COLOR CODING KEY

Characterization
Construct
Data Collection/Analysis
Improvement of Biobrick
Plant-Care
Plasmid
Plasmid/Construct Design
Preparatory Work

Sunday July 21st

- Plates:
  - Created and autoclaved agar
  - Poured 13 agar plates with 500uL of AMP
- Competent agrobacterium
  - Spectrophotometer results:
    - 0 with normal LB media
    - GV3101 had OD600 of .750,
    - EHA 105 had OD600 1.078
    - Used ten 100uL tubes of GV3101
    - Used ten 100uL tubes of EHA 105
- Liquid inoculation:
  - Controls are clear
  - Promoter 1 (x and y)
  - Promoter 2x
  - Promoter 3x
- Miniprep:
  - GFP y
  - Promotor 2x
  - Promotor 1 (x and y)
  - Promotor 3x
- RE digested the product
- Gel Electrophoresis:

    - Plasmid backbones are visible
    - Insert are not visible
    - GFP is very light

Monday July 22nd

- Liquid Inoculation:
    - Inoculated HiFi plasmids
    - 2 Prelim tubes
        - 1from 0.1x plate and 1 from normal PL plate
    - 2 Construct tubes
        - 1 from 0.1x plate and 1 from normal CS plate
    - 2 Controls tubes
        - 1 AMP and 1 KAN
- Miniprepped the liquid inoculations
- Transformation:
    - GFP did not work properly
    - Transformed again with DH5-alpha
- Ran a gel electrophoresis on the miniprep results

Tuesday July 23rd

- Liquid inoculation:
    - Good results
    - KAN tube grew faster than the AMP tube
    - Miniprepped the tubes after
- Gel Electrophoresis:
    - Prom2 and diluted final plasmid used 1uL DNA, 4uL water, and 1uL loading dye
    - GFP used 3uL DNA, 2uL water, and 1uL loading dye
    - Diluted final plasmid from 607.7ng/uL to 100ng/uL by adding 25uL of MilliQ water
    - Made four 1% gels
        - Used a total of 280mL TAE, 2.8g agar, and 7.2uL Ethidium bromide
- AMP stock solution
    - Prepared 100mg/mL AMP
    - Used 0.15g stock, 1.5mL MilliQ water
        - Filtered using 0.22um syringe filter
- Plates:
    - Created and autoclaved agar

    - Poured 13 agar plates with 500uL of AMP
    - Took a streak from the old PreLim HiFi plates and spread them on new AMP plates
    - Also made 21 KAN plates
- DH5-alpha liquid inoculations
    - 2 cultures and 1 control
- Liquid inoculation:
    - Inoculated in 100mL of LB
    - Promoter 2
    - GFP
- Plants:
    - Thinned, transplanted, and sowed a new batch of plants
    - Added sticky bug catching sheets

Wednesday July 24th

- Plates:
    - Placed the PL plates into the 4C fridge and left the 0.1x plates in the incubator
    - Made 16 plates of RIF + KAN
    - Created plates for GFP
- Competent cells:
    - Added 100mL of LB and 1 mL of liquid inoculation A
    - Added 100mL of LB and 1 mL of liquid inoculation B
- Gel Electrophoresis:
    - Made and ran a 1.75% gel so that it would run slower
    - Retry with a 1% gel
- Liquid inoculation:
    - 2 GFPs
    - 2 Ligation
    - 2 controls (KAN and AMP)
- Prepared 0.1M MgCl2
    - Used 0.48g of powdered MgCl2 and 50mL of autoclaved MilliQ water
- Spectrophotometer measurements of DH5-alpha cultures:
    - Wavelength set to 600nm
    - Zeroed/Set Reference with LB
    - 2 hours after inoculation:
        - A-.178
        - B-.250
    - 3.5 hours after inoculation:

        - A- .393
        - B - .470
- Making competent cells:
    - Inoculated DH5-alpha in 5mL of LB overnight
    - Inoculated 100mL of fresh LB with 1mL of overnight culture
    - Grow in shaking incubator to an OD 600 of 0.4-0.5
    - Then placed into a -20C fridge
- Testing competent cells:
    - Transforming the competent cell test kit into the DH5-alpha
    - Two transformations
        - 1 with SOC and 1 with LB
    - Plated 100uL of the transformation onto CAP plates
- Liquid inoculation:
    - Growth on AMP control only
    - Miniprepped the inoculations

Thursday July 25th

- Moved HD5-alpha into the -80C freezer
- Made two 1% gels
- Streaking:
    - Restreaked transformed agrobacterium onto fresh KAN plates and left in the incubator at 28C
- Liquid inoculation:
    - 1 from CS HiFi plate
    - 1 from PL HiFi restreak
- Miniprepped tubes 1-20
- Nanodropped results
- RE Digest:
    - Promoter
        - EcoRI
        - Spe1
    - GFP
        - Xba1
        - Pst1
    - Backbone
        - EcoR1
        - Pst1
- Ligation:
    - Ligated in a PCR tube

        - 2uL T4 buffer
        - 1.5uL vector
            - 2204 base pairs
        - 1.4uL GFP
            - 720 base pairs
        - 0.5uL promoter 2
            - 151 base pairs
        - 16.6uL water
        - 1uL T4 ligase
    - Left at 16C overnight

Friday July 26th

- Liquid inoculation:
    - Retry with same products
        - 1 from CS HiFi plate
        - 1 from PL HiFi restreak
- Streaking DH5-alpha cells:
    - 2 with loop and 1 with spreader
- Nanodrop:
    - Concentration: 607ng/uL
    - Diluted to ~100ng/uL
- Gel Electrophoresis:
    - Promoter 2 and diluted final plasmid used 1uL DNA, 4uL water, 1uL and loading dye
    - GFP used 3uL DNA, 2uL water, and 1uL loading dye
    - Dilute final plasmid from 607.7ng/uL to 100ng/uL by adding 25uL of MilliQ water
    - GFP did not show up
- Plant Inoculation:
    - 50uL of solution onto leaves
    - 4 Controls
        - 1x PBS
    - Three 1:100 dilution of TMV samples
        - 1uL TMV and 99uL of PBS
    - Three 1:10 dilution of TMV samples
        - 10uL dilution of TMV samples

Saturday July 27th

- Plates: placed 2 overgrown plates into the 4C fridge

- Miniprep:
    - Took both the CS to miniprep and then the PL from August 2nd
    - Eluted in 25uL
- Nanodrop:
    - CS HiFi had good results (150-160ng/mL)
    - PL HiFi had bad results
- Bleached the tubes in the hood
- Liquid inoculated the DH5-alpha
- Prepped 1M CaCl2 stock
    - 1.47g CaCl2 dihydrate + 10mL MilliQ water
- Plates:
    - Control is clear
- Gel Electrophoresis:
    - 1uL DNA, 4uL water, 1uL loading dye
    - Pipetted 4uL into each well
