PLOS BIOLOGY PREREGISTERED RESEARCH ARTICLE

GUIDELINES FOR AUTHORS

Preregistered Research Articles (aka Registered Reports) are a form of empirical article offered at *PLOS Biology* in which study rationale, methods and proposed analyses are reviewed prior to research being conducted. High quality protocols are reviewed for technical soundness of the proposed methodology, and provisionally accepted for publication before data collection commences. Refer to the Center for Open Science for more details.

This format of article is designed to minimise publication and reporting bias, while also maximising study quality by focusing peer review on the importance of the research question and rigour of the proposed methodology. It also allows complete flexibility to conduct exploratory (unregistered) analyses and report serendipitous findings. Preregistered Research Articles are offered across the full scope of empirical biological research at *PLOS Biology*.

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Editorial and Peer Review Process for Preregistered Research Articles

STAGE 1 PROTOCOL

Initial submissions will consist of the Stage 1 Protocol (please see below for the template). The editorial team will screen submitted protocols for importance of the research question using *PLOS Biology*'s general criteria for publication. Those that pass editorial screening will be invited to complete a full submission with manuscript details. The protocol will then be sent for in-depth peer review to further assess the importance of the research question and to evaluate the technical soundness of the proposed study design and methodology.

Following Stage 1 peer review, manuscripts will be rejected or offered the opportunity to revise the study proposal, if needed. Stage 1 Protocols that pass peer review and meet our high standards of importance and scientific rigour will be issued an in-principle acceptance decision, indicating that the article will be published pending completion of the study. Stage 1 Protocols are not published following upon an in-principle acceptance. Instead they are held and integrated into a single, completed 'Preregistered Research Article' following completion of the study, and peer review and acceptance of the final Stage 2 manuscript (see Stage 2 below for details).

Following a Stage 1 in-principle acceptance decision, authors are required to register their approved Stage 1 Protocol with the Center for Open Science or another recognised repository, either publicly or under private embargo until submission of the Stage 2 manuscript. Stage 1 Protocols can be quickly and easily registered using a tailored mechanism for Registered Reports.

STAGE 2 PREREGISTERED RESEARCH ARTICLE

Once given a Stage 1 in-principle acceptance decision, authors then proceed to conduct the study, adhering exactly to the peer-reviewed and approved Stage 1 Protocol and its study design. When the study is complete the authors will submit their finalised Stage 2 manuscript (including Results and Discussion sections), for review. Please see below for the Stage 2 template. Editorial decisions will not be based on the perceived importance or novelty of the results obtained when completing the study.

Any deviation from the approved Stage 1 Protocol (after in-principle acceptance), regardless of how minor it may seem to the authors, could lead to rejection of the manuscript at Stage 2. In cases where the pre-approved Stage 1 protocol is altered due to unforeseen circumstances (e.g. change of equipment or unanticipated technical error), the authors must consult the editors

immediately for advice, and prior to the completion of data collection. Minor changes to the protocol may be permitted according to editorial discretion. In such cases, the deviation must be reported in the Stage 2 submission.

Submitting a Stage 1 Manuscript

Stage 1 submissions should include a cover letter and the Protocol manuscript file (details below).

The COVER LETTER should include:

- A brief scientific case for consideration - what is the scientific question you are addressing and why is it important to the field?
- Information on recently published literature that is relevant to this research question.
- A statement confirming that all necessary support (e.g. funding, facilities) and approvals (e.g. ethics) are in place for the proposed research. Note that protocols will generally be considered only for studies that are able to commence immediately; however authors with alternative plans are encouraged to contact the journal editors for advice.
- An anticipated timeline for completing the study and proposed Stage 2 submission date, if given a Stage 1 in-principle acceptance. Extensions to this deadline can be discussed with the editor, if needed.
- A statement confirming that, if given a Stage 1 in-principle acceptance, the authors agree to register their Stage 1 Protocol in a recognised repository, either publicly or under private embargo until submission of the Stage 2 manuscript.
- A statement confirming that the authors agree to share their raw data, in accordance with the PLOS Data Availability Policy, and laboratory log, if needed, for all published results.

Stage 1 Protocol Manuscript Template

Stage 1 Protocol manuscripts should include the following sections:

- **Abstract**
  Brief explanation of research question, its relevance, and the proposed investigative approach. (Note: This section will have an additional outcome paragraph added in Stage 2 manuscripts).

- **Introduction**
  A review of the relevant literature that motivates the research question and a full description of the experimental aims and hypotheses. Please make sure to enumerate the specific hypotheses. (NOTE: This section cannot be edited after a Stage 1 in-principle acceptance).

- **Summary Table**
  Please include a summary table that aligns each research question with the hypothesis/es used to answer the question, the sampling plan for each hypothesis (e.g. power analysis, where applicable), the specific statistical analysis/es that will be used to test the hypothesis, and a pre-specification of which outcomes will confirm or disconfirm the hypothesis (to varying degrees of strength where multiple analyses with different possible outcomes are used to interrogate one hypothesis).

- **Materials and Methods**
  This section should include all the following, as relevant. (NOTE: This section cannot be edited after Stage 1 in-principle acceptance)

    - Protocol details regarding the following (repeat as necessary for all protocols being proposed): sampling, cell lines/organisms (i.e. experimental population), materials and reagents, procedure/intervention, deliverables. Experimental procedures and materials should be provided in sufficient detail to allow another researcher to repeat the methodology exactly, without requiring further information.

    - Sampling plan (e.g. power calculations or Bayesian sampling methods etc) should be included unless clearly not appropriate. Please include details of criteria for data inclusion and exclusion (e.g. outlier extraction). Procedures for objectively defining exclusion criteria due to technical errors or for any other reasons must be specified, including details of how and under what conditions data would be replaced. Please also detail when data collection would cease e.g. sample size, number of observations etc.

    - Proposed analysis pipeline, including all pre-processing steps, and a precise description of all planned analyses, including appropriate correction for multiple comparisons. Any covariates or regressors must be stated. Where analysis decisions are contingent on the outcome of prior analyses, these contingencies must be specified and adhered to. Only results from pre-planned analyses can be reported in the main Results section of Stage 2 manuscripts. However,

unplanned exploratory analyses will be admissible in a separate section of the Results (see Stage 2 details below).

- Statistics:
  - When relevant, studies involving Neyman-Pearson inference must include a statistical power analysis. Estimated effect sizes should be justified with reference to the existing literature. Since publication bias over-inflates published estimates of effect size, power analysis must be based on the lowest available or meaningful estimate of the effect size. The a priori power must be 0.9 or higher for all proposed hypothesis tests. In the case of highly uncertain effect sizes, a variable sample size and interim data analysis will be permissible but with inspection points stated in advance, appropriate Type I error correction for 'peeking' employed, and a final stopping rule for data collection outlined.
  - Methods involving Bayesian hypothesis testing are particularly encouraged. For studies involving analyses with Bayes factors, the predictions of the theory must be specified so that a Bayes factor can be calculated. Authors should indicate what distribution will be used to represent the predictions of the theory and how its parameters will be specified. For example, will you use a uniform up to some specified maximum, or a normal/half-normal to represent a likely effect size, or a JZS/Cauchy with a specified scaling constant? For inference by Bayes factors, authors must be able to guarantee data collection until the Bayes factor is at least 10 times in favour of the experimental hypothesis over the null hypothesis (or vice versa). Authors with resource limitations are permitted to specify a maximum feasible sample size at which data collection must cease regardless of the Bayes factor, however to be eligible for advance acceptance this number must be sufficiently large that inconclusive results at this sample size would nevertheless be of major importance.

- Full descriptions must be provided of any outcome-neutral criteria that must be met for successful testing of the stated hypotheses. Such quality checks might include the absence of floor or ceiling effects in data distributions, positive controls, or other quality checks.

- Any description of prospective methods or analysis plans should be written in future tense. For the Stage 2 manuscript, once the study is complete, these instances of future tense should be changed to past tense.

- **Timeline**
  Present an anticipated timeline for completion of the study.

- **Pilot Data** *(optional)*
  Can be included to establish proof of concept, effect size estimations, or feasibility of proposed methods. Pilot data can include details of any preliminary data that have already been obtained (approach, materials and methods, results, analytical observations etc.) Pilot data present at Stage 1 will need to be clearly distinguished from data subsequently obtained at Stage 2.

- **Secondary Analyses** *(optional)*
  If the study proposes secondary analyses of existing databases, please provide full details of the data to be analysed, its origin and any relevant information and citations with respect to the origin of the dataset and any previous analyses that have been performed. Please make clear what extent of prior observation you have had; secondary analysis of existing data may be bias-prone, which should be avoided. If this is a proposed replication study, full details of replication approach should be provided.

- **Data Availability Plan**
  Provide full details of where and how data and/or code produced will be shared (in line with the PLOS Data & Code sharing Policies).

- **Ethical Approval Plan**
  Provide details of ethical approval for animal and human subject research.

Stage 1 Review Criteria

Stage 1 Protocol submissions that are judged by the editors to be of sufficient quality and scientific importance will be sent for in-depth peer review. Reviewers will be asked to assess the following at Stage 1:

- The importance of the research question(s).
- The logic, rationale, and plausibility of the proposed hypotheses (does the manuscript provide a valid rationale for the proposed study, with clearly identified and justified research questions?)
- The soundness and feasibility of the methodology and analysis pipeline (including statistical power analysis where appropriate). Is the protocol technically sound and

planned in a manner that will lead to a meaningful outcome and allow testing of the stated hypotheses?
- Whether the clarity and degree of methodological detail is sufficient to exactly replicate the proposed experimental procedures and analysis pipeline.
- Whether the authors have pre-specified sufficient outcome-neutral tests for ensuring that the results obtained are able to test the stated hypotheses, including positive controls and quality checks.

Submitting a Stage 2 Manuscript

Stage 1 Protocols that pass peer review and meet our high standards of importance and scientific rigour will be issued an in-principle acceptance decision. Following Stage 1 in-principle acceptance, authors will proceed to conduct the study and submit their Stage 2 manuscript.

Incremental Registrations

Authors may sometimes wish to add experiments to pre-approved Stage 1 Protocols. In such cases authors can propose additional experiments for consideration. These new proposals will be reviewed using the same technical criteria as the regular Stage 1 review and, when possible, the editorial team will try to fast-track this evaluation. This option may be particularly appropriate where an initial experiment reveals a major serendipitous finding that warrants in-depth follow up within the same manuscript. For further advice on specific scenarios for incremental registration, please contact the editors at biology_editors@plos.org.

The Stage 2 manuscript consists of the approved Stage 1 Protocol manuscript, with the addition of Results and Discussion sections. Authors must collectively certify in the Cover Letter that all non-pilot data was collected after the date of Stage 1 in-principle acceptance (see below for details).

Stage 2 Preregistered Research Article Manuscript Template

Please follow the guidelines below when preparing the Stage 2 manuscript:

- **Abstract**

The Abstract in the Stage 1 manuscript should have an additional outcome paragraph added at Stage 2.

- **Introduction, Summary and Methods**
    - Apart from minor stylistic revisions, the Introduction and Methods section cannot be altered from the approved Stage 1 manuscript, and the stated hypotheses cannot be amended or appended. If Pilot Data and Secondary Analysis sections were present in the approved Stage 1 manuscript, those too should remain unaltered.
    - At Stage 2, any descriptions written in future tense within the Stage 1 manuscript should be changed to past tense. Any minor textual changes to the Introduction or Methods (e.g. correction of typographic errors) must be clearly marked in the Stage 2 submission.
    - Any relevant literature that appeared following the date of Stage 1 in-principle acceptance should be covered in the Discussion.
    - A URL to the (now public) Stage 1 Protocol, which was deposited in a repository, should be added to the Methods section.
    - An official Ethics Statement should also be added to the Methods section.

- **Results & Discussion**
    - The outcome of all pre-approved analyses from Stage 1 must be reported in the Stage 2 manuscript, except in rare instances where an approved analysis is subsequently shown to be logically flawed or unfounded. In such cases, the authors, reviewers, and editor must agree that a collective error of judgment was made and that the analysis is inappropriate. In such cases the analysis should remain in the Methods (as per the approved Stage 1 manuscript) but omitted from the Results with justification and discussion.
    - Authors may wish to include additional analyses that were not included in the Stage 1 submission. For instance, a new analytic approach might become available between Stage 1 in-principle acceptance and Stage 2, or a particularly interesting and unexpected finding may emerge. Such analyses are admissible but must be clearly justified in the text, and appropriately caveated as not having been approved in the Stage 1 Protocol. These analyses should be reported in a separate section of the Results titled “Exploratory analyses” Authors should be careful not to base their conclusions entirely on the outcome of such post-hoc analyses.

- **Data**
    - In according with the PLOS data availability policy, raw data must be made freely available in a public repository. Please note that for this article type, the raw data

itself should be archived in a public repository rather than submitted to the journal as supplementary material.
    - Raw data must be accompanied by guidance notes, where relevant, to assist other scientists in replicating the analysis.
    - Authors should also upload any relevant analysis scripts and other experimental materials that would assist in replication.
    - Data files should be appropriately time stamped to show that data was collected after Stage 1 in-principle acceptance and not before. Other than pilot data that were reviewed and approved at Stage 1, no data acquired prior to the date of Stage 1 in-principle acceptance is admissible in a Stage 2 manuscript. The authors must collectively certify in the Stage 2 Cover Letter that all non-pilot data was collected after the date of Stage 1 in-principle acceptance.

Stage 2 Review Criteria

The Stage 2 manuscript will most likely be evaluated by the same reviewers as in Stage 1, but could also be assessed by new reviewers if needed. Reviewers will be asked to assess the following at Stage 2:

- Whether the introduction, rationale and stated hypotheses are the same as the approved Stage 1 Protocol submission (required).
- Whether the authors adhered precisely to the approved Stage 1 experimental procedures.
- Whether the data are able to test the authors' proposed hypotheses by satisfying the approved outcome-neutral conditions (such as quality checks, positive controls).
- Whether the authors' conclusions are justified given the data.
- Whether any post-hoc analyses added by the authors are justified, methodologically sound, and informative.
- Whether, in accordance with the PLOS' data availability policy, data has been made freely available in a public repository. Data files should be appropriately time stamped to show that data was collected after Stage 1 Protocol approval and not before.

Reviewers are informed that editorial decisions will not be based on the perceived importance or novelty of the Stage 2 results. Thus while reviewers are free to enter such comments on the record, they will not influence editorial decisions. Reviewers at Stage 2 may suggest that authors perform additional post-hoc tests on their data; however, authors are not obliged to do so unless such tests are necessary to satisfy one or more of the Stage 2 review criteria.