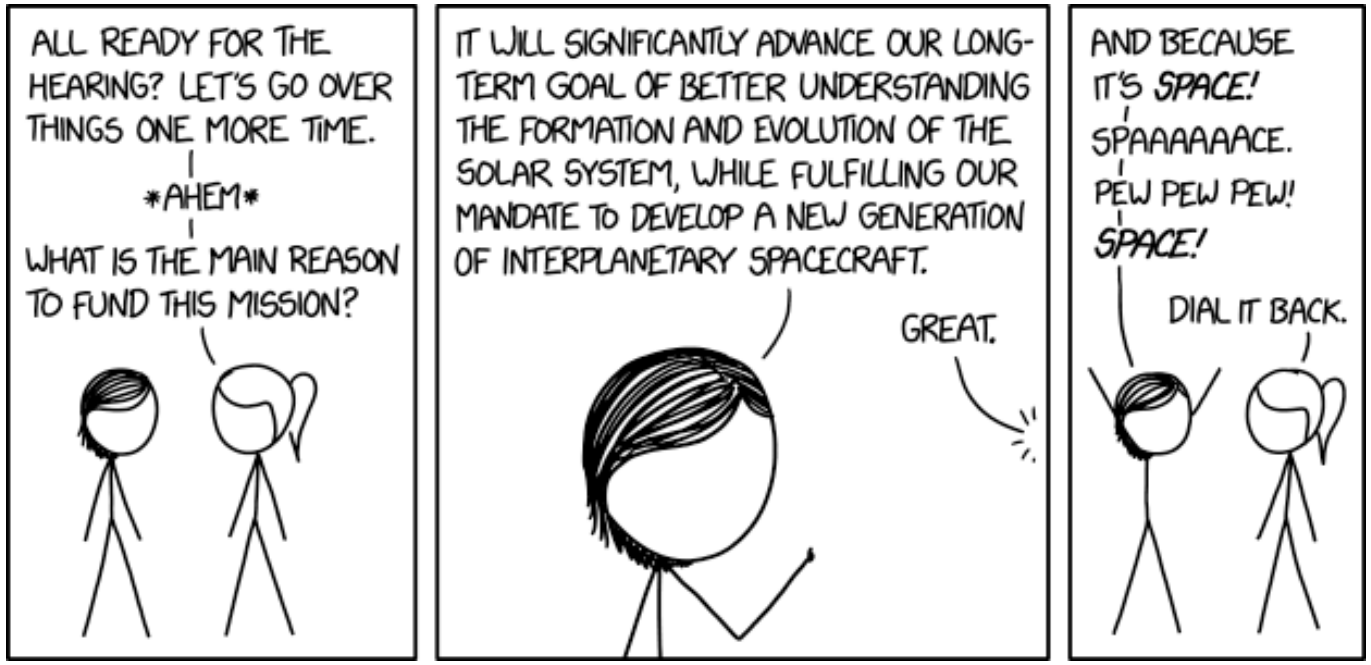
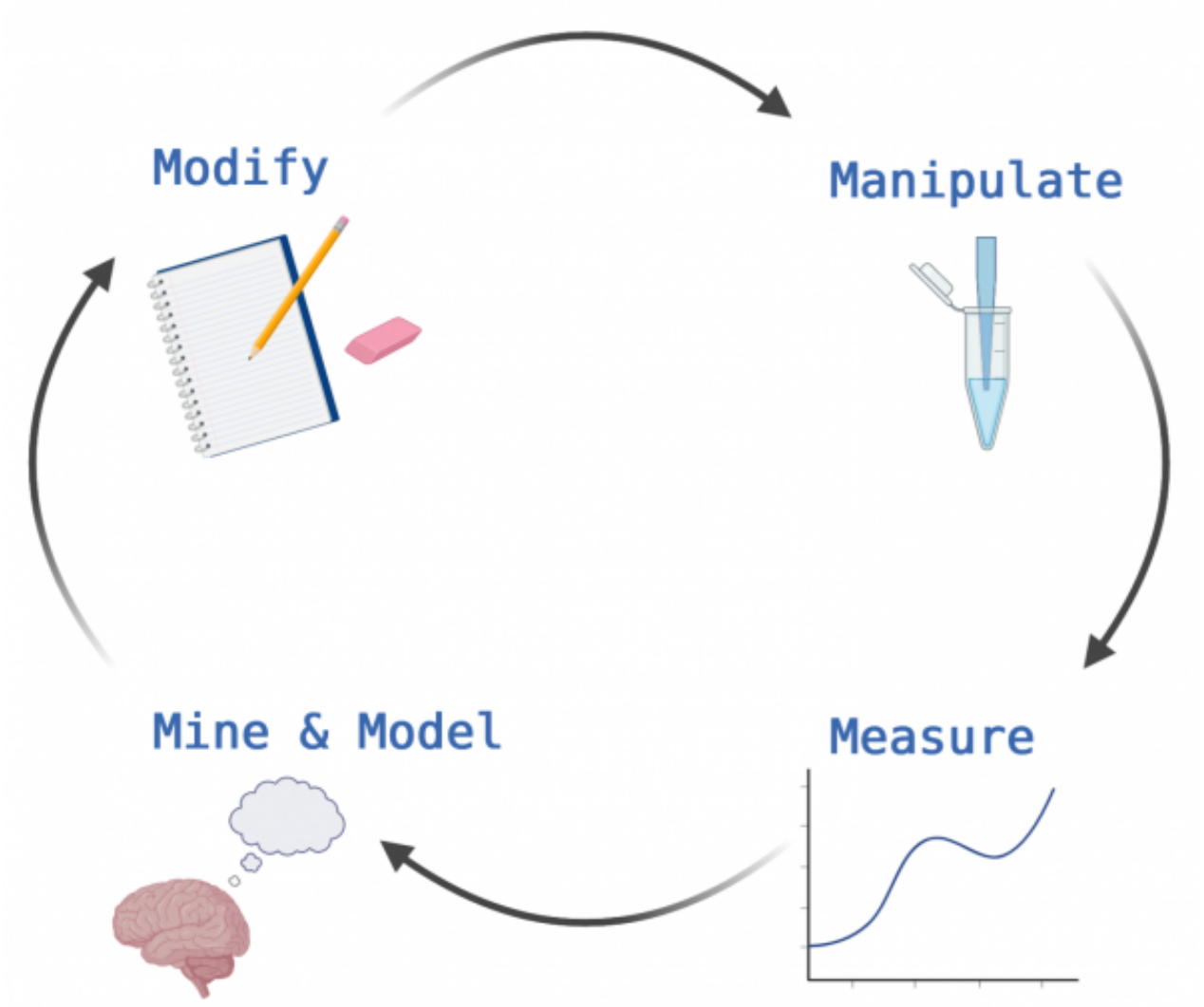
M3D4: Examine features in gRNA-targeted sequences

1. Prelab discussion
2. Examine sgRNAs for regulatory binding sites
3. Discuss your research proposal with a peer



Mod 3 Overview



Research goal: Improve ethanol yield in *E. coli* MG1655 using the CRISPRi system by expanding upon previous research to design an optimized sgRNA targeting sequence.

What have we done so far?

- Identified targets in MG1655 mixed-acid metabolic pathway
- Established CRISPRi system for our model
- Aligned previously used target RNA
- Examined Ethanol produced using CRISPRi system

What are we doing next?

- Evaluate sgRNA primers based on targets and ethanol production data
- Design improved sgRNA primers for increased ethanol production

Today in lab

PWM (species)	Start Position	End Position	Strand
AbrB | Bacillus subtilis (strain 168)	134	141	-
AbrB | Bacillus subtilis (strain 168)	798	805	+
AlgU (-10) | Pseudomonas aeruginosa (strain ATCC 15692 / PAO1)	957	965	+
ArcA | Escherichia coli (strain K12)	254	263	+
ArcA | Escherichia coli (strain K12)	285	294	-

- Determine the ability for sgRNA primers to inhibit transcription factor binding
  - Keep species, position of TFBS, and strand in mind!

- Work on research proposal details with group
  - We have an example here
  - Can resubmit your homework by 10pm tonight if you would like
    - Points will be deducted if some version was not submitted at the beginning of class

Example proposal details: background

M3D4 homework should include specific details necessary to understand your research proposal. References to research articles with similar techniques or approaches will provide support to the feasibility of the idea.

1) Brief project overview
   Alzheimer's affects 5.4 million Americans and a hallmark of this disease is β-amyloid plaques (**reference**). β-amyloid plaques contribute to degeneration of nerve function and cell death. This leads to loss of brain tissue and reduced brain function resulting in patient death. Novel amyloid-to-dark chocolate (ADC) enzyme recently discovered (**reference**). We propose the characterization of ADC enzyme, then the expression of this enzyme in a mouse model of Alzheimer's disease to determine if we can convert β-amyloid plaques to dark chocolate.

2) Sufficient background information for the audience to understand the <u>importance</u> and <u>novelty</u> of your research.
   If we can convert β-amyloid plaques to dark chocolate, next steps would determine if this enzyme could be delivered to the brain in larger animals and potentially be a treatment for Alzheimer's in humans.
   This enzyme was identified in Dr. X's laboratory using a yeast two- hybrid screen and has not been characterized (**Reference**).

Example proposal details: problem and goal

3) Statement that addresses the research problem and goal.
   Can purified ADC enzyme convert β-amyloid plaques to dark chocolate *in vitro* and if expressed in mouse brain tissue can the enzyme convert β-amyloid plaques to dark chocolate *in vivo*?

Example proposal details: specific aims and alternatives

4) Details regarding the project aims, including possible methods and technologies that will be used to complete the proposed experiments. Alternate experiments / methods / technologies in case unexpected results are observed should be included.

Aim1: Optimize the production of genetically engineered ADC using non-toxic *E. coli* strain.

- We will use BL21 *E.coli* and purify this protein using a his-tag and nickel agarose beads (**protocol here**.) We can use the positive control from the original assay this enzyme was identified in to determine optimal buffer conditions and stability (**reference**).
- If there are difficulties with the bacterial expression system or His tag purification, we would use a Baculovirus expression system to express ADC in a system that produces post-translation modifications commonly seen in mammalian cells (**reference**). If there is difficulty with His tag purification, we can use alternative tags such as GST, HA, or FLAG (**reference**)

Example proposal details: specific aims and alternatives

Aim2: Determine enzymatic efficiency of engineered ADC *in vitro* using harvested β- amyloid plaques

- We will harvest β-amyloid plaques using this technique (**protocol here**) then inclubate the plaques with increasing concentrations of ADC. We can monitor the conversion of plaques to dark chocolate using a fluorescence based assay and a spectrophotometer (**protocol here**.) If this approach doesn't work we could co-express two inducible plasmids (**plasmid details here**), one expressing ADC and one expressing β-amyloid, in cells in culture. We can induce expression of β-amyloid with INDUCER1 till plaques form then induce expression of ADC using INDUCER2 and monitor plaque levels via antibody staining (**protocol here**.)
- What do we learn if this doesn't work? What parameters can be changed in the next iteration?

Example proposal details: specific aims and alternatives

Aim3: Measure efficacy of engineered ADC *in vivo* using a mouse model of Alzheimer's disease.

- We chose to use the Alzheimer's disease mouse model J112345 with inducible β-amyloid plaques via INDUCER1(**reference**.) We will design the mouse experiment with the following parameters:
  - Group 1: no INDUCER1
  - Group 2: +INDUCER1 at Day 1, no treatment
  - Group 3: +INDUCER1 at Day 1 and injection of Adenovirus virus expressing ADC on day 10
  - Group 4: +INDUCER1 at Day 1 and injection of Adenovirus virus expressing ADC on day 20

10 mice in each group. Mice will all be sacrificed at day 60, 120, 180 and expression of ADC and β-amyloid plaques will be quantified using antibody staining and H&E staining respectively (**protocol or reference**.)

Reference for Adenovirus virus that should express ADC in the right neurons.

Alternative approach: different induction timing, expression of ADC via different approach. Harvest animals using timeline similar to different reference (include **reference**).

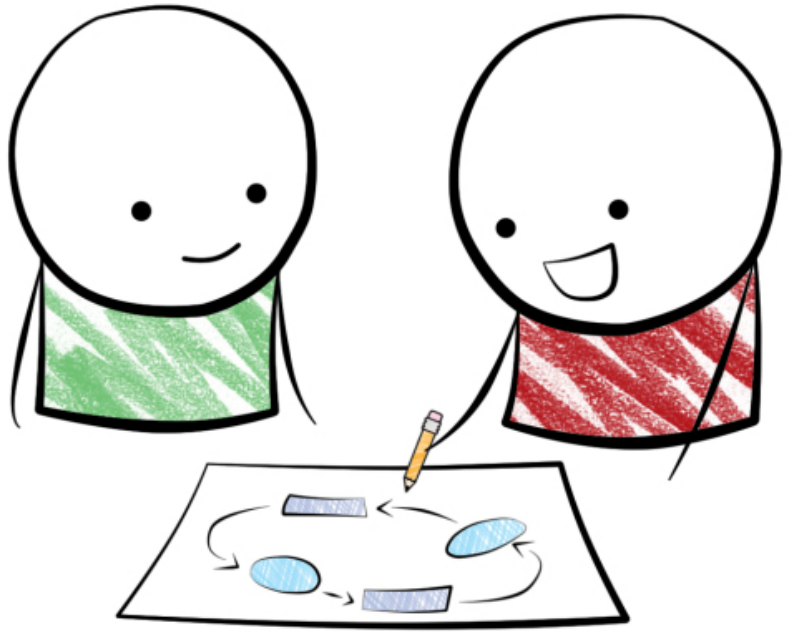
Societal impact of your research

We need to explore the possibility of a new ideal of impure science, in which scientists and engineers both educate and learn from others about the relation between science and society. Frodeman & Holbrook

- What populations/groups will benefit from your work?
  - Is there a potential for harm?
  - Is there a potential for population bias in your study– how will you mitigate it?
  - How will you select your sample pool?
- Are you producing potentially dangerous or harmful materials?
  - How will you handle these materials and secure them?
- Are you working with humans, human tissue, or animals?
  - How will you keep human data private?
  - How will you ensure humane treatment of all subjects?

Discuss research proposal with peer

- 4:30pm today
- Back in main room and reassigned to breakout rooms with someone outside your group
- Use wiki prompts to discuss your research proposal plans with someone outside your group in breakout rooms
  - 15 min per project available



For M3D5 (December 1)

- Outline the Mini-report
  - Progress report/state of project
  - See wiki for details
  - The mini-report is 5% of grade

- <u>With your lab partner!</u>