Hz, 1H), 4.03 (br d, *J* = 10.1 Hz, 1H), 4.23 (t, *J* = 6.9 Hz, 1H), 4.43 (m, 2H), 4.48 (m, 1H), 4.69 (d, *J* = 5.3 Hz, 2H), 5.26 (dd, *J* = 0.8 Hz, *J* = 10.5 Hz, 1H), 5.35 (dd, *J* = 0.8 Hz, *J* = 17.2 Hz, 1H), 5.79 (d, *J* = 7.4 Hz, 1H), 5.91 (ddt, *J* = 16.6 Hz, *J* = 11.0 Hz, *J* = 5.5 Hz, 1H), 7.32 (br t, *J* = 7.4 Hz, 2H), 7.41 (br t, *J* = 7.4 Hz, 2H), 7.61 (m, 2H), 7.77 (d, *J* = 7.6 Hz, 2H)
^{13}C NMR (125 MHz, $CDCl_3$) δ 47.30 (CH), 56.28 (CH), 63.48 (CH_2), 66.59 (CH_2), 67.42 (CH_2), 119.25 (CH_2), 120.21 (CH), 120.23 (CH), 125.30 (CH), 127.29 (CH), 127.32 (CH), 127.97 (CH), 131.51 (CH), 141.51 (C), 141.55 (C), 143.87 (C), 144.02 (C), 156.46 (C), 170.45 (C)
IR (CH_2Cl_2, cm^{-1}) 3425, 3064, 2952, 2892, 1725, 1513, 1198
FAB-LRMS (*m/z*)(relative intensity) 368 ($[M+1]^+$, 28 %)
Analysis (calculated, found for $C_{21}H_{21}O_5N$) C (68.65, 68.38) H (5.76, 5.59) N (3.81, 3.90)
(Taken from: 10.1038/nprot.2006.470)

Supplementary information

Each piece of supplementary information should have a title and a legend and be cited in order in the text. If any references are cited in supplementary information, an individually numbered reference list should be created for the supplementary

information and included with the supplementary information legends. References in the supplementary information should not be cited directly from main reference list. If any references are cited in both the main text and the SI, they can be repeated in the SI reference list. Please use one of our approved titles for your supplementary information.

File sizes should be as small as possible, with a maximum size of 30 MB, so that they can be downloaded quickly. The combined total size of all files must not exceed 150 MB.

Approved supplementary information file types

Common types:

- Supplementary Audio(s) (numbered)
- Supplementary Audio Legend(s) (optional, to accompany audios; posted in online Supp. Info. title page rather than as separate file)
- Supplementary Data (numbered if >1)
- Supplementary Discussion
- Supplementary Equation(s) (one file; use plural "Equations" in title if more than one equation is included in file)
- Supplementary Figure(s) (always numbered)
- Supplementary Figure Legend(s) (required, as part of figure or accompanying figure)
- Supplementary Methods
- Supplementary Note(s) (numbered if >1)
- Supplementary Table(s) (always numbered)
- Supplementary Video(s) (always numbered)
- Supplementary Video Legend(s) (optional, to accompany videos; posted in online Supp. Info. title page rather than as separate file)

Occasional types:

- Supplementary Manual
- Supplementary Results
- Supplementary Sequence Archive
- Supplementary Tutorial

Supplementary information file types must be one of the following: Adobe Acrobat file (.pdf), Audio Visual Interleave (.avi), Compressed Archive File (.zip), Encapsulated Postcript (.eps), Flash Movie (.swf), Graphics Interchange Format (.gif), HTML document (.html), JPEG image (.jpg), MPEG animation (.mpg), MS Excel spreadsheet (.xls, .xlsx), MS Power Point file (.ppt, .pptx), MS Word document (.doc, .docx), Plain ASCII text (.txt), PostScript (.ps), QuickTime movie (.mov), Rich Text Format (.rtf), Systems Biology Markup Language (.sbml, .xml, .owl), TAR archive file (.tar), TIFF image (.tif), Waveform audio file (.wav), WordPerfect document (.wpd).

Author contributions statements

Authors are required to include a statement of responsibility in the manuscript that specifies the contribution of every author. The level of detail varies; some disciplines produce manuscripts that comprise discrete efforts readily articulated in detail, whereas other fields operate as group efforts at all stages. Examples of published "author contributions" statements can be seen at this Nautilus post (http://blogs.nature.com/nautilus/2007/11/post_12.html). Nature journals also allow two coauthors to be specified as having contributed equally to the work being described (most often used for co-first authors), but prefer authors to use the "author contributions" style for reader clarity.

Acknowledgments

Please note an acknowledgments section can be included.

Competing financial interests

The published protocol will indicate if the authors have competing financial interests. Please include one of these statements in your manuscript:

The authors declare that they have no competing financial interests.

The authors declare competing financial interests (see the HTML version of this article for details).

Submission of a signed competing financial interests statement is required for all content of the journal. This statement will be published at the end of all papers, whether or not a competing financial interest is reported. In cases where the authors declare a competing financial interest, a short statement to that effect is published at the end of article, which is linked to a more detailed version available online.

References

List all references mentioned in the protocol. References are numbered sequentially as they appear in the text, figure legends, tables and boxes. Use superscript numbers to indicate a reference, for example [1]. Only one publication is given for each number, and footnotes are not used. Only papers that have been published or accepted by a named publication should be in the numbered list; meeting abstracts and papers in preparation should be mentioned in the text with a list of authors (or initials if any of the authors are co-authors of the present contribution). Patents should be included in the reference list. Published conference abstracts and URLs for web sites should be cited parenthetically in the text, not in the reference list; articles in formal, peer-reviewed online journals should be included in the reference list. Grant details and acknowledgments are not permitted as numbered references.

All authors should be included in reference lists unless there are more than five, in which case only the first author should be given, followed by '*et al.*'. Authors should be listed last name first, followed by a comma and initials of given names. Titles of cited articles are required and should be in Roman text and titles of books *in italics*; the first word of the title is capitalized, the title written exactly as it appears in the work cited, ending with a period. Journal names are *italicized* and abbreviated (with periods) according to common usage; refer to the National Library of Medicine for details. Volume numbers appear in **bold**. For book citations, the publisher and city of publication are required (e.g., John Wiley & Sons, Hoboken, New Jersey, USA, 2003).

List all references mentioned in the protocol. Use the format given in the examples below:

- Helms, C. *et al.* A putative RUNX1 binding site variant between *SLC9A3R1* and *RAT9* is associated with susceptibility to psoriasis. *Nat. Genet.* **35**, 349–356 (2003).
- Lovett, M. Direct selection of cDNAs with large genomic DNA clones. In *Molecular Cloning: A Laboratory Manual* Edn. 3 Vol. **2** (eds. Sambrook., J. & Russell, D.W.) 11.98-11.133 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA, 2001).
- Petroff, M.D. & Stapelbroek, M.G. Blocked impurity band detectors. US Patent 4,586,960 filed 23 Oct. 1980, and issued 4 Feb. 1986.

Statistical Guidelines

Every article that contains statistical testing should state the name of the statistical test, the n for each statistical analysis, the comparisons of interest, a justification for the use of that test (including, for example, a discussion of the normality of the data when the test is appropriate only for normal data), the alpha level for all tests, whether the tests were one-tailed or two-tailed, and the actual *P* value for each test (not merely 'significant' or '$P < 0.05$'). Randomization procedures, or other ways to eliminate bias in sampling (in particular for experiments involving animals), should be clearly described. It should be clear what statistical test was used to generate every *P* value.

Data sets should be summarized with descriptive statistics, which should include the n for each data set, a clearly labeled measure of center (such as the mean or the median), and a clearly labeled measure of variability (such as standard deviation or range). Ranges are more appropriate than standard deviations or standard errors for small data sets. Graphs should include clearly labeled error bars. Authors must state whether a number that follows the ± sign is a standard error (s.e.m.) or a standard deviation (s.d.).

Authors must justify the use of a particular test and explain whether their data conform to the assumptions of the tests. Three errors are particularly common:

- Multiple comparisons: when making multiple statistical comparisons on a single data set, authors should explain how they adjusted the alpha level to avoid an inflated Type I error rate, or they should select statistical tests appropriate for multiple groups (such as ANOVA rather than a series of *t*-tests).
- Normal distribution: many statistical tests require that the data be approximately normally distributed; when using these tests, authors should explain how they tested their data for normality. If the data do not meet the assumptions of the test, then a non-parametric alternative should be used instead.
- Small sample size: when the sample size is small (less than about 10), authors should use tests appropriate to small samples or justify their use of large-sample tests.

There is a checklist available to help authors minimize the chance of statistical errors.

Nomenclature

Authors should use approved nomenclature for gene symbols. The full name should be provided at first mention, the gene symbol in brackets, i.e., *titin* (*Ttn*). Thereafter, the gene symbol should be used. Please consult the appropriate nomenclature databases for correct gene names, symbols and formatting. A useful resource is Entrez Gene. Approved human gene symbols are provided by HUGO Gene Nomenclature Committee (HGNC), e-mail: hgnc@genenames.org. Approved mouse symbols are provided by The Jackson Laboratory, e-mail: nomen@informatics.jax.org. Another useful site is the Gene Ontology Project.

Avoid listing multiple names of genes (or proteins) separated by a slash, as in '*Oct4/Pou5f1*', as this is ambiguous (it could mean a ratio, a complex, alternative names or different subunits). Use one name throughout and include the other at first mention: '*Pou5f1* (also known as *Oct4*)'.

Guides for digital images

Please read the digital images integrity and standards policy before preparing your figures. When possible, we prefer to use original digital figures to ensure the highest quality reproduction in the journal. When creating and submitting digital files, please follow the guidelines below.

Submission of Figures, Tables, and other additional protocol components

Figures, tables, boxes and supplementary information must be cited in the text and numbered in the order in which they are cited.

Tables. Please submit tables in Word format at the end of your text document, or as a separate file. Tables should be created using the Microsoft Word table editor, where possible.

Chemical structure display items. Figures that contain chemical structures should be produced using ChemDraw or a similar program. Authors using ChemDraw should use the preferences below, submitting the final files at 100% as .cdx and .eps files. For more information, please also review the *Nature Chemical Biology* Chemical Style Guide.

- Drawing settings: chain angle, 120° bond spacing, 18% of width; fixed length, 14.4 pt; bold width, 2.0 pt; line width, 0.6 pt; margin width 1.6 pt; hash spacing 2.5 pt.
- Atom Label settings: font, Arial; size, 8 pt. "Show labels on Terminal Carbons" and "Hide Implicit Hydrogens" should be unchecked.

Figure format for initial submission. All figures should be uploaded separately via our online submission system, in one of our preferred formats [see below]. On initial submission all panels of a figure or table (e.g., Fig. 1a, b and c) should be combined into one file; please do not send as separate files. Please include a brief title and legend for every electronic figure submitted, and a title for every table. Please ensure that both axes of all graphs are labeled appropriately.

When a paper is accepted, we might request high-resolution files suitable for publication. Thus, please bear in mind our guidelines for the figures for accepted manuscripts [see below] when preparing your figures, so that it is easier to provide publication-standard figures when they are required.

Figure appearance and layout. Whilst *Nature Protocols* is an online product, many users will print your protocol prior to use. Thus figures should be sized to be legible to users and to facilitate printing. Unnecessary figures and parts (panels) of figures and tables should be avoided. Figures should not contain more than one panel unless the parts are logically connected; each panel of a multipart figure should be sized so that the whole figure can be reduced by the same amount and reproduced on the printed page at the smallest size at which essential details are visible. When a protocol is accepted for publication, we might ask for high-resolution figure files, possibly in a different electronic format. This information will be included in the acceptance letter.

Lettering on figures should be in a clear, sans-serif typeface (for example, Helvetica); if possible, the same typeface and approximate font size (no smaller than 7 point) should be used for all figures in a paper. Use symbol font for Greek letters. Figures should be on a white background, and should avoid excessive boxing, unnecessary color, spurious decorative effects (such as three-dimensional 'skyscraper' histograms) and highly pixelated computer drawings. The vertical axis of histograms should not be truncated to exaggerate small differences. Labeling must be of sufficient size and contrast to be readable after appropriate size reduction. The thinnest lines in the final figure should be no smaller than 0.5 point wide. Authors will see a proof of figures. Reasonable requests to enlarge figures will be considered, but editors will make the final decision on figure size.

Figures divided into parts should be labeled with a lower-case, bold a, b, and so on, in the same typesize as used elsewhere in the figure. Lettering in figures should be in lower-case type, with only the first letter of each label capitalized. Units should have a single space between the number and the unit, and follow SI nomenclature (for example, ms rather than msec) or the nomenclature common to a particular field. Thousands should be separated by commas (1,000). Unusual units or abbreviations should be spelled out in full or defined in the legend. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. In general, please use visual cues rather than verbal explanations, such as "open red triangles", in the legend.

Figure format

Image types. Images fall into two basic categories: photographic or scanned images, and graphs and schematic diagrams. Rasterized formats are best for photographs, scans, and composite figures containing photographic or scanned images with minimal or relatively simple labeling, whereas line or vector formats are best for graphs and schematic diagrams, and for composite figures containing a mixture of photographs or scans and detailed text or line artwork. The best format for any particular figure therefore depends partly on what kind of images it contains.

Electronic image formats. A wide variety of software is available to generate and manipulate images, and a huge range of graphics file formats exist. Rasterized (or bitmapped) formats such as TIFF are composed of an array of dots (pixels). The quality is determined by the resolution, usually measured in dots per inch (dpi; also referred to as 'pixels per inch'). For adequate reproduction we need a minimum of 300 dpi at the size the image is to appear. Size and resolution are linked so that, for example, enlarging an image to twice its original size will halve the resolution. If the resolution is too low, individual pixels become visible to the eye, the edges of lines begin to appear 'stepped' and the image may look blurred or pixelated.

Line (or vector) formats such as Postscript, EPS and PDF preserve individual lines and text as separate, editable components. This makes them easier and quicker to reletter or edit as necessary, reducing the chance of errors, and gives sharper results in print and online. Also, because these images have no 'resolution' as such, they may be enlarged without any reduction in quality.

Leave all layers intact in TIFF and EPS files.

Preferred formats

Adobe Photoshop (layered .psd file only) or **TIFF** format (high resolution, minimum 300 dpi) for photographic images.

Adobe Illustrator, Postscript, EPS or **PDF** format for figures containing line drawings and graphs, including figures combining text and line art with photographs or scans. If these formats are not possible, we can also accept Microsoft Word, Microsoft Excel, Microsoft PowerPoint or JPEG (high-resolution, 300 dpi, as separate files (not embedded in your text file)).

Please do **not** send figures prepared in the following formats, as we cannot use them: Canvas, Freehand, CorelDraw.

Movies and audio files. We welcome you to submit appropriate movies or audio files, if these would be useful for our users. Movies could include examples of the results obtained, or footage of researchers performing critical parts of the protocol. Please use QuickTime movie (.mov) or Flash movie (.swf) format for movies, or audio files (.wav). Movies should be less than 10 MB in size, however if your movie is larger than 10 MB please let us know and we will do our best to accommodate it.

Notes on particular formats

In **Photoshop**, please send the Photoshop file (.psd) with the layers intact. Individual components of the figure cannot be edited on flattened Photoshop files.

Although **PowerPoint** can export JPEGs, the resulting files are low resolution and not suitable for printing. The only way to obtain a high-quality graphics format from Microsoft Office applications is to generate Postscript using 'Print to file'.

JPEG is a compressed format, which achieves smaller file sizes by discarding information. As a result, saving in JPEG format may cause a noticeable reduction in quality, so we prefer Photoshop or TIFF format for rasterized images. When JPEG is the only option available, the quality should be set to the highest possible to minimize loss of information.

TIFF files are larger than JPEGs, but it is possible to reduce the file size by saving with compression (LZW compression is preferred), which does not degrade the quality of the image. Alternatively, compression software such as DropStuff or ZipIt can be used to reduce the file sizes.

How to send files

Should you have difficulties with our Manuscript Tracking System, we have an FTP site. If you wish to use this, please let us know so we can send you further details. Wherever possible, we prefer to use original digital figures to ensure the highest quality reproduction.

Other requirements

Authors are responsible for obtaining before publication permissions for tables, figures, images or movies previously published elsewhere. If your tables, figures, images or movies have been previously published, please cite their original place of publication in the legend. Citations of personal communications must be authorized by the correspondent involved, with signed permission to cite being sent to the editorial office by mail or fax. This is also relevant to figures that have been altered in any way. Please note that it is courteous to inform the author of the original material of your intent to use their published work, in addition to contacting the copyright holder.

Copyright. Authors of manuscripts accepted for publication will be sent a copyright transfer form, please contact us if you need further information at this stage.

Self-archiving. Authors are able to self-archive the author version of their accepted manuscript six months after publication. By "author's version" we are referring to Word or Text files produced by the author, not a PDF or HTML downloaded from nature.com. The article will have been developmentally edited and peer reviewed, but it will not have been copyedited into the final published version. This policy has been developed to meet the needs of authors and the evolving policies of funding agencies that may wish to archive the research they fund. It is also designed to protect the integrity of the scientific record, with the published version clearly identified as the definitive version of the article.

Abbreviation. The correct abbreviation for abstracting and indexing purposes is *Nat. Protoc.*

ISSN. The electronic international standard serial number (EISSN) for *Nature Protocols* is 1750-2799. The international standard serial number (ISSN) is 1754-2189.