SIALIC ACID ASSAY:

GOAL: Determine whether the gene has been successfully mutated based on the sialic acid content (If the fixed gene produced the same amount of sialic acid compared to the wild type gene)

Protocol:

Assay of Free Sialic Acid (NANA)

1. Setting up the Assay
    a. This method will measure 1 to 200 nmoles of NANA. We have to make sure our samples don't surpass this (research). If they do, we have to dilute our samples.
    b. For a 20 mg quantity of each, digest MM, ZZ, and CRISPR solutions by neuraminidase (0.7 mg, 6.7 units) in 0.05 M Acetate buffer at pH 5.0 for 4 hr at 37° → resulting in free NANA.
2. Material Prep
    a. ZZ, MM, and CRISPR solutions
    b. Tris Reaction Buffer - Dilute the 1 M Tris-HCl 40-fold with distilled water to make a 25 mM solution
    c. N-Acetylneuraminic Acid Aldolase – ready to use
    d. L-Lactic Dehydrogenase – ready to use
3. Procedure
    a. Add the sample to Tris Reaction Buffer to give a final volume of 980 µL, making the reaction mixture
    b. Pipette the reaction mixture into a cuvette and blank the spectrophotometer.
    c. Add 20 µL of the β-NADH Solution and mix by inversion several times.
    d. Read and record the initial β-NADH absorbance. Initial A340 should read ~1.25.
    e. Return the reaction mixture to the original tube. Add 1 µL of N-Acetylneuraminic Acid Aldolase and 1 µL of Lactic Dehydrogenase and mix by inversion several times.
    f. Incubate in a 37 °C water bath for a minimum of 1 hour.
    g. Pipette the reaction mixture back into the cuvette. Read and record the final A340. Calculate the nmoles of NANA.
4. Calculation
    a. nmoles NANA = [(A340 Initial – A340 Final) × 1,000] \ 6.22