iGEM 2018: NUS Singapore-A
WetLab Group 2_October 2018 Logbook

2018.10.01 (Mon)
- Luteolin 1.8
  - Decontamination test check
    - Only WT BL21* shows growth on No-Antibiotic plate
    - re-filtered & plated*
- NTU broth experiments
  - OD check
    - Growth observed in LB+G only, not G+K (photo taken)
- Pick colonies, 2per plates (6 plates)
  - Nanda's paptamers and laptamers*
  - picked & incubated in Big shaking incubator
- F3'H gblock has arrived (kept in -20 degree box)
- Glycerol stock for New BL21* from SynCti (no Streptomycin resistance*)

2018.10.02 (Tues)
- Cloning of parts into pSB1C3 (~6 constructions)
  - primers should be arriving
  - F3'H gblock has arrived (kept in -20 degree box)
    - Transformed into TOP10/Fake BL21*
    - pSB1C3-F3'H CDS
    - pSB1C3-FNS CDS
    - pSB1C3-Brep-F3'H
    - pSB1C3-Brep-FNS
    - pSB1C3-pBAD-FNS
    - pSB1C3-Brep-RFP-YbaQ
    - pSB1C3-Bind-RFP (to be transformed)
    - *SynCti's assembled plasmid size were checked under Gel Electrophoresis
      - gel is kept in 4 degree fridge
      - *need to further evaluate as bands observed were <3000bp
- Plasmid extraction of Nanda's paptamers and laptamers*
  - 12 tubes incubated in Big shaking incubator
- Make plates for NTU
- Keep Glycerol stocks for NTU
  - BW-sgRNA 1,2,3,4 (tubes are kept in 4 degree fridge)
- Samples inoculated for qPCR in 37 degree shaking
  - Brep-FNS Glycerol
  - pBAD-FNS Glycerol
  - Brep-F3'H Glycerol
  - Brep-F3'H co Brep-FNS
  - Brep-F3'H co pBAD-FNS

    - New BL21* from SynCti (no Streptomycin resistance*)
- Luteolin 1.8
    - Decontamination test check for WT BL21*
- Grow Old BL21* in LB+Strep

2018.10.03 (Weds)

- Check growth of Old BL21* in LB+Strep
    - no growth observed, old BL21* is not contaminated
- Make Competent cell
    - TOP10
    - B10
    - BW-sgRNA 3 for NTU
- Transformation
    - pSB1C3-Bind-RFP
    - pSB1C3-pA8C-FNS
    - pSB1C3-Brep-RFP-
- E6: Continue with Gel Extraction
- SynCti: Check Gel ran for your assembled plasmids (1,2,3,4,5)
    - gel is kept in 4 degree fridge
    - *need to further evaluate as bands observed were <3000bp
- Pick colonies (2 each)
    - pSB1C3-F3'H CDS
    - pSB1C3-FNS CDS
    - pSB1C3-Brep-F3'H
    - pSB1C3-Brep-FNS
    - pSB1C3-pBAD-FNS
    - pSB1C3-Brep-RFP-YbaQ

- Check Seq result
    - EL222-Brep-dRBS-FNS
    - Brep-dRBS-FNS-EL222
    - if Success-> Co-transform with Brep-F3'H-> Production of Luteolin

Luteolin 1.9

- SDS-PAGE, 2ml sample isolated out AFTER INDUCTION

2018.10.04 (Thurs)

- Plasmid extraction
    - pSB1C3-F3'H CDS
    - pSB1C3-FNS CDS
    - pSB1C3-Brep-F3'H
    - pSB1C3-Brep-FNS
    - pSB1C3-pBAD-FNS
    - pSB1C3-Brep-RFP-YbaQ
    - pSB1C3-Bind-RFP
- Checked paptamers and laptamers seq results
- Succeeded:

- lpp-spinach2.1
- lpp-ispinach
- phtpG1-spinach2.1 (1)
- Some mutations:
- phtpG1-ispinach -> mutations in the promoter
- all tdbroccoli have the same mutation in the scaffold at both ends
- NTU's experiment

2018.10.05 (Fri)
- Check NTU's plates and count colonies
- to repeat

2018.10.07 (Sun)
- Cotransformed Brep-FNS-CPR and Bind-RFP,
- Transformed pAC-EL222 into Brep-F3'H

2018.10.08 (Mon)
- Repeated NTU experiment by following their protocol strictly
- Inoculated Brep-FNS-CPR and Bind-RFP, Brep-F3'H and pAC-EL222, WT
- Sequencing results for pBAD-FNS is good -> added to submission kit
- Added phtpG1-RFP to submission kit
- Settled parts submission admin, submitted registration online

2018.10.09 (Tue)
- Biosynthesis 1.9
  - Wild-type BL21*
  - Flask 1: Brep-FNS and Bind-RFP, Brep-F3'H, co-culture under light
  - Flask 2: Brep-FNS and Bind-RFP, Brep-F3'H, co-culture in dark
    - OD of Overnight culture
      - WT: 4.00
      - FNS-CPR: 2.56
      - F3'H-EL222: 2.30
  - Initial OD in 50ml: 0.2
    - Flask 1 has lesser FC & F3'H-EL222 (insufficient)
    - 37 degree, started at 14:10
      - 16:25
        - WT: reach 1.25, diluted to 0.6 & kept in fridge
        - Flask 1: 0.55
        - Flask 2: 0.72
        - proceed to next step
  - Induction at OD 0.6
    - 30 degree, 3 hour
    - *no inducers were needed
  - Change medium to M9, at OD 1.8-2.0
    - centrifuge to collect cells (5000rpm, 6min), culture medium fresh M9

with Antibiotics
        - WT: 4.93 of 20ml = 30ml
        - Flask 1: 5.17 of 19ml = 31ml
        - Flask 2: 5.69 of 17.5ml = 32.5ml
    - Addition of Substrate (50ul 0.2mM Naringenin)
  - Continue Incubation at 30 degree, 36hr-> 11th Oct 9am
- Check and count NTU plates
  - photo taken
- prepare Naringenin
  - 2ml

2018.10.11

- Harvested Luteolin 1.9 and delivered to MD7