BEHAVIOR CONTROL LAB

Goal:
To observe the behavior of the MM protein and ZZ protein to give a basis for later labs (location, weight)
We are marking the proteins with different fluorescent proteins to see if they are inside the cell or in solution

Materials: (behavior control lab and sialic acid assay)

- GFP: E0040
- RFP: E1010
- Neuraminidase
- Acetate buffer
- 1.0 M Tris-HCl, pH 7.5 (1 mL)
- β-NADH Disodium Salt (15 mg)
- N-Acetylneuraminic Acid Aldolase (25 µL)
- L-Lactic Dehydrogenase (25 µL)

- Reporters (molecular weight of [1Da=1g/mol])
- GFP: E0040 (26909.4 Da)
- RFP: E1010 (25423.7 Da)

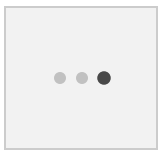
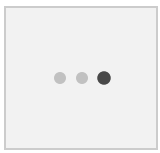
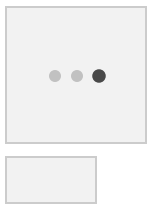
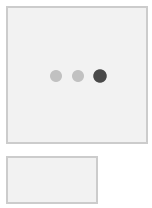
Method:

1. Assemble three different (MM-normal, ZZ-mutated, CRISPR-fixed) plasmids with Gibson Assembly (▪ 2µL PCR fragments + linearized vector ▪ 10µL Gibson Assembly Master Mix ▪ 20µL dH2O)
    a. Promoter: K525998 (T7 promoter + ribosomal binding site)
    b. Gene: normal DNA sequence/mutated DNA sequence/CRISPR DNA sequence
    c. Reporter: one with GFP, one with RFP
    d. Secretion Tag: osmY secretion tag
    e. Terminator: BBa_B00I0

2. Chemically Competent Cells Transformation Protocol

3. Analyze MM and ZZ plasmids for
    a. Secretion (location in relation to cell - inside vs outside)
        i. visible in test tube
    b. Polymerization (weight)?
        i. visible in conical tube, electrophoresis
    c. Secretion Ratios

Diagram:

Petri dish								
Conical Tube								

Rationale:

Pouring the products in the Petri dish and allowing them to grow over x hours, will allow us to count the number of colonies (measurement of how much the cells grow). Pouring the products in the conical tubes will separate things based on mass.

One reason behind doing this is just to see if the plasmids are functional, but we are also checking to see whether the normal gene produces proteins that are always secreted out of the cell while the mutated gene produces proteins that polymerizes always stays inside the cell. If this holds true, we can use this property in the future to test if our method works in fixing the DNA sequence and producing the correct alpha-1 antitrypsin proteins.

Rationale in details:

6 in the top left (MM, ZZ, MM, ZZ, CRISPR, CRISPR)

Since we are making the independent variable to be the difference DNA sequences (normal/mutated/CRISPR), other variables need to be controlled. This part of this Behavior Control Lab is to make sure the reporters (GFP/RFP) does not affect the growth of cells.

2 in the top right (MMZZ, MMZZ)

These are to see how the two interacts when they are placed next to each other (?)

6 in the bottom left (MM, ZZ, MM, ZZ, CRISPR, CRISPR)

These are also to make sure the reporters (GFP/RFP) does not affect the growth of cells. We can also determine whether the proteins are inside/outside the cells, because if the proteins are secreted, it would be at the top since they are lighter than cells. If it polymerizes and stays inside the cells, it would be at the bottom.

2 in the bottom right (MMZZ, MMZZ)

These are the ones we can do a qualitative analysis on to see if the mutated and the non-mutated proteins can be differentiated based on polymer/not polymer difference. If this clearly shows a difference, we can do a quantitative analysis in the future, using electrophoresis (see below) to confirm the results.