COLOR CODING KEY

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

Sunday August 4th

- Resuspended agrobacterium
  - OD600 of agro 25mL culture is 1.925
  - Pelleted the agrobacterium in a centrifuge for 5000RPM for 15 minutes
  - Resuspended pellet in agrobacterium resuspension buffer in a 15mL tube but then moved to a 250mL flask
  - Later on, adjusted OD600 to 0.4
  - Added 100uL of Acetosyringone into the buffer
  - Flask placed into shaking incubator for 28C at 200RPM until agroinfiltration
- Agroinfiltration of 10 plants:
  - 3 controls with PBS and agrobacterium resuspension buffer
  - 2 infected with TMV only
  - 2 infiltrated with agrobacterium only
  - 3 infected with TMV and infiltrated with agrobacterium
  - Experimented leaves are marked with nail polish

Monday August 5th

- Gel Electrophoresis:
  - Good results: bands are ~1kb

Tuesday August 6th

- Nanodrop 7 tubes
    - Average concentration around 450ng/uL

Wednesday August 7th

- Liquid inoculation of the following:
    - N gene
    - Silencing suppressor
    - Hifi restreak
- Made 7 KAN antibiotic plates
- Resuspended gold characterization materials
    - Followed protocol from IDT
- PCR'd gold characterization promoters
    - Used primer 67 and 72 (35s forwards and XRN1-2 reverse)
    - Followed protocol for Q5 mastermix
    - Left in thermocycler overnight

Thursday August 8th

- Nanodrop:
    - Nanodropped all gold characterization
    - Concentration ~400ng/uL
- RE digest (P2,T7, Backbone [-20])
    - Reagents
        - Xba1 - 1uL
        - Spe1 - 1uL
        - Cutsmart - 5uL
        - DNA - 1uL
        - Nuclease Free Water to a total of 25uL
- Ligation
    - Reagents
        - 10X T4 Ligase Buffer - 2uL
        - Backbone DNA - 1.5uL
        - Inserts (P1,P2,P3,T7) - 2uL
        - T4 Ligase - 1uL
        - Nuclease Free Water - 13.5uL
    - Same protocol
        - Used Xba1 an Spe1
- Transformation
    - Transformed 4 ligations
        - T7

            - with the first two enzymes
            - and the second two enzymes
        - P2
            - with the first two enzymes
            - and the second two enzymes

Friday August 9th

- Lab was cleaned
- Three 1% gel electrophoresis were ran on the liquid inoculated:
    - N gene
    - Silencing suppressor
    - Hifi restreak
- Used HiFi on 3 of 4 gold characterization promoters
    - Hifi of pcr'd backbone + promoter + mCherry + tNOS
    - PCR of backbone was used directly
    - All inserts were diluted to a point where 1uL was used
    - 5x concentration was used for any piece smaller than 250 bases
- Transformation:
    - Transformed E.Coli with HiFi products and plated
        - GC 1- 35s biobrick from registry
        - GC-3 stress response promoter
        - GC-4 heat shock response promoter
        - Positive control on AMP plates

Saturday August 10th

- Agroinfiltration with the following:
    - N gene
    - Silencing suppressor
- Miniprepped agrobacterium
- Transformed agrobacterium
    - Placed in a shaking incubator at 28C for 175RPM
- Agroinfiltrated:
    - 3 Controls
    - 2 TMV
    - 2 Agrobacterium
    - 3 Agrobacterium + TMV