A Checklist for Experimental Design in a Research Program

1. **Framework**: What is the broad, open-ended question that you want to answer? The framework is the philosophical construct in which the scientist performs the experiments to develop sufficient understanding to predict the future with statistical accuracy (26). The question is open ended rather than a more limited binary "yes/no" question, typical of a hypothesis, that may be a restrictive filter because it is a premature leap to a particular conclusion. Ex of binary questions: "Is the sky red?" vs. "What color is the sky?" and "Does enzyme X cause cancer?" (13)

2. **Inductive Space**: Decide what prior scientific knowledge informs your question and read this peer-reviewed scientific literature. Ex: "What is the function of an elephant’s trunk?" vs. "What is the function of a space alien’s trunk?" "Does MuRF1 do X?" vs. What is the function of a space alien’s trunk?" "Does MuRF1 resemble any proteins of known function?" > MuRF1 resembles E3 ubiquitin ligases > "Does MuRF1 function as an E3 ubiquitin ligase?" > "What are the protein substrates for MuRF1?" What constitutes a "representative case"? The caveats in "What visible color is the sky, at 10:00 AM, while standing at a particular point and directing one’s instrument of measurement 45° upward from the horizon toward Mars on December 15, 2007?" acknowledge a prior understanding the potential effects of relevant conditions. Included is the need to measure the subject at multiple points to develop an understanding of which results are "usual", what variability from the "norm" might be, and how that variability might be interpreted. In this case, the reason for measuring over these multiple points is not for increasing statistical precision; that is achieved by multiple measurements under fixed conditions.

3. **System**: What tools (methods) will you use to answer your question?

4. **System Controls**: How do you know your system works? How do you know your system can provide the type of data you require? Is the chosen system well matched to your question, or might a different system be better? Is the sample trustworthy and the measuring system reliable?

5. **Experiment**: What are you going to do to answer the question? Be sure that measurements are taken multiple times and that you are measuring the effect in a representative fashion. Study the effect over time and over a range of experimental conditions. Do a dose-response with any experimental agent. Try to determine the "representative case" for the subject if that is relevant to the question. Consult a statistician and discuss the mode of analysis for your data and how many data points are required.

6. **Establish criteria** for the effect in advance of the experiment.

7. **Negative controls**: What negative controls are required? Is there an "all but X" control available? Is the measurement system sufficiently specific to correctly detect the absence of "X", such as in the presence of potential cross-reactions?

8. **System-positive control**: How do you know that the system is still operational? What validated positive controls are necessary to prove that the thing you want to measure was actually measurable within the context of the experiment?

9. **Effect-positive controls**: How do you know that the effect you want to measure can be produced in your system? What positive controls are necessary to produce the effect?

10. **Assumption controls**: If X is being measured, what else can be measured that has been shown to occur when X occurs? If you think Y has happened as a result of X, is there something else you can measure that has been shown to also happen when Y happens? A clear understanding of where and when unwarranted assumptions may bias data and cause misinterpretation of results is needed and these unwarranted assumptions then turned into questions (171). Is the experimental material or subjects sufficiently representative of type? Of the subjects to which the model will be applied? Assumption controls are required when complex systems with thousands of variables and pathways are evaluated in a different species context (mice vs. humans), reduced to a tissue context (what influences from other tissues are eliminated?), further reduced to a cellular context (what influences from other cells are eliminated?) and finally to an isolated molecular context (what substrate effects are eliminated?).

11. **Do the Experiment**

12. **Experimentalist controls**: Analyze the data in a blind fashion; separate those doing subjective interpretation from knowledge of allocation or other potentially biasing information.

13. **Repetition**: Repeat the experiment using the same criteria and methodology.
14. **Model building:** What is the answer to the question? A model represents the pertinent features of the subject being analyzed.

15. **Model check:** Is the answer responsive to the question?

16. **Prediction:** Does the model predict what will happen again? Repeat the experiment.

17. **Extension:** Does the model hold in different circumstances?

18. **Change the system:** Approach the question in another way, establishing necessity and sufficiency. Can the experimental model be verified using an entirely different methodology than that used to derive the model? When dealing with complex systems, this step is very important because that two different methods will produce the same experimental artifact is very unlikely.

19. **Change the scientist:** If complex manipulations are involved, can others reproduce the effect?

20. **Present the data:** What do others think of the result? What do others think of the interpretation of the result?

21. **Predict the future:** Does the model continue to represent what will happen under various circumstances?

22. **Amend the model** as instances are found in which it is not predictive. Limit your claims as you discover limits to your claims.

23. **If the system is reductionist** in nature, realize that and apply the model in a non-reductionist or less-reductionist setting (e.g., isolated molecule > cellular context > tissue context > whole body context).

24. **A model that is limited but verifiable is superior** to a model that is comprehensive but not predictive.

25. **If an idea cannot be subjected** to experimentation and verification, it is not relevant to your project. Give the data the final word. Redesign the experiment and revalidate the model as knowledge is gained. When you obtain predictive power with your model, you have entered the on-going process of empiricism and discovery.