Rutgers Biomedical Rigor and Reproducibility Requirement Guidelines for PhD student Propositional Qualifying Exam B

In order to integrate the concepts and training of Rigor and Reproducibility throughout graduate education, Rutgers Biomedical PhD students are required to include the elements of Rigor and Reproducibility in their Propositional Qualifying Exams B as of January 2020. Below are the 4 areas that should be covered in the proposal either as a separate section or integrated into the proposal. There are also examples and resources in this document.

Source: https://grants.nih.gov/policy/reproducibility/index.htm
FAQ: https://grants.nih.gov/reproducibility/faqs.htm#II

Four key areas to be covered:

1. The rigor of the prior research

- A careful assessment of the **rigor of the prior research** that serves as the key support for a proposed project helps to identify weakness or gaps in a line of research. NIH expects applicants to describe the general strengths and weaknesses in the rigor of the prior research (both published and unpublished) that serves as the key support for the proposed project. It is expected that this consideration includes attention to the rigor of the previous experimental designs, as well as the incorporation of relevant biological variables and authentication of key resources. Applicants are expected to include plans to address any weaknesses or gaps identified.

2. Rigorous experimental design for robust and unbiased results

- **Scientific rigor** is the strict application of the scientific method to ensure robust and unbiased experimental design, methodology, analysis, interpretation and reporting of results. NIH expects full transparency in proposing and reporting experimental details so that reviewers may assess the proposed research and others may reproduce and extend the findings.

3. Consideration of relevant biological variables

- **Biological variables**, such as sex, age, weight, and underlying health conditions, are often critical factors affecting health or disease. In particular, sex is a biological variable that is frequently ignored in animal study designs and analyses, leading to an incomplete understanding of potential sex-based differences in basic biological function, disease processes and treatment response.
- NIH expects that sex as a biological variable will be factored into research designs, analyses, and reporting in vertebrate animal and human studies. Strong justification from the scientific literature, preliminary data or other relevant considerations must be provided for applications proposing to study only one sex.

4. Authentication of key biological and/or chemical resources

- **Key biological and/or chemical resources** include, but are not limited to, cell lines, specialty chemicals, antibodies and other biologics. Key biological and/or chemical resources may or may not be generated with NIH funds and:
  - may differ from laboratory to laboratory or over time;
  - may have qualities and/or qualifications that could influence the research data;
  - are integral to the proposed research.
- The quality of resources used to conduct research is critical to the ability to reproduce the results. Each investigator will have to determine which resources used in their research fit these criteria and are therefore key to the proposed research.

- **Scientific Rigor Examples from the NIH website.** Note that these examples each contain various parts of areas 1 – 4 above but not necessarily all 4 areas in each paragraph. The 4 areas can be addressed in other parts of the proposal besides specific aims including the intro and experimental plan so it is up to you where you put the content.

Example #1

Aim 3: Male and female mice will be randomly allocated to experimental groups at age 3 months. At this age the accumulation of CUG repeat RNA, sequestration of MBNL1, splicing defects, and myotonia are fully developed. The compound will be administered at 3 doses (25%, 50%, and 100% of the MTD) for 4 weeks, compared to vehicle-treated controls. IP administration will be used unless biodistribution studies indicate a clear preference for the IV route. A group size of n = 10 (5 males, 5 females) will provide 90% power to detect a 22% reduction of the CUG repeat RNA in quadriceps muscle by qRT-PCR (ANOVA, α set at 0.05). The treatment assignment will be blinded to investigators who participate in drug administration and endpoint analyses. This laboratory has previous experience with randomized allocation and blinded analysis using this mouse model [refs]. Their results showed good reproducibility when replicated by investigators in the pharmaceutical industry [ref].

Example #2

Aim 1: Primary screen: In this high throughput screening assay, we combined the SMN promoter with exons 1-6 and an exon 7 splicing cassette in a single construct that should respond to compounds that increase SMN transcription, exon 7 inclusion, or potentially stabilize the SMN RNA or protein [refs]. The details of the assay and the SMN2-luciferase reporter HEK393 cell line have been extensively validated [refs]. Each point is run in triplicate, the compounds are tested on three separate occasions, and the results are averaged to give an EC50 with standard deviation. Secondary screen: …We analyze SMN protein levels by dose response in quantitative immunoblots with statistical analysis by one-way ANOVA with post-hoc analysis using Dunnett or Bonferroni, as appropriate.

Aim 2: Each set of compounds will include a blinded negative control compound that has been determined to be inactive and that is solubilized in the same manner as test compounds. Mice will be randomly assigned within a litter, and data will be collected and submitted to the PI. For compounds that demonstrate extended survival, the PI will be sure to have these tested in {the collaborators'} labs, and data will be merged and evaluated. To calculate the number of the experimental mice, we will perform an SSD sample size power analysis to ensure that the

appropriately minimal number of mice is used in each experimental context. Typically for each compound in life span studies, we will need ~20 SMA animals in the treated group; ~20 SMA animals in the vehicle treated group; ~20 SMA animals in the untreated group. If we can administer the compound in aqueous solution without expedient, the vehicle and untreated groups might be combined, as these should have identical survival. Therefore, no more than 80 SMA animals will be needed per compound.

Example #3
Aim 2: Intensity signal data will be transformed into log values and then modeled by longitudinal methods (reference cited). Specifically, the composite difference in mean intensity signals over time between the bi-specific T cells vs. control groups is assumed to be 2.8 logs with a composite standard deviation of 2.2 logs. Furthermore, we will assume at least five repeated measurements per mouse after T cell infusion and a within-mouse intra-correlation coefficient equal to 0.50. Thus, a sample size of 10 mice per group will provide at least 80% power to detect the above difference between treated versus control group with a 5% significance level. Log-rank test will be used to compare the survival distribution between groups.

VAS: Animal numbers are based on the requirement to perform each experiment (power and sample size calculations are described in the Research Strategy), which includes an independent experimental repeat.

Example #4
Aim 1: Statistical considerations: In our preliminary studies consisting of this same cohort of DFUs (n=100) and utilizing 16S rRNA sequencing, we were able to detect dimensions of DFU microbiome, including microbial diversity, that were significantly associated with DFU outcomes. We therefore anticipate that the sample size will provide sufficient power to detect significant differences using metagenomic sequencing, as this is a more sensitive and less-biased assay of microbial identification and diversity.

Aim 3: Random Forests, a machine learning approach for classification, will be used to determine which metagenome features differentiate groups (e.g., antibiotics vs. no antibiotics; pre- vs. post-debridement). Random Forest uses a bootstrap method to assess test error, ideal in our situation of small sample size (n=18). For diversity and load measures, significance between groups will be assessed using non-parametric Wilcoxon rank-sum tests.

- ## Resources and Tools for Rigorous Experimental Design

The Experimental Design Assistant – EDA (https://www.nc3rs.org.uk/experimental-design-assistant-eda)
The Experimental Design Assistant (EDA) is a free online tool from the NC3Rs, designed to guide researchers through the design of their experiments, helping to ensure that they use the minimum number of animals consistent with their scientific objectives, methods to reduce subjective bias, and appropriate statistical analysis. Additional guidance on experimental design, including sample size estimation is provided here (https://eda.nc3rs.org.uk/experimental-design-group).

protocols.io
A free, up-to-date, crowd-sourced protocol repository for researchers.

EQUATOR Network Reporting Guidelines (http://www.equator-network.org/)
The EQUATOR (Enhancing the QUAlity and Transparency Of health Research) Network is an international initiative that seeks to improve the reliability and value of published health research literature by promoting transparent and accurate reporting and wider use of robust reporting guidelines. The Library contains a comprehensive searchable database of reporting guidelines and also links to other resources relevant to research reporting.

- ## Authentication Plan Examples

Chemicals
All acquired compounds and reagents will be authenticated for both identity and purity using standard techniques and methods for the characterization of small molecules according to the guidelines of the American Chemical Society (http://pubs.acs.org/page/jacsat/submission/authors.html). Experimental techniques employed routinely in the lab for the authentication of chemicals include NMR, EPR, and UV-visible absorption spectroscopy, mass spectrometry, X-ray diffraction analysis, cyclic voltammetry, magnetometry, and combustion analysis. This information will be included in peer-reviewed publications on the project, along with details on commercial sources for precursors and any necessary purification, handling and storage of reagents and products (e.g., under an inert atmosphere).

Plasmid DNA
Plasmid NNNN-NNN for the expression of NNNN in mammalian cells was obtained from Addgene (#NNNN) and validated through Sanger sequencing.

Antibodies
Antibodies for proteins associated with NNNNN were obtained from commercial manufacturers providing hybridoma clone identification, lot number and appropriate references. The specificity of the antibodies employed in this study will be authenticated by immunoblot analysis (including knockdown samples when possible) and appropriate controls are included in every experiment. In addition, we will monitor the Antibody Registry database (http://antibodyregistry.org/) to be aware of any issues observed by other investigators with antibodies in use in our laboratory.

Cell lines
The breast and colon adenocarcinoma cell lines (MCF7, MDA-MB-231, Caco-2) and the normal fibroblasts (MRC5 and CCD18-co) employed in this project so far were purchased from the American Tissue Culture Collection (ATCC). The pancreatic cancer cell lines to be used for testing of constructs targeting NNNN are available in the lab and have been authenticated by sequencing. Additional cell lines for the project will be obtained from ATCC or from the National Cancer Institute through the RAS Initiative. All our cell lines are authenticated periodically at the University of Arizona Genetics Core facility by Short Tandem Repeat (STR) profiling. Our cell cultures are continuously monitored for doubling times and morphology, and they are tested periodically for contamination from mycoplasma and bacteria using standard detection kits. Each culture is passaged less than 20 times. We typically assess the quality of commercially obtained reagents for cell-based assays (e.g., MTT, 2',7'-dichlorodihydrofluorescein) by NMR spectroscopy and/or liquid chromatography/mass spectrometry (LC-MS).