NIH's Policy on Rigor and Reproducibility

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Introduction

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Outline

- Background
  - NIH Initiatives
  - Awards affected
  - General application information
- NIH's Rigor and Reproducibility Requirement Overview
- Breakdown of 4 Key Areas
  - The 'what' and 'why'
  - Resources
  - Application instructions
  - Questions
- More Resources

The majority of the content in this presentation comes from NIH

Reproducibility Initiatives

October 2013: NIH introduced initiative with emphasis on unbiased experiments and reproducible results

January 2014: Dr. Francis Collins and Dr. Lawrence Tabak published commentary in Nature

June 2014: workshop hosted by NIH with Nature publishing group and Science for preclinical reporting guidelines (currently, 135 journals endorse reporting guidelines)

January 2016: NIH Rigor & Reproducibility policy takes effect

December 2016: Section 2039 of 21st Century Cures Act Requires NIH to develop policies for Enhancing the rigor and reproducibility of scientific research

- Includes establishment of Advisory Committee to the Director (ACD) working group

May 2019: National Academy of Sciences publishes Reproducibility and Replicability report

September 2019: National Academies hosts Reproducibility and Replicability Symposium with several key stakeholders

https://www.niams.nih.gov/about/about-the-director/letter/nih-initiative-enhancing-research-reproducibility-and-transparency
Collins FS, Tabak LA. NIH plans to enhance reproducibility. *Nature* 2014;505(7485):612-13. doi: 10.1038/505612a
https://www.congress.gov/bill/114th-congress/house-bill/34/text;

Type of FOA	Description
Parent Announcements	•Broad FOAs allowing applicants to submit an investigator-initiated application for a specific activity code •Many NIH institutes and centers (IC) participate •Usually ongoing (3 yrs) •Use standard due dates List of Parent Announcements
Program Announcements (PAs) NOSIs	•FOAs issued by one or more Institutes and Centers to highlight areas of scientific interest •Encourage applications for a new or ongoing program •Usually ongoing (3 years) •Use standard due dates •Types of PAs: PAS: with set-aside funds PAR: special receipt, referral, and/or review considerations **Note: There is growing use of "Notices of Special Interest", rather than topic-specific PAs, to highlight areas of scientific interest. The notices designate existing FOAs to use for application submission.**
Requests for Applications (RFA)	•FOAs issued by one or more Institute or Center to highlight well-defined areas of scientific interest to accomplish specific program objectives (Make sure the science you are proposing fits the scope of the RFA!) •Indicate the amount of set-aside funds •Indicate anticipated number of awards •Usually single due date •Institute/Center usually convenes review panel

Understand Funding Opportunities | grants.nih.gov. (n.d.). Retrieved December 31, 2019, from https://grants.nih.gov/grants/how-to-apply-application-guide/prepare-to-apply-and-register/understand-funding-opportunities.htm

Parent Announcements (For Unsolicited or Investigator-Initiated Applications)

Parent announcements are broad funding opportunity announcements allowing applicants to submit investigator-initiated applications for specific activity codes. They are open for up to 3 yea and use standard due dates.

Not all NIH Institutes and Centers participate on all parent announcements. Before submitting your application, make sure the NIH Institute or Center that might be interested in your research i listed as a participating organization in the announcement.

The following Parent Announcements are available (sorted by Activity Code):

[Research (R) | Research Training (T) | Career Development (K) | Fellowships (F) | Admin Supplements | Post-award Administrative Action]

Research (R) Announcements

Activity Code(s)	Title	Announcement Number	Issuing Organization	Release Date	Opening Date (SF424 Only)	Expiration Date
R01	NIH Research Project Grant (Parent R01 Basic Experimental Studies with Humans Required)	PA-19-091	NIH	11/28/2018	01/05/2019	01/08/2022
R01	Research Project Grant (Parent R01 Clinical Trial Required)	PA-19-055	NIH	11/05/2018	01/05/2019	01/08/2022
R01	Research Project Grant (Parent R01 Clinical Trial Not Allowed)	PA-19-056	NIH	11/05/2018	01/05/2019	01/08/2022
R03	NIH Small Research Grant Program (Parent R03 Clinical Trial Not Allowed)	PA-19-052	NIH	11/05/2018	01/16/2019	01/08/2022
R13	NIH Support for Conferences and Scientific Meetings (Parent R13 Clinical Trial Not Allowed)	PA-18-648	NIH	02/09/2018	03/12/2018	01/08/2021
R21	NIH Exploratory/Developmental Research Grant Program (Parent R21 Basic	PA-19-092	NIH	11/28/2018	01/16/2019	01/08/2022

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Parent Announcements (For Unsolicited or Investigator-Initiated Applications). (n.d.). Retrieved December 31, 2019, from https://grants.nih.gov/grants/guide/parent_announcements.htm

Phase I – went into effect 1/25/16

What Changed

- Impacted most RESEARCH and CAREER DEVELOPMENT grants – but that is about to change…
- Enforced increased scientific rigor and transparency in the application instructions for writing the Research Strategy.
- New "Authentication of Key Biological and/or Chemical Resources" attachment.
- Additional rigor and transparency peer review questions.
  - See NOT-OD-16-011 and NOT-OD-16-012

Phase I - Progress Reports (RPPRs)
Section B – Accomplishments*

B.2 What was accomplished under these goals?

- Include the approaches taken to ensure robust and unbiased results.

B.6 What do you plan to do for the next reporting period to accomplish these goals?

- Discuss efforts to ensure that the approach is scientifically rigorous and results are robust and unbiased.

*"NOT-OD-16-031: Updates to NIH & AHRQ Research Performance Progress Reports (RPPR) to Address Rigor and Transparency." n.d. Accessed January 20, 2018. https://grants.nih.gov/grants/guide/notice-files/NOT-OD-16-031.html.

Special Notes and Exceptions

Research grants excluded

- C06, G08, G11, G12, G13, G20, R13, S06, S10, S21, SB1, U13, U55, UB1, UC6, UC7, UG4, UH4, X02, and 333

Career Development Awards excluded

- K02, K05, and K24, as candidates for these awards are expected to have independent, peer reviewed research support at the time the career award is made.
- NOT-OD-16-012

Special Note on Research Resource and Related grants

- P30, P40, P41, P2C, R24, R28, U24, U41, U42, and U2C may have slightly revised review language
- Refer to the Funding Opportunity Announcement (FOA).

*R25: not subject at this time, but must read FOA carefully!

"NOT-OD-16-011: Implementing Rigor and Transparency in NIH & AHRQ Research Grant Applications." n.d. Accessed January 20, 2018. https://grants.nih.gov/grants/guide/notice-files/NOT-OD-16-011.html.

Phase II - Formal Instruction on Rigor

- See notice NOT-OD-16-034 issued 12/17/15
- **Advance notice:** NIH & AHRQ to **require formal instruction** in scientific rigor and transparency to enhance reproducibility for all individuals supported by:
  - Institutional training grants: D43, T15, T32/TL1, T34, T35, T36, T37, T90/R90, and U2R
  - Institutional career development awards: K12/KL2
  - Individual fellowships: F05, F30, F31, F32, F37, F38, and FI2
- **Seen examples for specific FOAs:**
  - NIGMS T32 PAR-17-341
  - NINDS T32 PAR-19-211

Phase II – UPDATE! Formal Instruction on Rigor

- Update per NOT-OD-20-033, issued 12/3/2019
- Effective for proposals submitted for due dates on or after **May 25, 2020**

Institutional Training Grants (i.e., T32, K12, etc)

- The **Program Plan** section of the application will be expected to include a description of **how the program and faculty will provide training** in rigorous research design and relevant data science and quantitative approaches.

- The requirement to include a **Plan for Instruction in Methods for Enhancing Reproducibility** attachment will be expanded to all applicants.

Upcoming Changes for Fellowships

Applicant's Background and Goals for Fellowship Training - limited to 6 pages

Research Strategy – limited to 6 pages

Changes:

- In describing their training **goals** and objectives in the **Program Plan** attachment, fellowship candidates will be expected to address, as applicable, **any new research skills they plan to acquire in the areas of rigorous research design, experimental methods, quantitative approaches, and data analysis and interpretation.**

- In the **Research Strategy** section of the Program Plan attachment, fellowship candidates will be expected to describe (a) the strengths and weaknesses in the rigor of the prior research that serves as the key support for the proposed project, (b) plans to address any weaknesses in the rigor of the prior research, (c) how the experimental objectives proposed will achieve robust and unbiased results, and (d) how relevant biological variables are factored into research designs and analyses.

Upcoming changes for Career Development Awards

- In describing their **career development plans** in the **Program Plan** attachment, candidates for career development awards will be expected to address, as applicable, any **new research skills they plan to acquire** in the areas of rigorous research design, experimental methods, quantitative approaches, and data analysis and interpretation.

Typical Research Strategy

- **Divided into**
  - Significance
  - Innovation
  - Approach

- **Research grant**: 12 page limitation

- **Career Development (K):** Candidate Information and Goals for Career Development and Research Strategy: combined cannot exceed 12 pages

- **Note for Applicants with Multiple Specific Aims:** You may address the Significance, Innovation, and Approach either for each Specific Aim individually or for all of the Specific Aims collectively.

Four Key Areas to Address: Research and Career Development Applications

Key Area	Application Instructions
Rigor of Prior Research	Research Strategy: Significance (scored) and Approach (scored)
Scientific Rigor	Research Strategy: Approach (scored)
Consideration of Relevant Biological Variables, such as sex	Research Strategy: Approach (scored)
Authentication of Key Biological and/or Chemical Resources	**Separate Attachment (not scored):** • to be saved as a single file named "Authentication of Key Resources Plan" in the "Other Research Plan Section" • Required if project involves key biological and/or chemical resources. Recommend 1 page.

Calling out the Review Criteria – Typical Research Grant

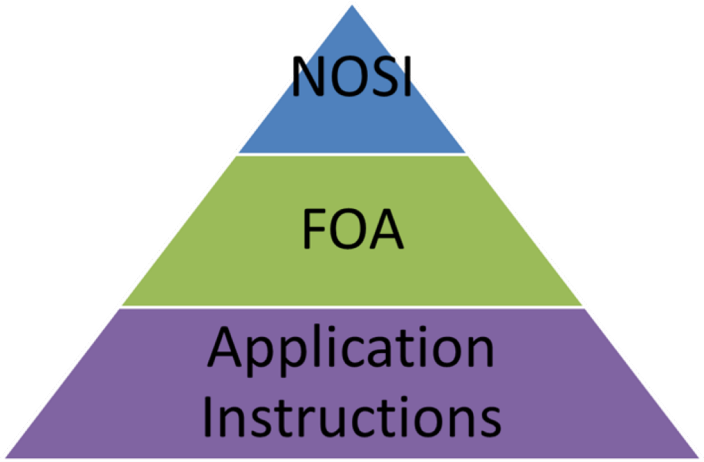
Review Criterion	Proposal Sections
Significance	Research Strategy
Investigator	Biosketch
Innovation	Research Strategy
Approach	Research Strategy
Environment	Facilities and Other Resources

Calling out the Review Criteria – Typical Career Development Award

Review Criterion	Proposal Sections
Candidate	Biosketch, Candidate section, Reference letters
Career Development Plan/ Career Goals & Objectives	Candidate section: Career Goals and Objectives, Candidate's Plan for Career Development/Training Activities During Award Period
Research Plan	Research Plan (one score whole thing)
Mentor(s), Co-Mentor(s), Consultant(s), Collaborator(s)	Letters of Support; Plans and Statements of Mentor and Co-Mentor(s)
Environment & Institutional Commitment to the Candidate	Description of Institutional Support, Institutional Commitment to Candidate's Research Career Development

Page Limits

- With all these R&R requirements, the page limits stayed the same.
- However, things began to shift with the NIH's Human Subject & Clinical Trial policies.
- Watch out for page limitations (or, the circumvention of page limitations)
- Note that the application instructions in specific Funding Opportunity Announcement (FOA) *supersedes* the SF 424 Application Instructions, in case there are conflicts.
  - And NOSIs supersede FOAs.



Research Strategy and Proposed Clinical Trials

- **Note for Applications Proposing the Involvement of Human Subjects and/or Clinical Trials:**
- Although some overall information may be duplicative between the Research Strategy and PHS Human Subjects and Clinical Trials Information form, it is usually inappropriate to copy/paste large blocks of text.

- Use the Research Strategy attachment to discuss the **overall strategy, methodology, and analyses of your proposed research.**
- Use the PHS Human Subjects and Clinical Trials Information form **to provide detailed information** for human subjects studies and clinical trials.

- The PHS Human Subjects and Clinical Trials Information form will capture detailed study information, including eligibility criteria; inclusion of women, minorities, and children; protection and monitoring plans; and statistical design and power.

- You are encouraged **to refer to information** in the PHS Human Subjects and Clinical Trials Information form as appropriate in your discussion of the Research Strategy (e.g., see Question 2.4 Inclusion of Women, Minorities, and Children).

NIH Clinical Trial Protocol Templates

- E-Protocol Writing Tool:
- https://e-protocol.od.nih.gov/#/home

- Applicants conducting phase 2 or 3 clinical trials that require Investigational New Drug applications (IND) or Investigational Device Exemption (IDE) applications can use a NIH-FDA template with instructional and sample text to help write protocols.

- A separate template is available for applicants conducting behavioral and social sciences clinical trials.

- Use of these templates is optional.
- Questions? SciencePolicy@od.nih.gov

More Resources for Clinical Trial Protocols

https://research.columbia.edu/ReaDI-Program

Clinical and Health Sciences

Expand all | Collapse all

- Functional MRI
- Mixed Methods and Qualitative Research
- Patient-Centered Outcomes Research and Observational Studies
- Clinical Trial Design, Techniques and Methodology
- Clinical Trial Protocol Development

https://research.columbia.edu/clinical-and-health-sciences

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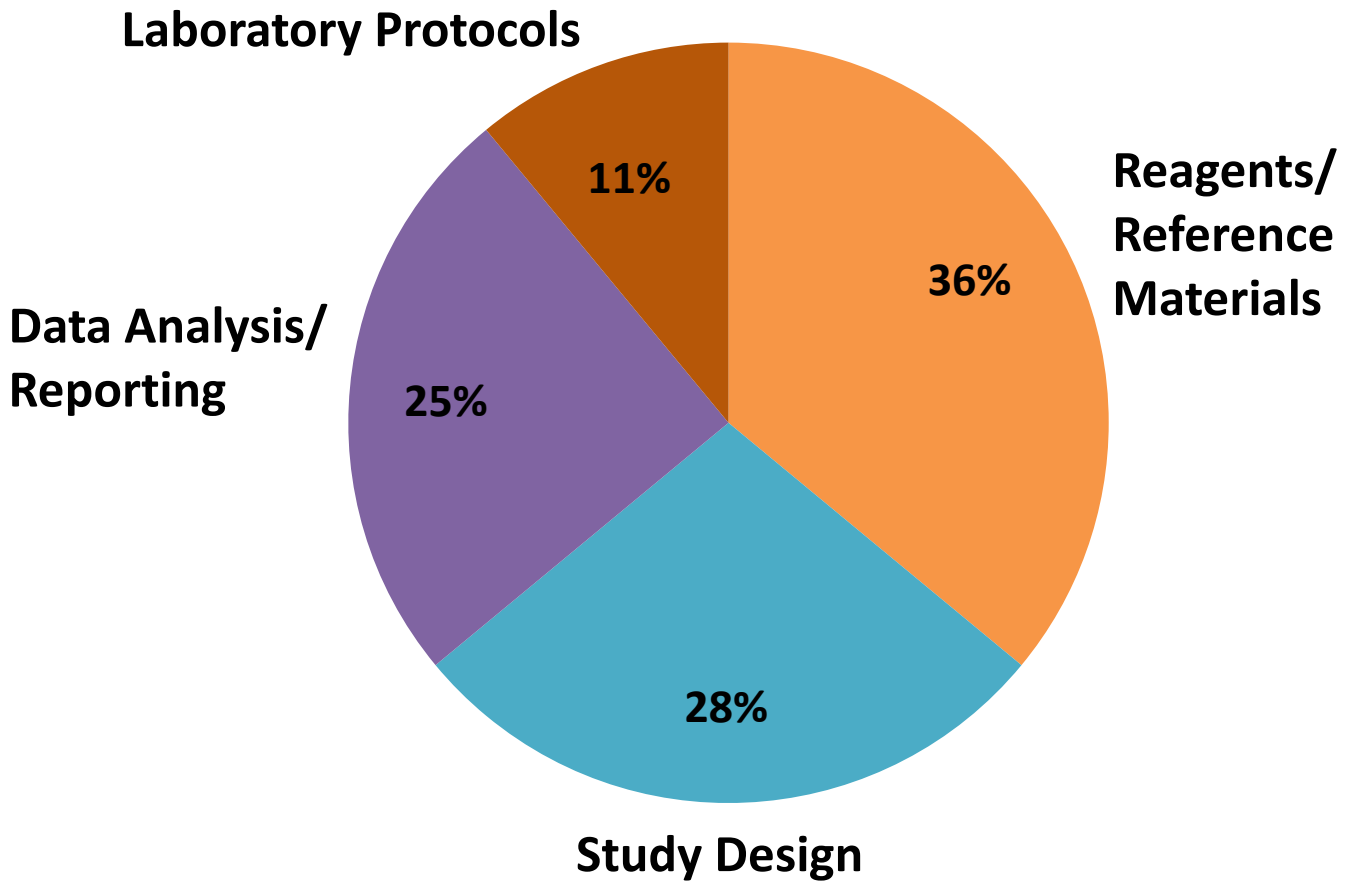
Four Areas of Irreproducibility

A 2012, retrospective analysis shows >50% of preclinical research results are not reproducible = ~$28 billion/year spent



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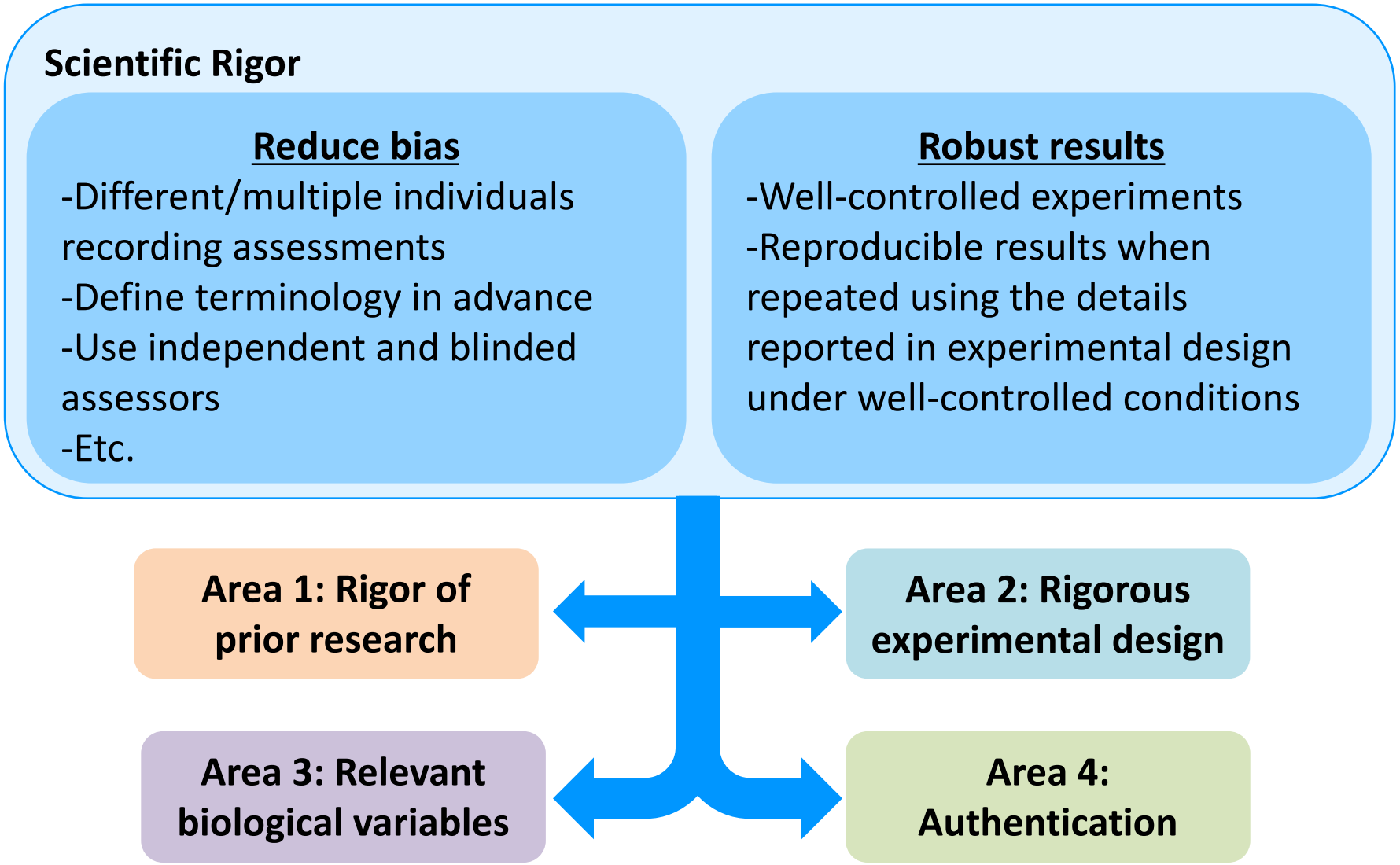
Freedman LP, Cockburn IM, Simcoe TS. The Economics of Reproducibility in Preclinical Research. *PLOS Biology* 2015;13(6):e1002165. doi: 10.1371/journal.pbio.1002165

NIH Introduced Four Areas to Address Scientific Rigor



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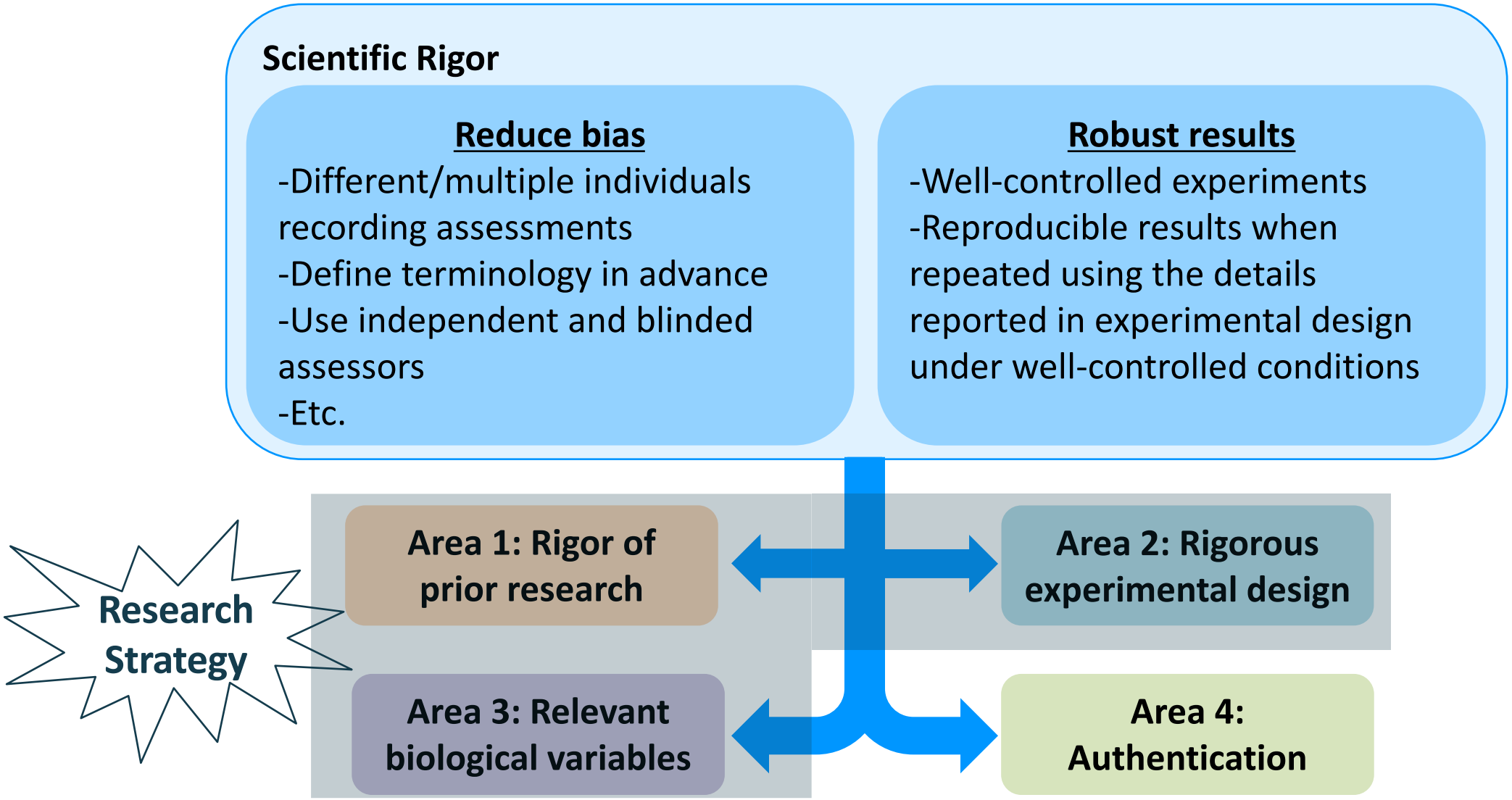


Area 1: Rigor of Prior Research | Background

- Often times, cited literature demonstrates the feasibility of the proposed experimental approach (positive)
  - Wasted resources
  - Incorrect conclusions
  - Unnecessary risks for trial subjects/unjustifiable clinical trials
- Researchers are missing the “whole picture” when they fail to seek or acknowledge literature that both negates and/or confirms a proposed study

https://grants.nih.gov/reproducibility/faqs.htm#4825

Area 1: How Rigor of Prior Research Changes Significance Section

Prospective analysis

- Importance of problem
- Critical barriers
- Improve scientific knowledge
- Affect field of study

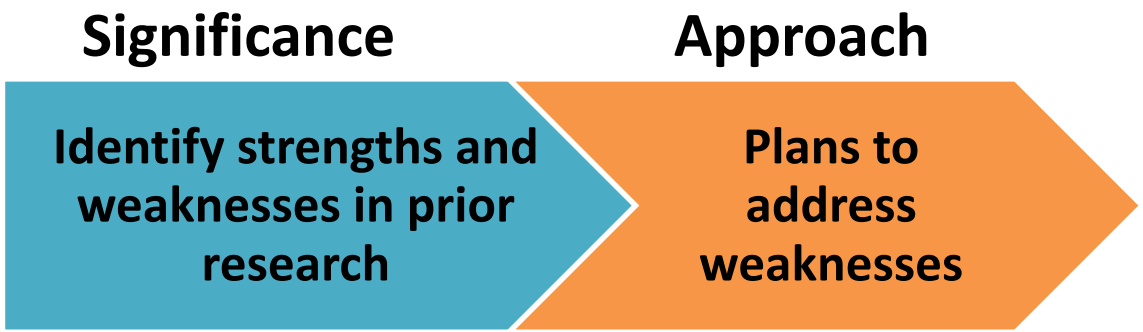
Retrospective analysis

- Identify strengths and weakness of prior research

Rigor of Prior Research

Area 1: Addressing Rigor of Prior Research

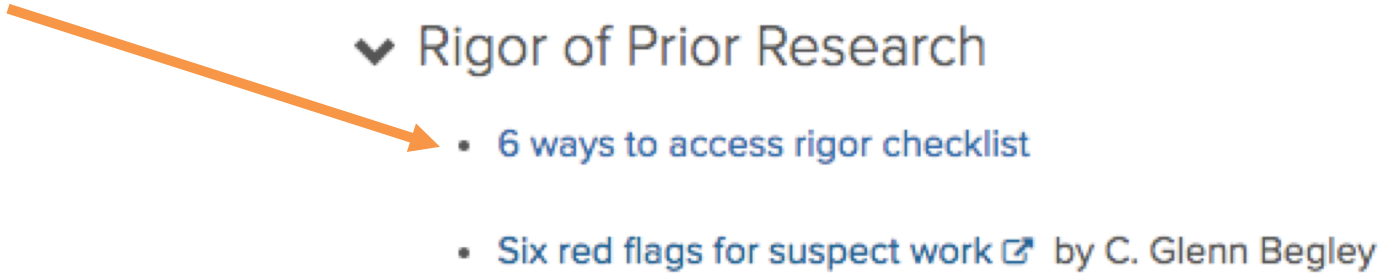
- Assessment of the rigor applied to previous studies (including own research—published or unpublished)
  - Identify and acknowledge shortcomings in rigor (including reporting on rigor)
  - Shortcomings could include:
    - No or insufficient authentication of key resources
    - Not considering relevant biological variables
    - Weak statistical analyses/experimental designs
- Approach includes strategies to address identified shortcomings
- Exploratory grant applications (with limited preliminary data) should include a critical assessment of the literature that either supports or contradicts research question



https://grants.nih.gov/reproducibility/faqs.htm#4825

Area 1: Rigor of Prior Research Checklist

- ❑Were the studies blinded?
- ❑Were all the results shown?
- ❑Were experiments repeated?
- ❑Were positive and negative controls shown?
- ❑Were reagents validated?
- ❑Were the statistical tests appropriate?



https://research.columbia.edu/reproducibility-resources-and-guidelines-topic

Begley CG. Six red flags for suspect work. *Nature* 2013;497:433. doi: 10.1038/497433a

Area 1: Rigor of Prior Research | Application Instructions

Research Strategy – Significance:

- "Describe the strengths and weaknesses in the **rigor of the prior research** (both published and unpublished) that serves as the key support for the proposed project."

"G.400 - PHS 398 Research Plan Form." n.d. Accessed January 20, 2018. https://grants.nih.gov/grants/how-to-apply-application-guide/forms-e/general/g.400-phs-398-research-plan-form.htm#3.

Area 1: Rigor of Prior Research | Reviewer Criteria

Latest 3/18/2019:
https://grants.nih.gov/grants/peer/guidelines_general/Reviewer_Guidance_on_Rigor_and_Transparency.pdf

- "The applicant should discuss the strengths and weaknesses of the prior research used to support the application and describe how the proposed research will address weaknesses or gaps identified by the applicant. This may include the applicant's own preliminary data, data published by the applicant, or data published by others. The NIH expects this consideration to include attention to the rigor of the previous experimental designs, as well as relevant biological variables and authentication of key resources."

- Reviewers will evaluate the rigor of the prior research as part of the **Significance** and **Approach** criterions for research grant applications or the **Research Plan criterion for mentored career development award applications**.
  - Consider whether the prior research that serves as the key support for the proposed project is rigorous.
  - Consider whether the investigators **included plans to address weaknesses** or gaps identified in the rigor of prior research.

- Weaknesses or gaps in the rigor of the prior research that serves as the key support for the proposed project, or the failure to address those weakness or gaps, may affect criterion and overall impact scores.

Area 1: Rigor of the Prior Research | Writing Resources

Check out NIAID's *Apply for a Grant*:

https://www.niaid.nih.gov/grants-contracts/apply-grant

Contains sample applications and strategy.

Other writing resources:

VP&S Grant Starter Kit: https://www.ps.columbia.edu/research/funding/grant-resources/grant-toolbox/grant-starter-kit

Check out several of the NIH grant writing books: https://pileader.com/collections/all

The Grant Application Writer's Workbook:
http://www.grantcentral.com/workbooks/national-institutes-of-health/

Questions on Area 1: Rigor of the Prior Research?

Next:

Area 2: Rigorous Experimental Design

Area 2: Rigorous Experimental Design

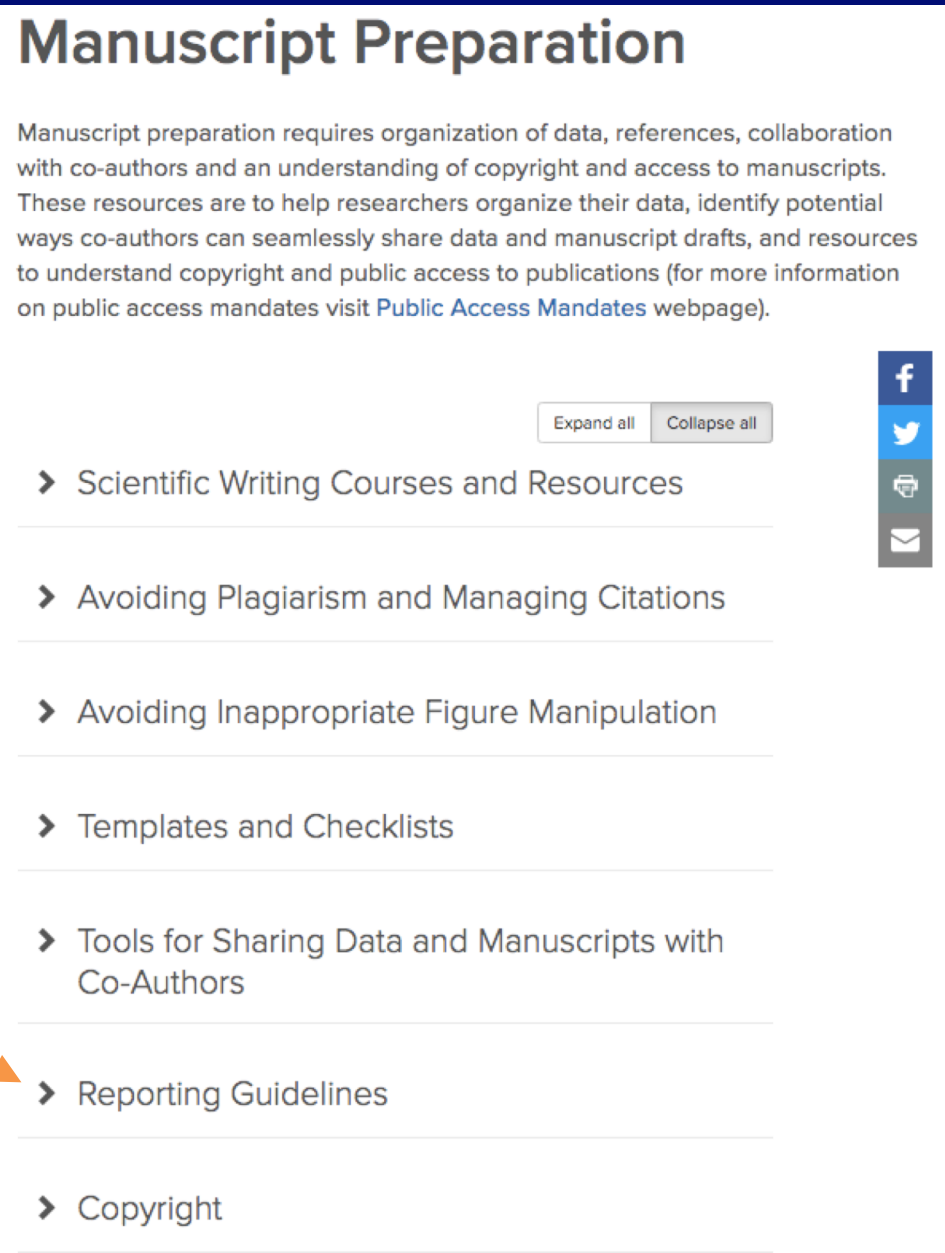
- Full transparency of experimental details are expected in grant applications
  - Reviewers need to know all details to assess the rigor
  - Researchers (should) already be writing transparently in publications
- Experimental design is discipline and project specific and might include descriptions of the following:
  - Use of standards
  - Sample size estimation
  - Randomization
  - Blinding
  - Appropriate replicates
  - Controlling for inter-operator variability
  - Statistical methods planned
  - Inclusion and exclusion criteria
  - Subject retention and attrition
  - How missing data will be handled
  - Any other information as appropriate to the science

Transparency and consideration on how to avoid biases is key!

https://grants.nih.gov/reproducibility/index.htm
https://nexus.od.nih.gov/all/2016/01/28/scientific-rigor-in-nih-grant-applications/

Area 2: Tips for Writing Transparently

- Consider the details included for publication
- Reporting checklists
  - "A call for transparent reporting to optimize the predictive value of preclinical research"
    - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3511845/
  - Questionnaire from Penelope:
    - https://www.penelope.ai/equator-wizard
  - EQUATOR Network:
    - https://www.equator-network.org/library/



https://research.columbia.edu/manuscript-preparation

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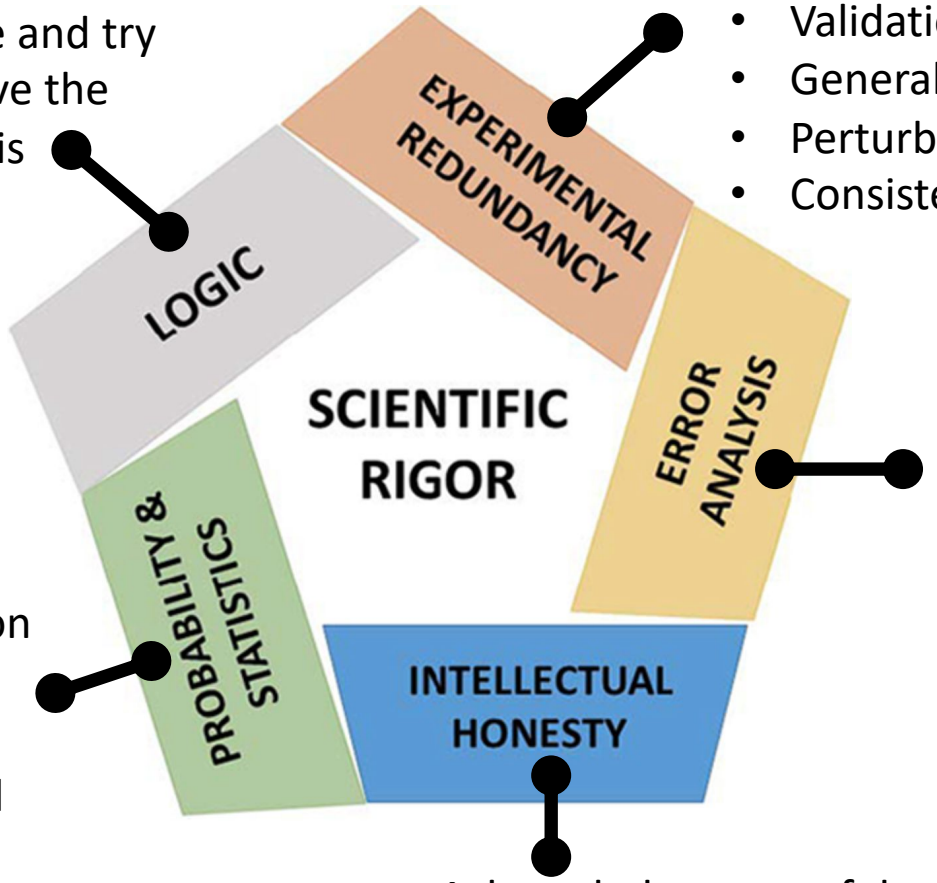
Area 2: Tips for Writing Rigorously



- Challenge and try to disprove the hypothesis

- Replication
- Validation
- Generalization
- Perturbation
- Consistency

- Consideration of introduction of errors
- Sensitivity analysis

- Power calculation
- Other statistical considerations
- Size of observed effect

- Acknowledgement of data that do not meet hypotheses
- Acknowledgement of others' work
- Corroborate with others

Arturo Casadevall, and Ferric C. Fang mBio 2016; doi:10.1128/mBio.01902-16

Area 2: Tips for Addressing Bias

- Consider and test alternative hypotheses
- Seek literature that supports and contradicts hypothesis (rigor of prior research). Be wary of published studies, consider rigor of previously published studies
- Rigorously check and repeat both expected and unexpected results (use of controls, blinding, etc.)
- Ask a colleague to repeat experiments
- Recognize 'cherry-picking' behaviors

Nuzzo R. How scientists fool themselves - and how they can stop. *Nature* 2015;526(7572):182-85. doi: 10.1038/526182a

Area 2: More Resources for Experimental Design

https://research.columbia.edu/ReaDI-Program

Area 2: Rigor | Application Instructions

Research Strategy – Approach – some quotes

- Describe the experimental design and methods proposed and how they will achieve robust and unbiased results.

- For trials that randomize groups or deliver interventions to groups, describe how your methods for analysis and sample size are appropriate for your plans for participant assignment and intervention delivery.

"G.400 - PHS 398 Research Plan Form." n.d. Accessed January 20, 2018. https://grants.nih.gov/grants/how-to-apply-application-guide/forms-e/general/g.400-phs-398-research-plan-form.htm#3.

Area 2: Rigor | Review Criteria

The applicant should describe experimental controls, plans to reduce bias (blinding, randomization, inclusion and exclusion criteria, etc.), power analyses, and statistical methods, as appropriate.

Reviewers will assess **scientific rigor** as part of the ***Approach*** criterion for research grant applications and the Research Plan criterion for mentored career development award applications, as well as the overall impact score..

- The Vertebrate Animal Section no longer requires a justification of animal numbers (NOT-OD-16-006). Inadequate vertebrate animal numbers should be reflected in the score and will not result in a block to funding.
- Reviewers will assess information concerning numbers of animals according to the section where it is included in the application.

- **HS/CT Form, Sec 4.4 – Statistical Design and Power** – application instructions "Specify the number of subjects you expect to enroll, the expected effect size, the power, and the statistical methods you will use with respect to each outcome measure you listed in 4.3 Outcome Measures." – not duplicative!

"G.500 - PHS Human Subjects and Clinical Trials Information." n.d. Accessed January 20, 2018. https://grants.nih.gov/grants/how-to-apply-application-guide/forms-e/general/g.500-phs-human-subjects-and-clinical-trials-information.htm#4.4.

Area 2: How much detail should I include in my application regarding rigor?

- *Comes from NIH FAQ, Section III: Scientific Rigor, FAQ#6*
- Every detail is not expected.
- State succinctly what is planned.
  - For example: "10 males and 10 females will be randomized to blinded treatment and control groups, giving 80% power to detect a treatment effect size of 65% compared to a baseline response of 5% at a significance level of 0.05."
- Investigators should be aware of the guidelines for publishing preclinical research in journals, which are similar in intent to the new application instructions.

"Frequently Asked Questions. Rigor and Transparency." 2016. February 1, 2016. https://grants.nih.gov/reproducibility/faqs.htm.

Area 2: Rigor | See NIH Examples in Awarded Applications

- NIH provided four examples (Biomedical/lab examples)
- Selected based on high overall impact scores and positive reviewer comments specific to rigor.
- Show how elements of rigor and transparency have been *succinctly* provided in applications.
- May not represent all of the aspects and may still have room for improvement, recognizing that many things go into the full review of applications.
- https://grants.nih.gov/policy/reproducibility/resources.htm

"Rigor and Reproducibility | Grants.Nih.Gov." n.d. Accessed January 20, 2018. https://grants.nih.gov/reproducibility/index.htm#resources.

Questions on Area 2: Experimental Rigorous Design?

Next:

Area 3: Relevant Biological Variables

Area 3: Relevant Biological Variables

- Choice of animal model or human population to be included will vary with the scientific topic of the proposed research
- Relevant biological variables (such as sex and age) are to be *considered* in research design, analyses and studies for vertebrate animals and humans
- Biological variables that may affect the outcome should be considered
  - Sex
  - Age -> NOT-OD-18-116 (https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-116.html)
  - Life stage
  - Weight
  - Underlying health conditions
- Applies to basic, preclinical, and clinical research
- It is expected that sex as a biological variable will be factored into research designs, analyses, and reporting in vertebrate animal and human studies

https://nexus.od.nih.gov/all/2016/01/29/consideration-of-relevant-biological-variables-in-nih-grant-applications/
http://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-102.html

Area 3: Sex as a Biological Variable (SABV) Background

- Preclinical research historically has focused mainly on male animals[1]
- The results of mostly single-sex studies contributes to ambiguous information on how sex-based differences may influence outcome[2]
- There is increasing evidence of sex-based differences in basic genetics, cellular and biochemical organization[1,2]
- Exclusion of females from preclinical studies has led to treatments with adverse events that are more common or severe in women than men[3]

1: Janine Austin Clayton. Studying both sexes: a guiding principle for biomedicine *FASEB J February 2016 30:519-524*

2: Brian J. Prendergast, Kenneth G. Onishi, Irving Zucker, Female mice liberated for inclusion in neuroscience and biomedical research, Neuroscience & Biobehavioral Reviews, Volume 40, March 2014, Pages 1-5,

3: W.A. Rogers, A.J. Ballantyne Australian gender equity in health research group 2008. Exclusion of women from clinical research: myth or reality? Mayo Clin. Proc., 83 (2008), pp. 536–542

Area 3: Strategies for Accounting for SABV in Research Strategy

"Considering sex as a biological variable" is not the same as "sex differences research"

- Literature review on the influence of biological sex (add search terms: sex, gender, male/female, etc.)
- Formulation of research questions
- Take into account the influence of sex in study design
- Include males and females into studies or provide justification for a one sex study
- Stratified randomization of males and females into experimental conditions
- Characterize and report study results separately for males and females
- Generalize research findings, when appropriate
- Examine the treatment or toxicity effects for each sex separately
- Consider influence of sex in the interpretation of study results
- Rationale for number of study subjects now to be explained in Research Strategy

https://nexus.od.nih.gov/all/2015/12/11/what-does-it-mean-to-consider-sex-as-a-relevant-biological-variable-in-your-nih-grant-application/
Janine Austin Clayton. Studying both sexes: a guiding principle for biomedicine *FASEB J February 2016 30:519-524*
https://genderedinnovations.stanford.edu/methods/SABV_checklist.html

Area 3: Do I Need More Animals/Human Subjects?!

- At a minimum, develop a data analysis plan that provides for the collection of data disaggregated by sex
- Investigators may need larger sample sizes, especially **if** expecting sex to influence the results
  - In general, studies have preliminary data/hypothesis that hint that the results may be influenced by sex
  - Differentiate sex effects: **MAY** require larger numbers of animals, or equal numbers of both sexes to ensure adequate statistical power
- FREE online sample size and power calculators are available

https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-102.html

Janine Austin Clayton. Studying both sexes: a guiding principle for biomedicine *FASEB J February 2016 30:519-524*

Area 3: Strategies for Reporting SABV

Reporting of Results

- Report the sexes of animals
- Characterize and report study results separately for males and females
- Generalize research findings, when appropriate
- Avoid using terms like: better, improved or worse when describing sex differences

Reporting one Sex

- Provide justification from the scientific literature, preliminary data, or other relevant considerations
- Without strong justification, it is expected that both males and females will be included in research

Janine Austin Clayton. Studying both sexes: a guiding principle for biomedicine *FASEB J February 2016 30:519-524*
Brian J. Prendergast, Kenneth G. Onishi, Irving Zucker, Female mice liberated for inclusion in neuroscience and biomedical research, Neuroscience & Biobehavioral Reviews, Volume 40, March 2014, Pages 1-5,

Area 3: What About Cell Lines?

- Sex should be considered when using cells or tissues taken **DIRECTLY** from the animal or human
- *Consider* the possible role of sex in research
- Established cell lines:
  - NIH recognizes the difficulty in determining sex
  - Continuing to work on enhancing strategies and techniques to address challenges
  - "At this time, cell lines are **not** explicitly covered by this policy **BUT** NIH encourages investigators to consider SABV and be transparent in reporting of cells (when known) and relevant sex-specific data"

https://grants.nih.gov/reproducibility/faqs.htm#5016

Area 3: Special Considerations for Animal Research

- Justification of species for the proposed research in vertebrate animals section
- Report on the characteristics of the research animal's environment[1,2]
  - E.g. temperature, group housing, etc.
- Clearly describe study population and do not generalize findings (ex: adult animals vs. young/juvenile adults and aged adults)[1]
- Non-human primates are considered a scarce resource[3]
- IACUC is not required by federal regulations to request justification of the choice of sex(es) proposed in studies, but may ask for justification in studies with only one sex[4]

1: Janine Austin Clayton. Studying both sexes: a guiding principle for biomedicine *FASEB J February 2016 30:519-524*
2: Brian J. Prendergast, Kenneth G. Onishi, Irving Zucker, Female mice liberated for inclusion in neuroscience and biomedical research, Neuroscience & Biobehavioral Reviews, Volume 40, March 2014, Pages 1-5,
3: http://orwh.od.nih.gov/sexinscience/overview/pdf/NOT-OD-15-102_Guidance.pdf
4: https://grants.nih.gov/reproducibility/faqs.htm#4844

Area 3: SABV FAQs

Higher prevalence in one sex?

- Acceptable justifications may include the study of sex-specific conditions or phenomena, or investigation in which the study of one sex is scientifically appropriate

Small sample/population availability?

- Scarce resources may be considered adequate justification based on evidence of scarcity

Secondary Analysis? (such as a dataset i.e. Clinical Data Warehouse)?

- Be aware the limitations in the data available which thereby influence the types of questions that can be asked along with the generalizability of the research
- Limitations in existing clinical data sets, grantees should provide strong justification including evidence of the scarcity of this type of data
- *Consider* relevant biological variables when possible

https://grants.nih.gov/reproducibility/faqs.htm

Area 3: SABV Resources

COLUMBIA | RESEARCH

https://research.columbia.edu/ReaDI-Program

Area 3: Application Instructions: Also in *Approach*

- Explain how relevant biological variables, such as sex, are factored into research designs and analyses for studies in vertebrate animals and humans.

- For example, strong justification from the scientific literature, preliminary data, or other relevant considerations, must be provided for applications proposing to study only one sex. Refer to the NIH Guide Notice on Sex as a Biological Variable in NIH-funded Research for additional information.

https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-102.html
https://grants.nih.gov/grants/guide/notice-files/NOT-OD-16-011.html
"G.400 - PHS 398 Research Plan Form." n.d. Accessed January 20, 2018. https://grants.nih.gov/grants/how-to-apply-application-guide/forms-e/general/g.400-phs-398-research-plan-form.htm#3.

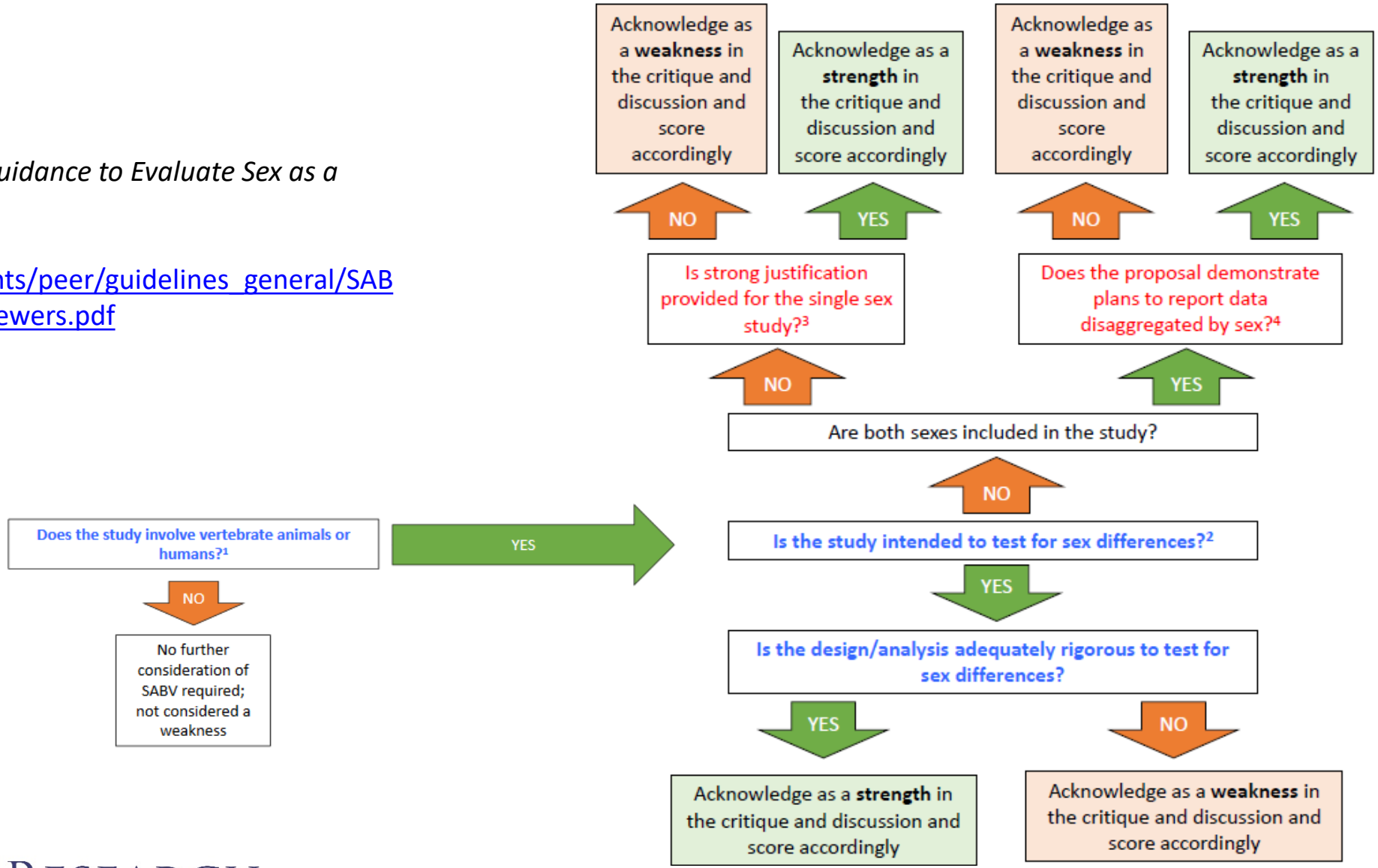
Area 3: Review Criteria

- Consideration of SABV does not necessarily mean sex differences research. See Figure 1 in "Studying both sexes = A guiding principle for biomedicine" for further detail.
  - Clayton, Janine Austin. 2016. "Studying Both Sexes: A Guiding Principle for Biomedicine." *The FASEB Journal* 30 (2):519–24. https://doi.org/10.1096/fj.15-279554.
- A justification is expected if the application proposes to study one sex, for example in the case of a sex-specific condition or phenomenon (e.g., ovarian or prostate cancer), acutely scare resources, or sex-specific hypotheses when there are known differences between males and females.
- Cost and absence of known sex differences are inadequate justifications for not studying both sexes.
- If the project involves human subjects and/or NIH-defined clinical research, are there plans to address:
  - 1) the protection of human subjects from research risks, and
  - 2) the inclusion (or exclusion) of individuals **on the basis of sex/gender, race, and ethnicity, as well as the inclusion (exclusion) of individuals of all ages** (including children and older adults), justified in terms of the scientific goals and research strategy proposed?

Area 3: Review Criteria | Decision Tree

Captured from *Reviewer Guidance to Evaluate Sex as a Biological Variable (SABV).*

https://grants.nih.gov/grants/peer/guidelines_general/SABV_Decision_Tree_for_Reviewers.pdf



Area 3: How do I write about it?

- Refer to Slide #45!
- Can pull ideas from here, and just explain it.
- Can be an expansion of your rigor description.
- Demonstrate you have reviewed literature that supports how you considered sex and/or other biological variables in the design of your study.

Reduced Criteria for Vertebrate Animals Section (VAS)

- A description of veterinary care is no longer required
- Justification for the number of animals has been eliminated
- A description and justification of the method of euthanasia is required only if the method is not consistent with AVMA Guidelines for the Euthanasia of Animals

See VAS Worksheet and Checklist:
http://grants.nih.gov/grants/olaw/vertebrate_animal_section.htm

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VAS: Only state the sex of the animals
Research Strategy (Approach): must address how sex is factored into the research design
VAS: only state total # of animals proposed
Research Strategy (Approach): justification on # of animals is an element of rigor

More on VAS

Typically, all of the required elements for the VAS can be addressed within 1-2 pages. **The VAS must not be used to circumvent page limits.**

- Source: https://grants.nih.gov/grants/olaw/vertebrate_animal_section.htm

Questions on Area 3: Relevant Biological Variables?

Next:

Area 4: Authentication of Key Resources

Area 4: Authentication of Key Resources

- Investigator determines what is a “key resource”
- Describe methods to ensure the identity and validity of key biological and/or chemical resources used in the proposed studies, including frequency of authentication
- What is a key resource?
  - May differ from laboratory to laboratory, over time
  - May have qualities or qualification that could influence results
  - Integral to the proposed research
  - Includes resources not generated by NIH funds
  - Ex: specialty chemicals, cell lines, antibodies, other biologics, etc
  - Standard laboratory reagents that are not expected to vary do not need to be included in the plan.
    Ex: buffers, common chemical or biological reagents

https://grants.nih.gov/reproducibility/faqs.htm#4846

Area 4: Cell Line Authentication and Antibody Validation | Background

Cell Lines

- Subject to many potential issues
  - ~15-35% of cell lines are contaminated by Mycoplasma[1]
  - Contamination w/ other cells: 2003 study of 550 cells leukemia-lymphoma showed ~15% contaminated[2]
- Obtain cell lines from a trusted vendor, use a fresh cell line before starting a series of experiments
- Check to see if cell line has been reported as contaminated: http://iclac.org/databases/cross-contaminations/

Antibodies

- Frequently used tool but can vary: batch-to-batch, non-specific binding[1]
  - 2011 analysis found ~25% of 246 antibodies used in epigenetic studies bound to more than one target[2]
- Use a trusted manufacturer
  - But STILL authenticate them!

1: Marx V. Cell-line authentication demystified. *Nature Methods* 2014;11:483. doi: 10.1038/nmeth.2932
2: Drexler HG, Dirks WG, Matsuo Y, et al. False leukemia-lymphoma cell lines: an update on over 500 cell lines. *Leukemia* 2003;17(2):416-26. doi: 10.1038/sj.leu.2402799

Baker M. BLAME IT ON THE ANTIBODIES. *Nature* 2015;521(7552):274-76. doi: 10.1038/521274a
Egelhofer TA, Minoda A, Klugman S, et al. An assessment of histone-modification antibody quality. *Nat Struct Mol Biol* 2011;18(1):91-+. doi: 10.1038/nsmb.1972

Area 4: NIH Provides Some Authentication Plan Guidance

- Cell line authentication might include short tandem repeat (STR) profiling and mycoplasma testing
- Chemical authentication might include liquid or gas chromatography, or mass spec, NMR, etc.
- Genetically modified animals or cells might include PCR amplification or Southern blot to confirm genome modification

https://grants.nih.gov/grants/guide/notice-files/NOT-OD-17-068.html

Area 4: Authentication Plan FAQs

Key resources purchased or obtained from outside source?

- It is *expected* to include a plan to independently verify the identity and activity of product before use
- If product is used long-term, consider the stability of the product and how validity of the product will be assessed over time
- Data sets and databases are not a "key resource" (see below)

An outside party is performing analyses? (Centers, LabCorp, etc.)

- If they're using a "key resource," may request information of authentication and include within own authentication plan

Proposing to establish a new resource?

- Research conducted for resource development, including plans for validating the resource, should be described in Research Strategy section

Secondary analysis of data collected through means of a "key resource?"

- NO- data sets, databases, machinery, or electronics are not a "key resource"

https://grants.nih.gov/reproducibility/faqs.htm

Area 4: Authentication Plan FAQs

Primary cell cultures?

- Proposing to collect primary cells for short-term culture as part of research, the activities (including plans for authentication identity of cells) should be described in Research Strategy
- If obtained from another laboratory, an authentication plan should be provided

Collecting biologics as part of research?

- One-time analysis/sample? Do **not** need authentication plan
- Storing samples for repeated use/using stored samples? Authentication plan needed

Imaging a key part of research?

- Using a "key resource" as part of imaging process? Authentication plan needed
- Otherwise, the parameters to ensure reproducibility of imaging needs to be addressed as part of rigorous experimental design in Research Strategy

Meritorious applications with concerns on adequacy of a authentication plan should be resolved by program official before proposal awarded

https://grants.nih.gov/reproducibility/faqs.htm

Area 4: Resources

COLUMBIA | RESEARCH

https://research.columbia.edu/ReaDI-Program

Area 4: Resource Authentication | The Attachment

- "If applicable to the proposed science, briefly describe methods to ensure the identity and validity of key biological and/or chemical resources used in the proposed studies. A maximum of one page is suggested."
  - https://grants.nih.gov/grants/how-to-apply-application-guide/forms-e/general/g.400-phs-398-research-plan-form.htm#11
- Key biological and/or chemical resources may or may not be generated with NIH funds and: 1) may differ from laboratory to laboratory or over time; 2) may have qualities and/or qualifications that could influence the research data; and 3) are integral to the proposed research. These include, but are not limited to, cell lines, specialty chemicals, antibodies, and other biologics.

- Standard laboratory reagents that are not expected to vary do not need to be included in the plan. Examples are buffers and other common biologicals or chemicals.

"NOT-OD-17-068: Reminder: Authentication of Key Biological and/or Chemical Resources." n.d. Accessed January 20, 2018. https://grants.nih.gov/grants/guide/notice-files/NOT-OD-17-068.html.

Area 4: Resource Authentication | The Attachment

- Information in this section **must focus only** on authentication and/or validation of key resources to be used in the study as described above.

- All other methods and any data must be included within the page limits of the research strategy.

- ***Applications identified as non-compliant with this limitation will be withdrawn from the review process***

"NOT-OD-17-068: Reminder: Authentication of Key Biological and/or Chemical Resources." n.d. Accessed January 20, 2018. https://grants.nih.gov/grants/guide/notice-files/NOT-OD-17-068.html.

Area 4: Reviewer Criteria

- Applicants should provide a brief plan (one page or less).
- The plan should not include authentication data.
- The plan may reflect existing guidelines or standards for authentication of a resource when such standards exist.
- Reviewers will discuss the authentication plan after scoring; comments on key resource authentication should not affect scores.
- Reviewers will comment in their written critiques and discussion at the meeting on the adequacy of the plan for key resource authentication; comments can be addressed by the applicant prior to award for meritorious applications.
- Reviewers should note if the authentication plan is missing from the application.

https://grants.nih.gov/grants/peer/guidelines_general/Reviewer_Guidance_on_Rigor_and_Transparency.pdf

Area 4: Reviewer Criteria continued

- Review of this attachment will occur after scoring; comments on key resource authentication **should not affect scores**. Reviewers will comment on the **adequacy** of the plan for key resource authentication; comments can be addressed by the applicant prior to award for meritorious applications.
- After scoring of the application is complete, Scientific Review Groups (SRGs) will comment on the plans for resource authentication in a manner consistent with the scientific goals of the research. Any concerns raised about the adequacy of the plans for resource authentication should be resolved by the program official before the application/proposal is funded.
- Best practices have started to emerge. See ReaDI resources.
- **NIH has Authentication Plan Examples:** https://grants.nih.gov/policy/reproducibility/resources.htm#authentication

Questions on Area 4: Authentication of Key Resources?

Next:

Resources

NIH Rigor and Reproducibility Training Modules

"These modules, developed by NIH, focus on integral aspects of rigor and reproducibility in the research endeavor, such as bias, blinding and exclusion criteria.

The modules are not meant to be comprehensive, but rather are intended as a foundation to build on and a way to stimulate conversations, which may be facilitated by the accompanying discussion materials. Currently, the modules are being integrated into NIH training activities."

https://www.nigms.nih.gov/training/pages/clearinghouse-for-training-modules-to-enhance-data-reproducibility.aspx

Rascal Courses

Five new Rascal courses of videos obtained from *A University Symposium: Promoting Credibility, Reproducibility and Integrity in Research (PCRI)* held on March 29, 2019. Each Rascal course includes an attestation to enable documentation of "credit" for watching the video.

TC4900	Recognizing Influences and Biases in Research
TC4901	Robust Science: Problems and Solutions
TC4902	"Put the Ph. Back in the Ph.D." and Other Novel Approaches to Scientific Training
TC4903	Journal Editor Perspectives on Rigor and Transparency
TC4904	Wrap-up: Observations and Lessons Learned

Research and Data Integrity (ReaDI) Program

COLUMBIA | RESEARCH

https://research.columbia.edu/ReaDI-Program

Boilerplate Text for Proposals

Boilerplate text on Columbia's Research and Data Integrity (ReaDI) and the PCRI Symposium that can be included in institutional letters of support or elsewhere in the proposal, depending on the requirements of specific Funding Opportunity Announcements (FOAs) and the new application instructions.

https://research.columbia.edu/nih-institutional-training-grants

Participate in Peer Review

- https://grants.nih.gov/grants/peer/becoming_peer_reviewer.htm
- Contact: ReviewerVolunteer@mail.nih.gov

- NIH Center for Scientific Review's Early Career Reviewer (ECR) Program.
- https://public.csr.nih.gov/ForReviewers/BecomeAReviewer/ECR

QUESTIONS?