The scientific method & experimental design

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The synthetic biology design cycle

Design a DNA/genome

Design a pathway

Design experiments

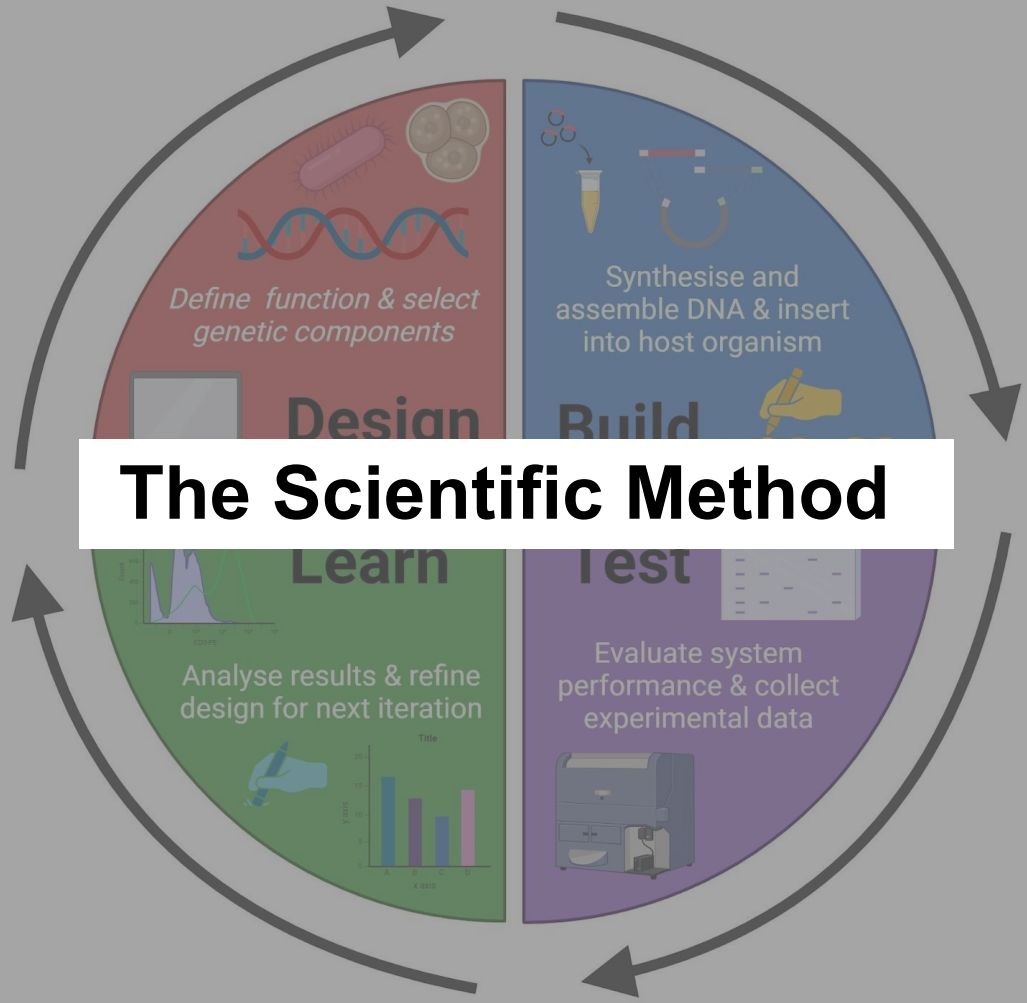
Select a chassis

Data analysis

Trouble Shooting

Modeling/Simulation

Re-Design



The Scientific Method

DNA assembly

Gene editing

Synthetic chromosomes

Introduce into chassis

PCR/qPCR

Protein biochemistry

Next-Gen-Sequencing

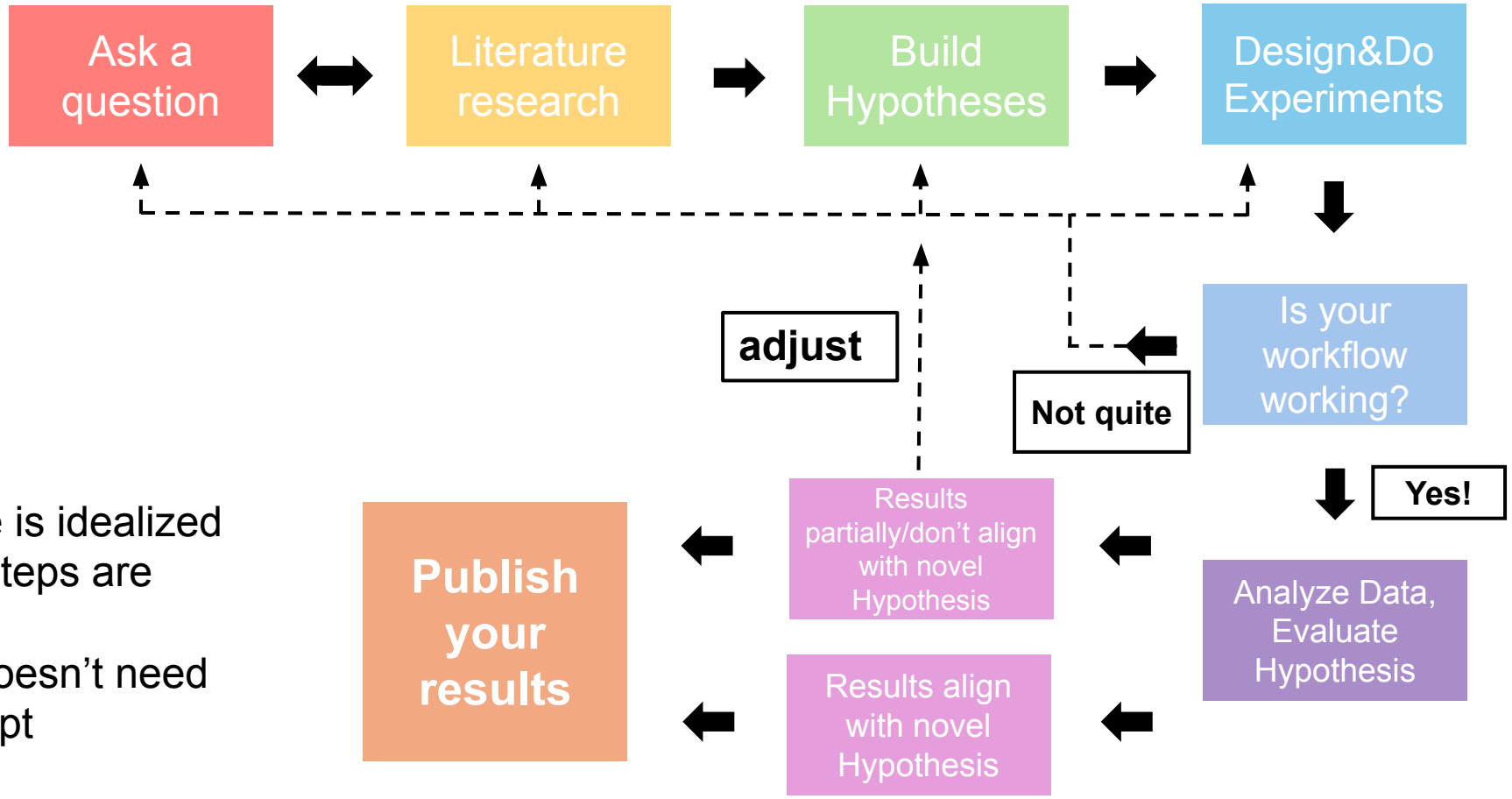
Flow-cytrometry

Mass spectrometry

Microfluidics

What is the scientific method? An overview

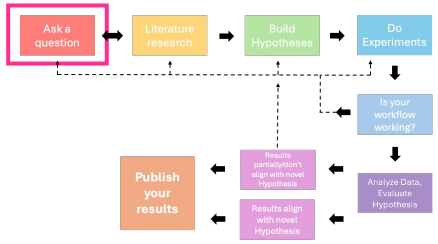
→ Allows for the acquisition of empirical knowledge



This scheme is idealized

- Not all steps are needed
- Order doesn't need to be kept

Everything starts with a question



Interest

A question/interest you always had

Affects/specific to your immediate/wider environment

A problem you want to solve

Something you want to create

Something that is new/unexplored/untested

Something you want to work on in the next weeks/months/(years?)

Feasibility

Can you build/**grow**/test/measure/quantify/model it?

Are you achieving something novel?

Do you have the equipment & material?

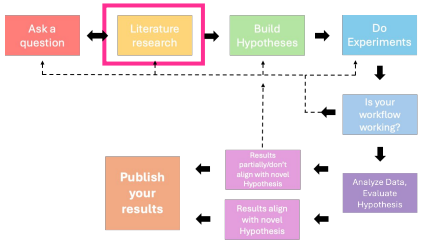
Do you have time to do it?

Is there literature/previous work that proves feasibility?

Is it safe/ethical to do?

- Answer a How…? What…? If ...? Does…? When..?, Which, Why, or Where? Question
  - Can be quantitative: How does changing…affect…?
  - In HTGAA: Can be explorative: Is it possible to..?
- The more specific the question, the better.

Literature research



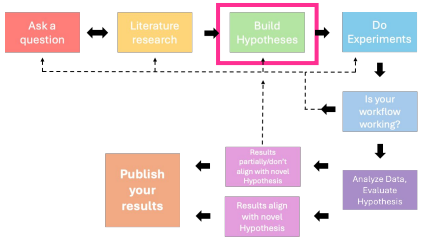
→ Avoid reinventing the wheel and repeating mistakes!

→ Create a research plan

- Keywords
- Determine your (basic) knowledge gaps
- What is the status quo on this topic/goal?
- Which labs/authors are important in this field?
- What are the standard experiments of this field/state-of-the art methods?
- What do you need to answer your research question/grow your project?

That sounds like a lot. How do I do this the best?

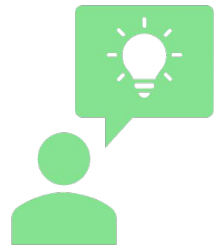
→ **How to do Literature Research** by Anastasia Bernaz right after this talk!



Build your hypothesis

- You have defined a question ☑
- You did your literature research ☑

→ What do you think?



The scientific hypothesis:

→ An educated guess of an answer to your question, from which you predict logical consequences which you can test experimentally

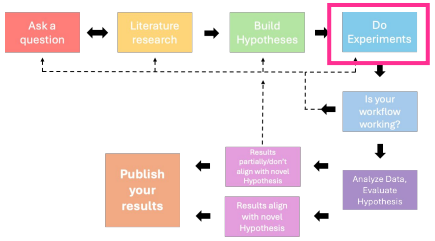
- Can be broad or specific
- Must be falsifiable (there exist the potential of possible outcomes of an experiment or observation that conflict with predictions)

Example Hypotheses:

- It is possible to change the fluorescence intensity of a fluorescent protein by changing the amino acids in its fluorophore
- It is possible to increase host-resistance of bacteriophages by introducing changes to their genomes

→ See yesterday's talks about some of our hypotheses and how you can help to test them!

Design and perform experiments



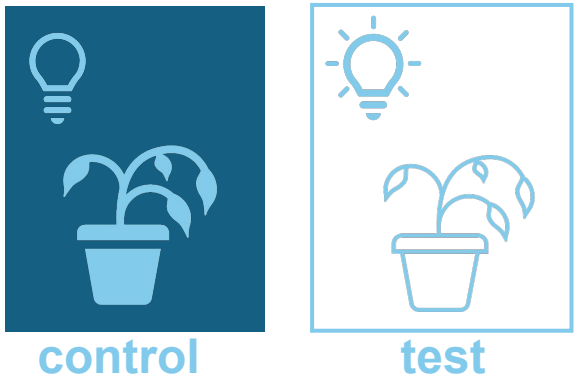
→ Put your hypothesis and the deduced predictions to the test

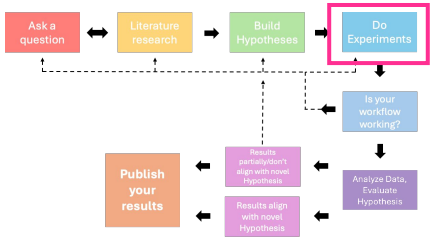
Good scientific practices to design your experiment

Later today:

- **Tools for molecular biology** by Adrian Filips
- **Wetlab Methods** by Dr. Jieming Chu, Adrian Filips, Martina Armas, Benjamin Arias

- The experiment has to be fair: You test one independent variable at a time
    - All controlled variables remain constant
    - Dependent variables are measured
    - The Independent variable can take on
        - A binary value (experiment vs control)
            - Experimental group (change of independent variable)
            - Control group (reference value, natural state/untreated/wild type)





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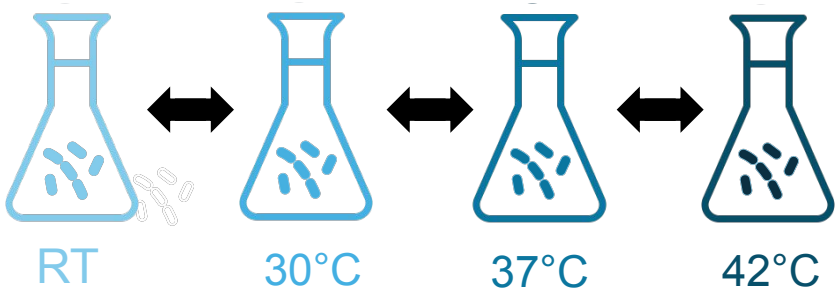
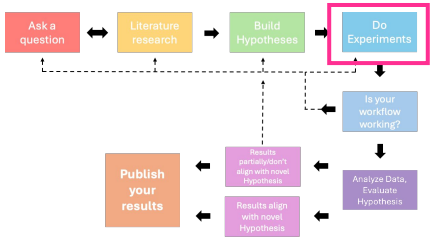
Good scientific practices to design your experiment

- The experiment has to be fair: You test one independent variable at a time
    - All controlled variables remain constant
    - Dependent variables are measured
    - The Independent variable can take on
        - A binary value (experiment vs control)
        - or multiple values (experimental groups compared to each other)



→ You determine which experimental set up is appropriate to test your hypothesis

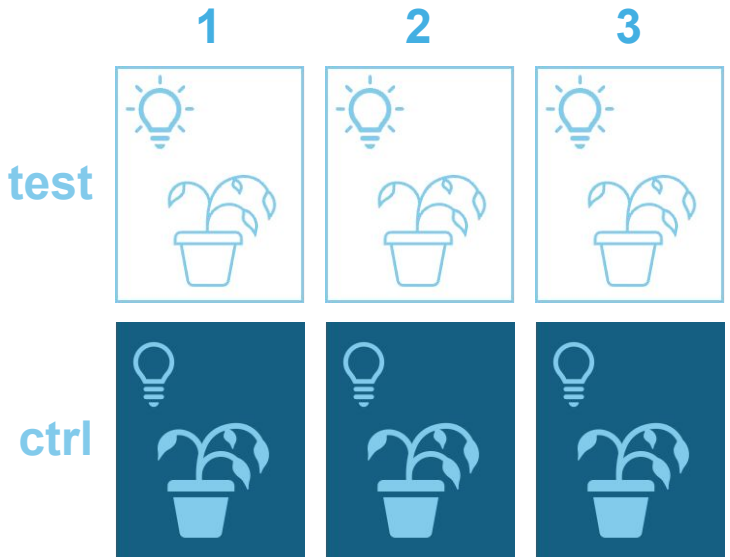


Design and perform experiments

→ Put your hypothesis and the deduced predictions to the test

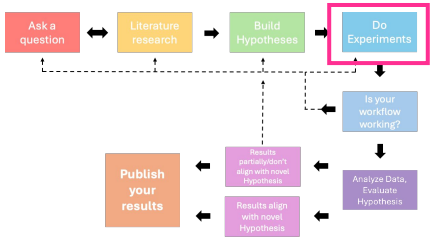
Good scientific practices to design your experiment

- The experiment has to be fair: You test one independent variable at a time
- The experiment has to be replicable: You repeat your experiment at least three times
    - Experiment can be repeated subsequently (trials)
    - Or sometimes, you can perform experiments on three replicates at the same time (biological replicate)



→ Careful: Biological replicate vs Technical replicate

- Biological replicates show biological variability
    - You want to capture natural biological variation of your test subject
    - Three independent samples, from different biological source materials (different animals/donors, cultures, sections, tissues, plant individuals)



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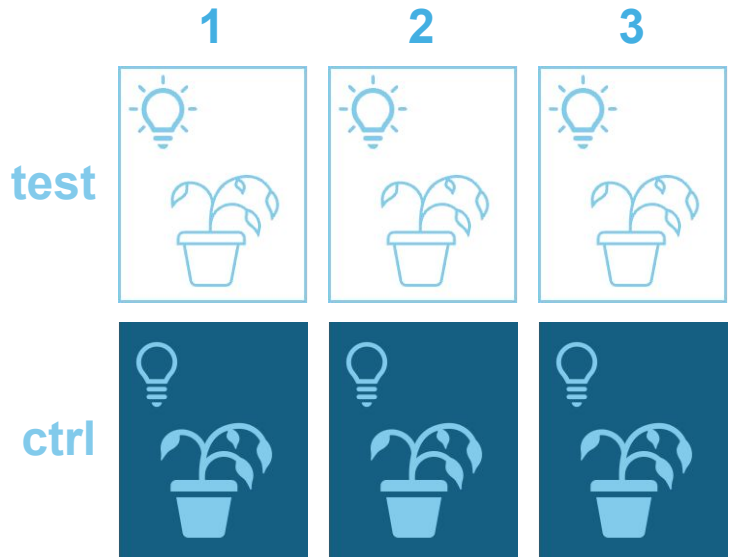
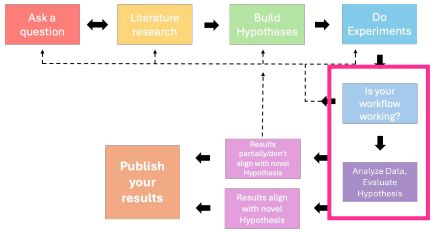
→ Careful: Biological replicate vs Technical replicate

- Biological replicates show biological variability
- Technical replicates show variability of the experimental process
    - You want to assess the precision and reproducibility of the lab procedure, equipment, or reagent
    - Need to use the same source sample, repeat experimental procedure three times (leaves from one plant, cuttings from one plant, same culture, same tissue sample etc.)



Plants of each group have same modification but grew from different seeds

Document, analyze and evaluate results



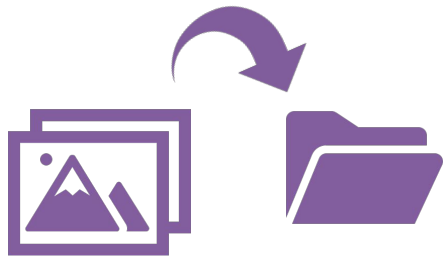
Scientific documentation

- All experiments, procedures and results need to be documented in your labbook.
  - Can be analogue, handwritten
  - Or digital/online → Day 3, **Benchling Basics** by Dr. Cholpisit (Ice) Kiattisewee
- Some information you need to document
  - Date!!!
  - Project/Context
  - Experimental procedures
    - Material, Equipment, Reagents incl. manufacturers
    - Methods/Protocols/Kits
    - Spontaneous adjustments/changes
    - Even code/simulation/program runs could be worth it
  - Results/Data
    - Images/Photos
    - Measurements
    - Calculations/Data Processing (e.g. Excel Sheets)

    → All with labels, legends and notes!

→ The more information you document, the better

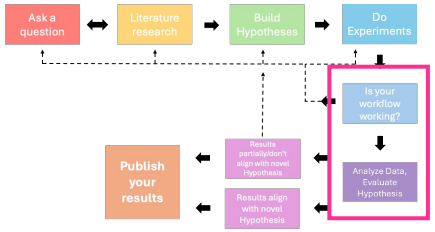
- Other electronic lab notebooks: SciNote ELN
- Experimental protocols: Protocols.io



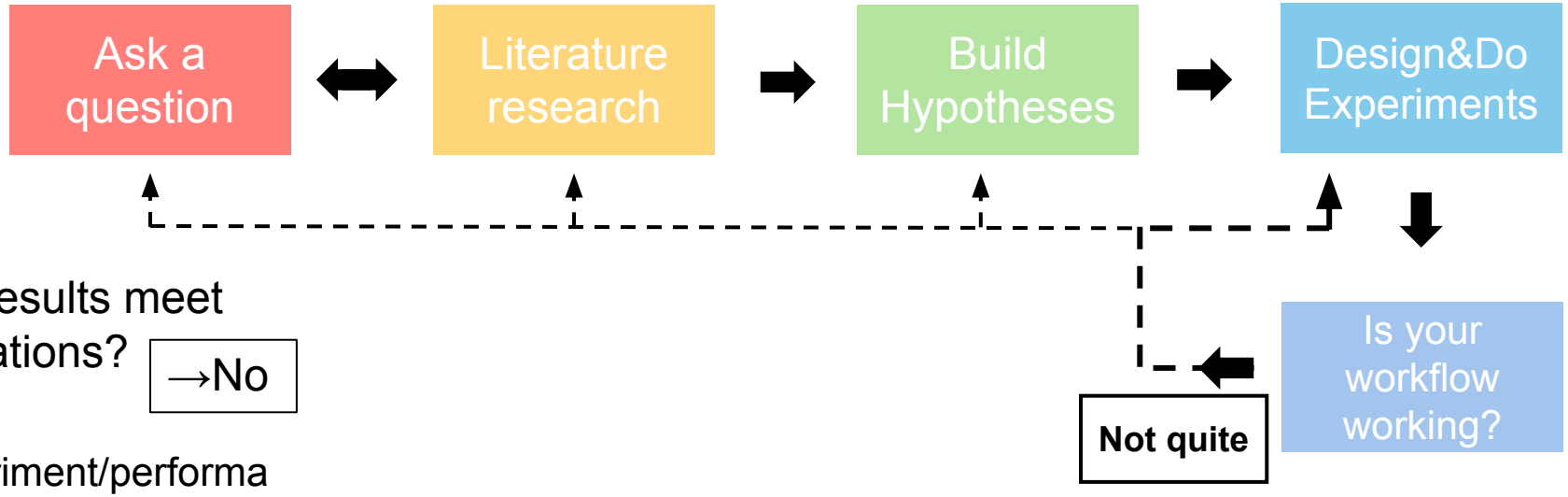
Keep your raw data safe and & secure!

→ Later today: **How to make Scientific Figures**

Document, analyze and evaluate results



Analyzing experiments and results



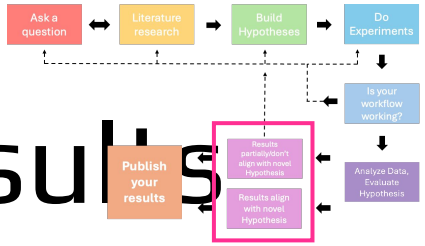
→ Do your results meet your expectations? →No

→ Was your method/experiment/performance appropriate to test your hypothesis in the first place? (check documentation) →No

→ Troubleshooting and experimental optimization/re-adjustment, repeat

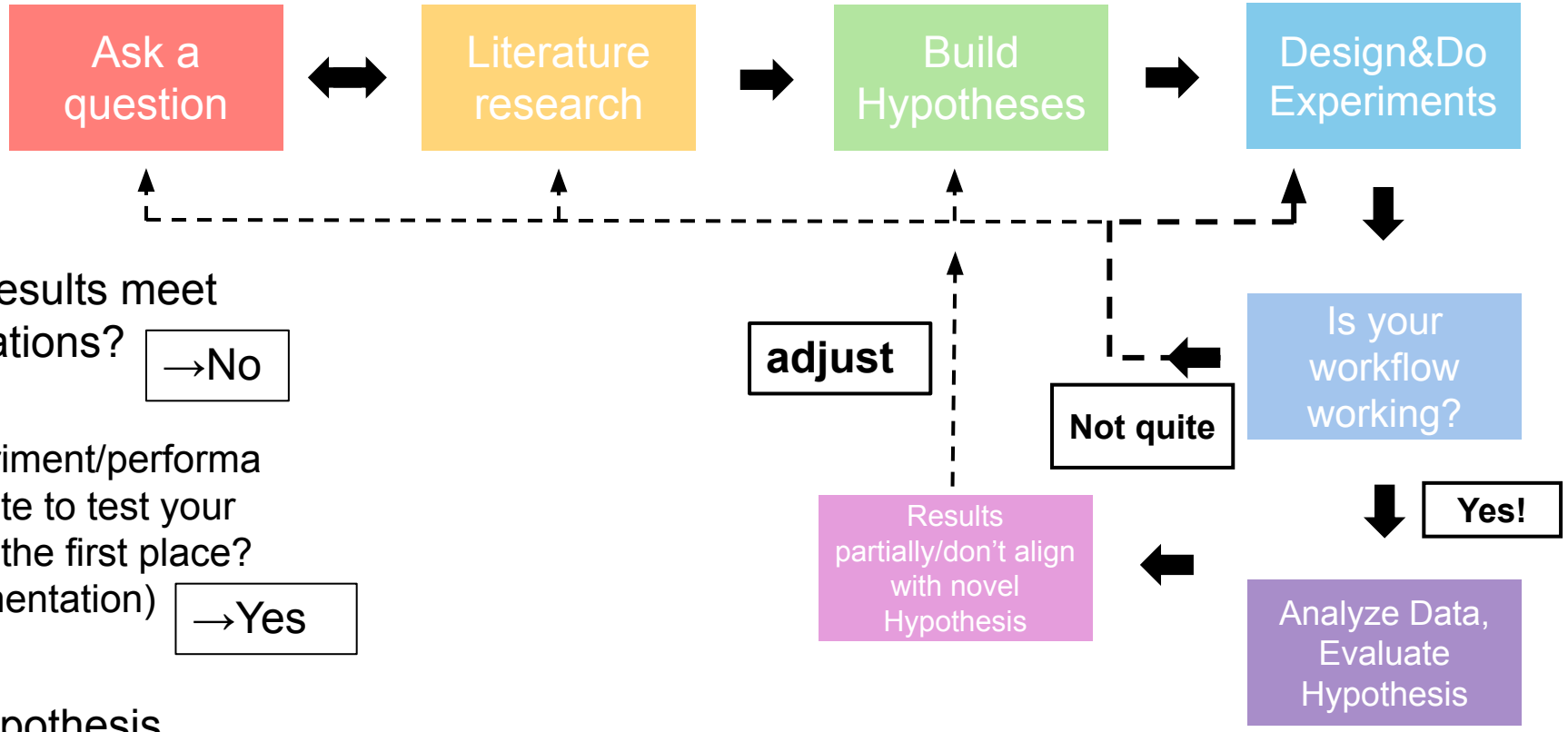
→ Ask for help! Cooperation and scientific discussion can lead to better experiments

→ See yesterday's talk: **How to onboard on Discourse**, by Dr. Derek Jacoby



Document, analyze and evaluate results

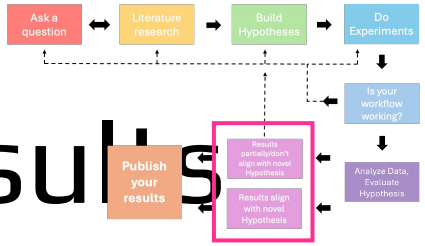
Analyzing experiments and results



→ Do your results meet your expectations? →No

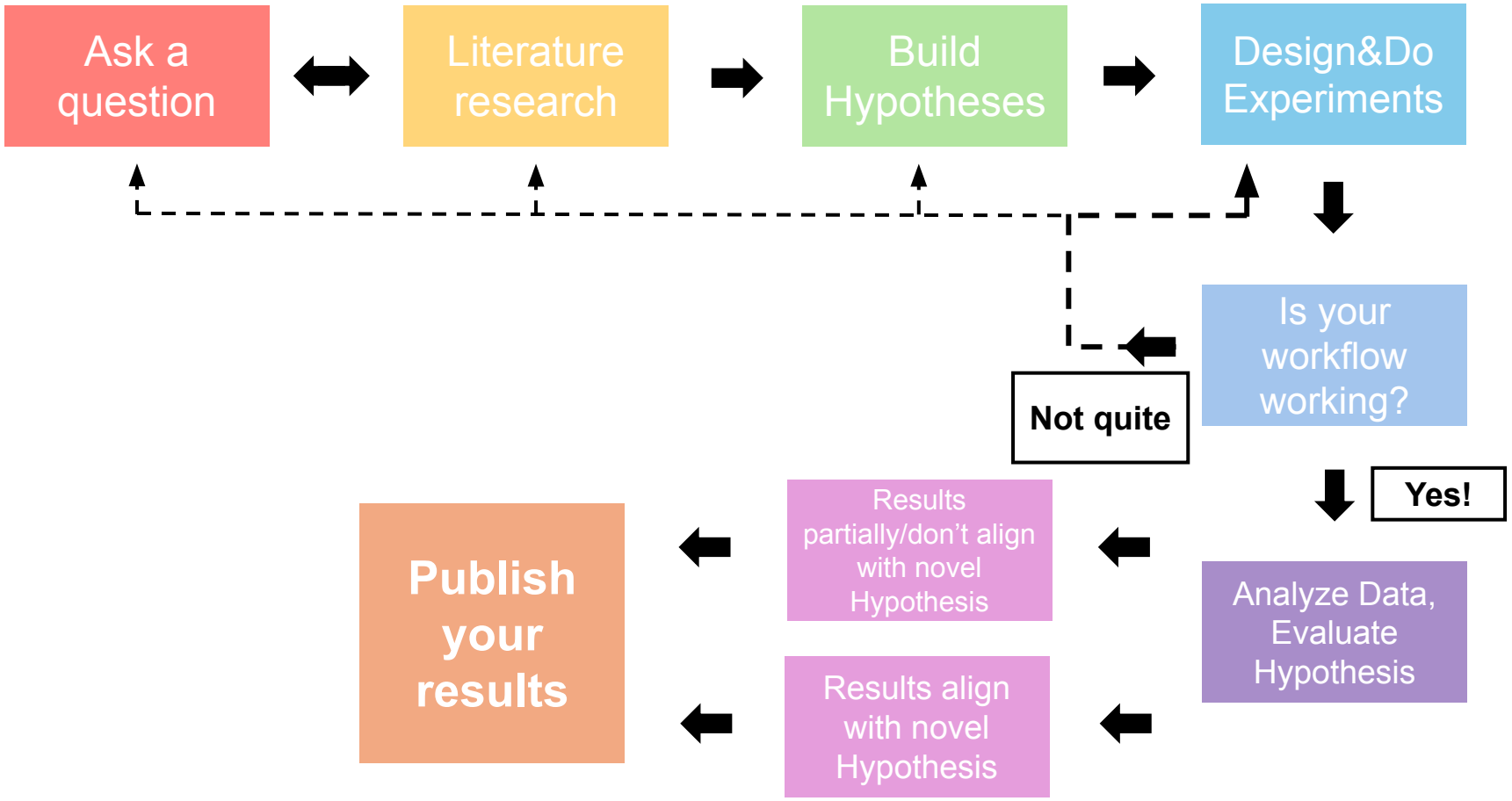
→ Was your method/experiment/performance appropriate to test your hypothesis in the first place? (check documentation) →Yes

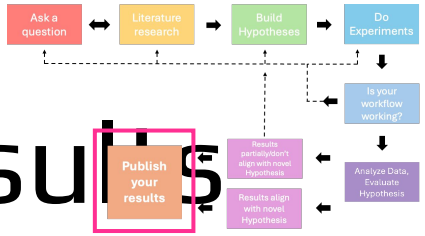
→ Adjust hypothesis



Document, analyze and evaluate results

Evaluating results





Document, analyze and evaluate results

Publication of your results

- Create a story line
  - IRL: Often not chronologically accurate
  - For HTGAA: Your choice. But stories are more fun.
- Decide on the format of your publication
  - IRL: Pick and choose data, choose number of publications, the appropriate journal etc.
  - For HTGAA: Your personal website!

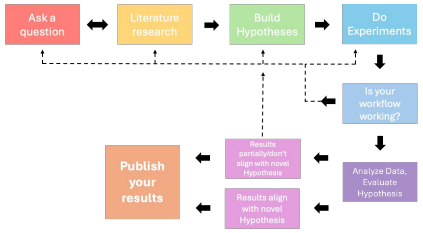
→ Day 3: **How to navigate the HTGAA website** by Greg Galparin

- Decide who contributed
  - IRL: Ideally everyone who contributed to data generation + the PI. Reality can be sometimes nasty or very generous.
  - For HTGAA: It's always good practice to mention if other students significantly helped you or contributed to your project! This is pretty low-stakes.
- Prepare your data
  - IRL: Your main results are in the paper as figures, everything else, including all your raw data need to be documented and accessible (e.g. supplements) fpr maximum transparency and scientific rigor.
  - For HTGAA: Document all your efforts, however far you got, however it suits your story. But be scientifically honest.

→ Later today: **How to make scientific Figures**

- Have your project reviewed
  - IRL: The journal picks a limited number of experts of your field to evaluate your work. You might have the choice to rank potential reviewers by your preference. Once you get pass the reviewers you're good.
  - HTGAA: TAs evaluate your work, but your work is online no matter what. In the best case scenario, you have already discussed your results with other students and the TAs beforehand.

Some additional things



- At all times during your research: Honesty is key, always show your results as they come.
- Pursue multiple ideas and projects when you start out. Continue with those that catch on and show the most promise. It's always good to have a back-up project
- Keep track of where you are getting your assumptions from: which literature is behind it? Is it really what the findings of the publication say? Always check in
- If you can, black-box your experiments to avoid bias during experimentation (e.g. reversibly cover the labels on your samples)
- Always document everything (!)
- Publish your research in such a way that it is easy to follow and replicate. Obfuscating your results and methods will prevent people from finding, replicating, improving upon and citing your research at best and can be scientifically dishonest at worst.
- Collaboration is key: You oftentimes get farther and more creative with a project, when working in a duo/group




Thank you for your attention & have fun designing research!