

Title is a maximum of 145 characters, including spaces, and avoids the use of jargon and uncommon abbreviations

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SUMMARY

- The summary is a paragraph no longer than 80 words and written in the active voice and present tense.
- Any background information should be limited to one sentence. The summary should focus on the details of the major steps of the protocol, techniques involved, and model organisms used.
- Avoid the use of the word “method” in your summary. Acceptable substitutions are “protocol”, “technique”, “assay” or “approach”. Do NOT include references in the summary.
- Summaries should include the statement as the last sentence: For complete details on the use and execution of this protocol, please refer to X et al.¹
 - Only reference published, peer-reviewed and associated manuscripts in this statement. If you do not have an associated published manuscript, do not include this statement, and make note in your submission cover letter.
 - This statement does not count towards the 80-word limit for the Summary.

Graphical abstract

- The GA is a graphical description of the protocol that highlights the time for each major step. Click here to see examples. We strongly encourage you to submit a GA with your initial submission, but it is not required; however, it will be required if your paper is accepted.
- We have created a graphical abstract template (**if the link is not working, please copy and paste this link to your web browser to help you create a GA.
- Programs such as Biorender may also be used with licensing and citation to create the Graphical Abstract.
- Please ensure that the GA is in jpg format.

Before you begin

37

- Describe the protocol for a specific usage case (ideally what was done in your associated research paper). You may also describe alternate applications of the protocol.

– For example: “The protocol below describes the specific steps for using HeLa cells. However, we have also used this protocol in HEK293 cells and NIH3T3 cells.”
- Keep background information related to the protocol to a minimum. Introductory text should not exceed 1 page.
- Provide instructional steps for what needs to be set up or prepared before a researcher begins the protocol. Please **use numbering** (up to three levels: 1,2,3; a,b,c; i,ii,iii) to list steps. **Do not use bullet points** in this section.

– Avoid including more than 15 sub-steps for an individual step.
- **All materials recipes should be listed in the Materials and Equipment Setup section. Do not place them here.**
- For explanatory text related to the steps, please use the following callouts that can be inserted after the relevant numbered step.

– Call outs are not to contain instructional steps.

– **Optional:** [Call out optional steps in this format; optional steps are not numbered.]

– **Note:** [Please include general notes or explanations as a note. Notes are not numbered and should be near the relevant step.]

– **Pause point:** [If relevant, list potential pause points with time intervals in this format; pause points are not numbered.]

– **CRITICAL:** [when applicable, note steps that are critical for success of the protocol; critical steps are usually not numbered.]

Institutional permissions (if applicable)

60

- Any experiments on live vertebrates or higher invertebrates must be performed in accordance with relevant institutional and national guidelines and regulations.
- In the before you begin section, a statement identifying the committee approving the experiments and confirming that all experiments conform to the relevant regulatory standards must be included.

– In addition, you should provide a statement reminding readers that they will need to acquire permissions from the relevant institutions.

Preparation one

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Timing: s, min, h, days, or weeks.

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1. First step of preparation one. Use numbers to order steps.

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2. Second step of preparation one. 71
 - (a) Sub-steps under first step. Use letters for ordering of sub-steps. 72
 - (b) Sub-step two, as needed. 73
 - i. Third-level sub-steps under second-level sub-steps. Use lowercase Roman numerals for ordering. 74
 - ii. Third-level sub-step two, as needed. 76

CRITICAL: when applicable, note steps that are critical for success of the protocol. 77

Preparation two 78

1. First step of preparation two. Use numbers to order steps. 79
 - (a) Use letters for ordering of sub-steps. 80
 - (b) Sub-step two, as needed. 81
2. Second step of preparation two. 82

Key resources table 83

To create the KRT using word, please use the KRT webform or this table template. 84

Materials and equipment setup (optional) 85

- This section is for providing additional information regarding equipment setup, details about custom software used, and notes on alternative materials and equipment. This optional section is a complement to the key resources table. 86
- ALL EQUIPMENT SHOULD BE LISTED IN THE KRT UNDER “OTHER,” NOT HERE. 87
- All recipes should be in this section; these tables do not require legends or call outs and can stay in this section. 88
- Please use bullet points (up to three level) to list items. Do not use numbering (1,2,3; a,b,c; i,ii,iii) in this section. 89
- Recipes with 3 or more ingredients should be presented as tables. All solutions should include storage conditions (temperature and maximum store time). 90

Step-by-step method details 96

- This section lists the major steps and provides step-by-step details and timing for each major step. 97
- Please use continuous numbers for this section through Major Steps. Do not continue numbering from the Before You Begin section. 98

- Please use numbering (up to three levels: 1,2,3; a,b,c; i,ii,iii) to list steps. Do not use bullet points in this section. 101
- Avoid including more than 15 sub-steps for an individual step. 102
- DO NOT number the Major Steps. 103
- When describing the protocol steps, write in the present tense and use active and imperative verbiage throughout. See authors instructions page for an example. 104
- For steps using manufacturer’s protocols, please include link(s) to the protocols. 105
- Whenever possible, include links between troubleshooting sections and major steps. 106

Your major step one 109

1. First step of major step one. Use numbers to order steps 110
 - (a) Sub-steps under first step. Use letters for ordering of sub-steps. 111
 - (b) Sub-step two, as needed. 112
 - i. Third-level sub-steps under second-level sub-steps. Use Roman numerals for ordering. 113
 - ii. Third-level sub-step two, as needed. 114
2. Second step of major step one. 115

Your major step two 117

Include a brief description about what this major step accomplishes. This will help other researchers repeat and troubleshoot the protocol. 118

1. First step of major step two. Use numbers to order steps. 120
 - (a) Sub-steps under first step. Use letters for ordering of sub-steps. 121
 - (b) Sub-step two, as needed. 122
2. Second step of major step two. 123

Expected outcomes 124

This section should be written in paragraph form and detailed the expected results from the protocol and what a researcher can expect to produce for data. Include information about anticipated outcomes of the protocol, e.g., estimated yield of DNA extraction, images of protein expression pattern. We encourage authors to provide figures to illustrate the expected outcomes (see examples here) and describe the expected results in paragraphs. If you have no associated primary research manuscript, you are REQUIRED to provide evidence to validate the data generated by your protocol. DO NOT directly insert relevant results sections OR directly reproduce figures from a primary research paper. Find additional instructions for figure preparation in our instructions for authors here. 125

Quantification and statistical analysis (optional)

134

This section should be written in paragraph form and detail the analysis pipeline. Avoid placing extensive stepwise instructions in this section; if the analysis methods require multiple stepwise instructions, add them as a detailed analysis major step to the protocol. Either numbering (up to three levels: 1,2,3; a,b,c; i,ii,iii) or bullet points (up to three levels) can be used to list steps in this section. Provide details about the methods used for data processing, quantification, and statistical analysis of the data generated in this protocol. Include criteria for data inclusion/exclusion. If the protocol outcome requires a complex statistical or computational analysis, include a sample data set to allow others to repeat your approach, and provide instruction on the interpretation of the raw data. You may provide sample data or reference supplementary data files here.

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Limitations

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In paragraph form, describe possible limitations of the protocol, including any situations where the protocol may be unreliable or unsuccessful. Describe environmental factors or mechanical limitations that might affect the validity of the results.

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Troubleshooting

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- Describe any problems that might arise from running the protocol and provide possible solutions.
- Make sure to flag them in the corresponding steps of the protocol and refer to this section.
- If applicable, this section can also be the space to include information about alternatives, i.e., what reagents and/or equipment has some flexibility and what cannot be changed.
- If you wish to include a list for each problem/potential solution, please use bullet points (up to three levels) make a list.
- DO NOT use numbering (1,2,3; a,b,c; i,ii,iii) in this section.
- We recommend including a minimum of 5 Troubleshooting points.
- Link all Troubleshooting points to the appropriate Steps in the manuscript. Provide the step number in the Troubleshooting point.

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Problem 1:

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When illustrating problems, we encourage the use of figures/videos to indicate a good outcome versus a bad outcome. See examples here and here.

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Potential solution:

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Describe the details of the solution to resolve this problem.

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Resource availability

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Lead contact

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Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, [lead contact's name] (lead contact's email).

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Technical contact

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Technical questions on executing this protocol should be directed to and will be answered by the technical contact, [technical contact's name] (technical contact's email).

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Materials availability

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Provide information regarding availability of newly generated materials associated with this protocol, including any conditions for access.

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Plasmids generated in this study have been deposited to [Addgene, name and catalog number or unique identifier].

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Data and code availability

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State whether the protocol includes all datasets generated or analyzed during this study. Provide information about data and code availability.

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Figures

180

Do not include figures in the main text document. All figures should be uploaded as separate high-resolution JPG. All captions for tables should also be included here.

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Citations

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To cite/link to display items (figures and tables) and/or sections of the manuscript, simply write, for example, "(Figure 1)" or "see discussion." Our team will do the rest. Please note that we do not number sections or subsections.

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To cite references, you may use the cite command, e.g., "Recent articles in *Matter* and *Cell*^{1–3} have shown ..." or "Many interesting discoveries have been reported,^{4–7} which ..."

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Equations

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Simple formulae should appear in line with the text whenever possible. You can write inline math by enclosing it between $\backslash($ and $\backslash)$, as in this example: $x^2 + y^2 = z^2$. You can also enclose it between dollar signs (\$), as in this example: $E = mc^2$.

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Larger, more complex formulae may appear on a new line, either by enclosing them between $\backslash[$ and $\backslash]$ or by using the `displaymath` environment:

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$$x^n + y^n = z^n$$

$$\sqrt{x^2 + 1}$$

If any equations or formulae need to be referred to or cited again later in the text, use the `equation` environment to number them. Later, you can cite these as "Equation 1," "Equation 2," etc.

$$f(x) = \sum_{i=0}^n \frac{a_i}{1+x} \tag{1}$$

Supplemental information index (contact your handling editor) 198

Figures S1-S5 and their legends in a PDF	199
Table S1. A descriptive title for an Excel file that was too large to appear in the PDF	200
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Data S1. Raw data on x, y, and z	202

Acknowledgments 203

This work was funded by [FUNDER] via grant [GRANT NO.]. The authors thank all members of the lab for their support. 204
205

Author contributions 206

Conceptualization, S.C.P. and S.Y.W.; methodology, A.B., S.C.P., and S.Y.W.; investigation, M.E., A.N.V., N.A.V., S.C.P., and S.Y.W.; writing – original draft, S.C.P. and S.Y.W.; writing – review & editing, S.C.P. and S.Y.W.; funding acquisition, S.C.P. and S.Y.W.; resources, M.E.V and C.K.B.; supervision, A.B., N.L.W., and A.A.D. 207
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Declaration of interests 211

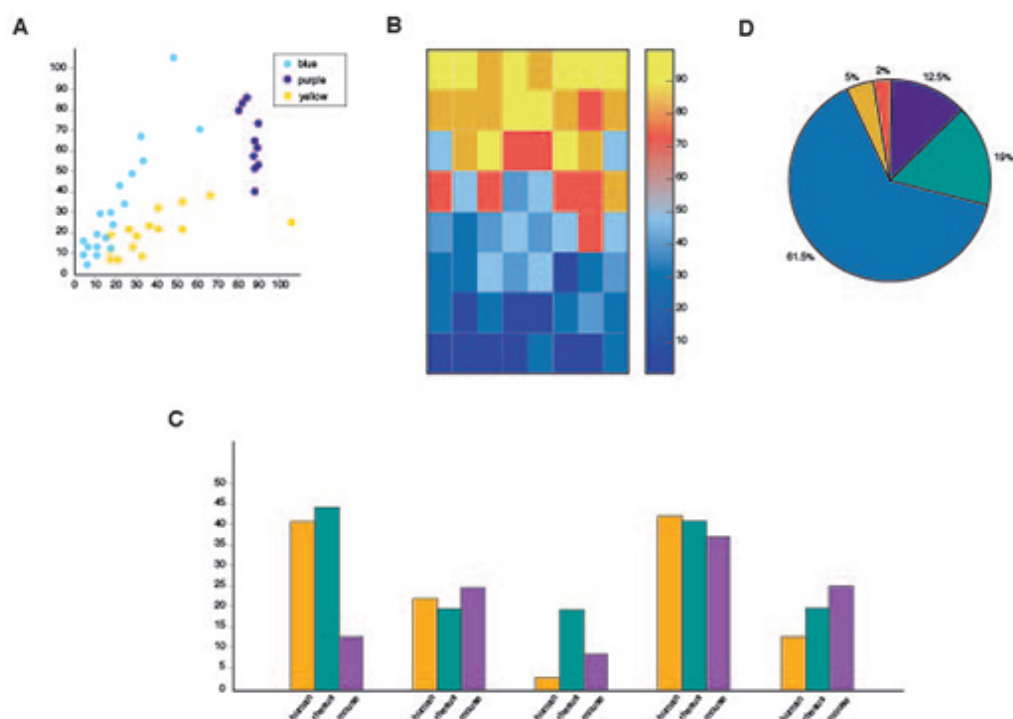
S.Y.W. is an employee and shareholder of COMPANY. M.E.V. is a founder of COMPANY and a member of its scientific advisory board. 212
213

Declaration of generative AI and AI-assisted technologies 214

During the preparation of this work, the author(s) used [NAME OF TOOL OR SERVICE] in order to [REASON]. After using this tool or service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication. 215
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MAIN FIGURE TITLES AND LEGENDS

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Figure 1. A brief title that describes the entire figure without citing specific panels

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The figure legend can be all one paragraph and describe the images (A), graphs (B), and plots (C), etc., together.

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(A) Or each panel or group of panels can be described separately, as shown here and below.

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(B) Graph of X, Y, and Z.

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(C and D) If panels are grouped like this, please explicitly describe each panel, e.g., “Images showing SEM (C) and TEM (D).”

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Please define all scale and error bars, and please review the Cell Press figure guidelines before submission: <https://www.cell.com/figureguidelines>. Example figure created by Cassie Comeau, Cell Press.

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MAIN TABLES, INCLUDING TITLES AND LEGENDS

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Table 1. A table with clear organization of data

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Column 1	Column 2	Column 3	Column 4
Row A ^a	6	87,837	787
Row B	7	78	5,415
Row C	545	778	7507
Row D	545	18,744	7,560
Row E	88	788	6,344

233

The table legend (optional) follows the table itself. The legend should be used to provide additional info that relates to the table as a whole.

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^aFootnotes can be used to provide additional info on specific content within the table, such as this footnote to the first row (row A). Do not use footnotes in the table title.

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References

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