COLOR CODING KEY

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

Sunday July 28th

- Prepped DH5-alpha competent cells by sub culturing
  - 1mL of DH5-alpha in 99mL of fresh LB media overnight
  - Spectrometer measurements of sub culture were measured hourly for 3 hours; results increased from 0.139 to 0.241 to 0.387
- Competent cell prep
  - 1mL of DH5-alpha in 99mL of fresh LB media overnight
  - Cells were not washed with MgCl2
  - 15% glycerol and 0.1M CaCl2 were used instead of 20% glycerol
  - Snap frozen in ethanol from -80C freezer
  - Immediately placed in -80C freezer afterwards
- Competent cell test
  - Old and new competent cells were transformed using heat shock
  - 4 plates used to test:
    - Old cells transformed in LB
    - New Cells transformed in LB
    - New cells transformed in SOC
    - New cells transformed in SOC and concentrated
- Plates: some growth
- Liquid inoculations:
  - Promoter 1,2,3
  - Ligation 1 has growth
  - Both GFP have growth

    - Only miniprepped the GFPs
- Nanodrop:
    - Concentration for GFPs were around 100ng/uL
- Gel Electrophoresis:
    - Made with 2uL DNA, 3uL MilliQ water, and 1uL loading dye

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Monday July 29th

- Made LB plates and 1% gels
- Liquid inoculations were miniprepped, nanodropped, ran through a gel, and gel extracted
- RE digested promoter 1
- Liquid inoculation:
    - GFP
    - Promoter 1,2,3

Tuesday July 30th

- Sequencing results arrived
    - Not so good
- Miniprep:
    - Promoter 2 and 3 and Exp. Characterization
    - Eluted in 25uL
- Nanodrop:
    - Good curves, low concentration
- Plant:
    - Decreased light intensity

Wednesday July 31st

- Processed some liquid inoculations and miniprep
- Nanodrop:
    - Used CS HiFi
    - Low concentration
- Gel Electrophoresis:
    - Used 5uL of ladder in the 3rd column
    - Used 5uL of CS HiFi in the 5th column
- Ran a gel electrophoresis: GFP did not show up
- Pipetted 100uL of the experimental characterization into a 96 well plate with OD620
- Plated and liquid inoculated GFP and Promoter 1,2,3

Thursday August 1st

- Miniprepped promoter 2
- Resuspended DNA in 100uL TE buffer

Friday August 2nd

- New batch of liquid agrobacterium
    - For RIF + KAN antibiotics
    - Placed into incubator set to 28C and 175RPM

Saturday August 3rd

- Made resuspension buffer for agroinfiltration
    - 10mMol MES (1.92g per Liter)
    - 10mMol MgCl2 (2.03g Per Liter)
    - Procedure
        - Dissolved above in 800mL MilliQ water
        - Adjusted pH with KOH
        - Added milliQ water up to 1 Liter
    - Split into two bottles and autoclaved
    - Later stored in 4C fridge
- Obtained 100mMol of Acetosyringone
    - Placed into -20C fridge
- Agroinfiltration:
    - Used 2-day old agrobacterium culture
    - Used 1mL of Liquid agrobacterium culture in 25mL LB with antibiotics and 20uMol acetosyringone (5uL of 100mMol stock) in an erlenmeyer flask
    - Placed in a shaking incubator at 28C
- Resuspended primers to 100uM
- Dilute in a separate tube to 10uM
    - 10uL of 100uM primer + 90uL of nuclease free water
    - Put 100uM stock to -20C fridge
- PCR
    - 20uL reactions for each one (P1,P2,P3,T7)
    - Reagents
        - 5x Phusion Buffer - 4uL
        - 10mM dNTPs - 0.4uL
        - 10uM Forward Primer - 1uL
        - 10uM Reverse Primer - 1uL
        - DNA (P1,P2,P3,T7) - 2.5uL

        - Phusion Polymerase - 0.2uL
        - Nuclease free water - 10.9ul
    - nanodropped , ran a gel electrophoresis, PCR purify, and nanodropped the results
- RE Digest (P2,T7)
    - Reagents
        - Ecor1 - 1ul
        - Pst1 - 1ul
        - Cutsmart - 5ul
        - DNA - 10ul
        - Nuclease Free Water to a total of 8ul
