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

2018.09.01 (Sat)
- Harvest Luteolin 1.6 (BL21* & Co-factors)
  - 36hr at 1st Sep, ~11am
  - Measure OD600:
    - Brep-F3'H-pTet-FNS: 5.30
    - Brep-F3'H: 3.96
    - pA8C-FNS: 4.93
    - Wild type: 3.86
- Plasmid extraction
  - pLasR-F3H-DFR and pBad-ANS-3GT in BL21* 1&2
- Glycerol stock
  - Brep-F3H-DFR-BL21*
- Competent cells
  - M9 Adapted BL21*, refreshed in LB (named BA)
- pT7-RFP *repeat* (plate in 4 degree)
  - repeat by re-picking colonies
- Mini-prep *numerous*
- Co-transformation
  - (Prem's plasmids) Bind-RFP & R-EL222, TOP10, plated on K/C

2018.09.03 (Mon)
- Sequencing result check*
  - pAC-EL222
  - ANS-3GT-B from Yan Ping
- Samples to be sent for Sequencing
  - ready in keep in 4 degree
- Luteolin 1.6
  - SDS-PAGE on pellets
  - Passed HPLC samples to Tvarita (Supernatant (Luteolin & Naringenin))
- Competent cells
  - M9 Adapted BL21* (30 degree incubator), to be refreshed in M9 (named BMA)
  - *ask Prof, from Dr LingHua: NTU's BW wild type
- pT7-RFP *Repeat*
  - pT7-RFP + (red) & -(not so red): in 30 degree incubator
  - IPTG added
- Pick colonies & inoculate in 50ul K+50ul C
  - Bind-RFP & R-EL222, TOP10

2018.09.04 (Tues)

- Plasmid extraction
  - Bind-RFP & R-EL222, TOP10 (NOT SENT FOR Sequencing as Plasmid map is not available)
- pT7-RFP *Repeat* (in 37 degC shaking incubator): 5000RPM, 5min
  - Spin down cells to observe whether there's change in colour of pellet
- Gel extraction & Assembly & Transformed into Top10 & Plated
  - Bind-AAV (F fragment~1.5k bp, [46]; R fragment ~1.3k bp, [73])
  - Bind-DAS (F fragment~1.5k bp, [67]; R fragment ~1.3k bp, [56])
- Make 1 more bottle of M9 and 1 more tube of Naringenin (follow previous concentration)
- Sequencing results for F3H-DFR, F3'H gblock, and...

2018.09.05 (Weds)

- Morning measurement* David is going to use Micro-plate reader in the afternoon
  - pT7-RFP with/without IPTG, using 96-well plate kept in 4 degree, samples are loaded
- Pick colonies & Inoculate
  - Bind-AAV
  - Bind-DAS
- Plasmid construction
  - one-gene-one-plasmid: pT7-Brep-F3'H (since there is premature stop codon)
  - pT7 & new RBS
- Search/Check papers for Plasmid design in Luteolin-synthesis
  - can write to them to request
- Prepared M9, cells and Naringenin for NTU
- Inoculated Brep-F3'H, pA8C-FNS and F3'H-FNS for induction of enzymes (6 hours)

2018.09.06 (Thurs)

- Induction of enzymes & OD measurement for Growth curve
  - Refresh each overnight culture in 50ml LB (inoculate to new media) with respective antibiotics (wrap all flasks/falcons in aluminium foil):
    - Kanamycin (final concentration of 50ug/ml) for Brep-F3'H-pTet-FNS & Brep-F3'H
    - Chloramphenicol (final concentration of 25ug/ml) for pA8C-FNS
    - No antibiotics for WT
  - Initial OD to be 0.09 (11:30am)
    - *Correct way of Measuring OD600
    - ON culture tends to be high OD~4, prior 2x dilution & then Nanodrop measure
    - Do calculation & Perform subsequent dilution to obtain desired OD
    - (Nanodrop tends to be insensitive when OD gets too low)
      - WT: 500ul ON into 50ml LB
      - FNS: 313ul into 50ml LB+C
      - FF: 500ul into 50ml LB+K
      - F: 500ul into 50ml LB+K

- *Hourly OD reading
- At OD 0.6 (dilute to OD 0.6 if OD gets too high), add (1st induction):
  - ATC (final concentration of 200nM) into
    - Brep-F3'H-pTet-FNS
    - Brep-F3'H
    - WT
  - 0.2% Arabinose
    - pA8C-FNS
- Incubate at 30°C for 3 hours, wrap all flasks/falcons in aluminium foil

- Glycerol stock
  - Brep-F3'H, pA8C-FNS and Brep-F3'H-pTet-FNS
- Plasmid extraction and sent for sequencing
  - Bind-AAV and Bind-DAS
- Sequencing results
  - EL222 ok
  - F3'H amplified gblock ok
  - F3'H in double gene single plasmid ok

2018.09.07 (Fri)
- Plasmid construction
  - Brep-FNS (pBad is replaced with Brep), keep in -20 box
- Retrieve Naringenin-OD data for Marcus tmr
- Retrieve BL21* Overnight growth in LB, 96-well plate data
- Make modified M9
- Real BL21* arrives
  - previous "BL21*" are just BL21
- Sequencing result check*
  - Co-transformed pLasR-F3H-DFR and pBad-ANS-3GT in BL21* 1&2
    - 1: several point mutations at the end of DFR gblock, F3H is intact; ANS-3GT FAILED
    - 2: several point mutations at the end of DFR gblock, missing pLasI promoter, F3H is intact; ANS-3GT FAILED

2018.09.08 (Sat)
- Competent cell preparation
  - BL21* DE3
  - BW25141 for NTU
- Removal of WRONG BL21 tubes
  - cells (e.g. Glycerol stocks), plasmids… that used WRONG BL21

2018.09.10 (Mon)
- E6's Construction tasks
  - A & C
- Co-transform F3H-DFR & ANS-3GT in BL21*
- Co-transform cells for bind characterisation (all tags and EL222 cells) on K/C

plates
    - with Prem's co-transformed Bind-RFP & EL222 as Control (not sequence checked)
- Co-transform NTU plasmids into BW25141 competent cells & Plated on respective plates
- Go through necessary Sequencing results with Jingyun
    - *Confirm the right ones*
    - Brep-F3'H
    - pA8C-FNS
    - Brep-F3'H-pTet-FNS (all have mutations)
    - Brep-F3H-DFR
    - CPR

2018.09.11 (Tues)
- NTU's plate colony count
    - results & photos in Ting2's phone
- Pick colonies for Co-transformed Bind-RFP and various Deg tags

2018.09.12 (Weds)
- NTU's plasmid are insufficient
    - transformation for plasmid replication/ inoculation for extraction
- Construction of Plan A and Plan F (PCR)
- Kept culture for EL222/Bind-RFP and tags

2018.09.13 (Thurs)
- Characterisation of bind-RFP & various tags
- Miniprep of Bind-RFP
- Continue construction of plan A and F

2018.09.14 (Fri)
- Pick colonies
    - NTU's
    - Brep
- Plasmid Construction

2018.09.18 (Mon)
- Picked 3 colonies each for Brep-FNS-CPR and Brep-CPR-FNS
- Made competent cells for pA8C-EL222
- Inoculated BW SgRNA in G+50K
- Prepared 2 G+K plates each for 3 diff conc: 50, 200, 300

2018.09.19 (Tues)
- Miniprep for Brep-FNS-CPR and Brep-CPR-FNS & Ses result

    - Brep-FNS-CPR: Primer (Chloro FWD, CPR FWD)
    - Brep-CPR-FNS: Primer (FNS FWD1, CPR FWD)
- Transform pA8C-FNS into BL21* & Plated on K plate
- Naringenin growth expt on WT BL21*
    - WT BL21* inoculated from Glycerol stock (8/9/18)
- Inoculate BW SgRNA into K200 and K300 with Gentamycin
- Plate BW SgRNA onto K50, K200 and K300 plates
    - Overnight culture 10,000x dilution (100 in 900, 2 times)
- Sequencing result CHECK (Bind-RFP, EL222)

2018.09.20 (Weds)

- Pick colonies
    - Brep-FNS and Brep F3'H cotransform in BL21*
    - NTU BW2451 on K200 & K300 plate
- Naringenin growth expt on WT BL21*
- Sequencing result CHECK
    - Brep-FNS-CPR
        - FC1: missing middle part of CPR
        - FC2: low concentration, re-doing
        - FC3:
            - FNS (several point mutations at the end), bp2071 Valine-> Glycine, bp2077 Glycine silence mutation, bp2094 Arginine-> Proline;
            - CPR, several point mutations at the beginning & end, 1 missing nucleotide in the middle (bp2854): ATG->AT(missing)
    - CPR-Brep-FNS
        - CF1: missing CPR
        - CF2: missing CPR
        - CF3: missing CPR
- Brep-F3'H and Brep-FNS competent cells
- Talk about BL characterisation with Prof
    - Brep old result
    - Bind cotransformation

2018.09.20 (Thurs)

- Biosynthesis 1.7 with real BL21*
    - cells collected (5000rpm, 6min) & resuspended using New M9 to OD2.00, ~40ml
    - all added with Naringenin; pBad-FNS is induced with 0.2% Arabinose (400ul of 20% stock into 40ml culture)
    - *co-cultures were not added with Antibiotic
    - Production started at ~21:00
- Blue light characterisation for bind with spacer
- Made new M9

2018.09.21 (Fri)

- Made competent cells

    - Brep-F3'H and Brep-FNS
    - Top10
- Miniprep for:
    - 1) cotransformation of Brep-F3'H and Brep-FNS
    - 2) Brep-F3'H-pTet-FNS
- Check Naringenin growth experiment
- Analysis of Characterisation data of EL222 Competent cell transformed with Bind-RFP
- 3 tubes of Bind-RFP (no spacer) co-transformed with r-EL222 (Prem's) were centrifuged, pellets & supernatant were not red

2018.09.22 (Sat)

- Harvesting of Luteolin biosynthesis 1.7
    - success
- Overlap PCR for phtpG-aptamers
    - success
- Transformation
    - pBAD-FNS and Brep-FNS into Brep-F3'H competent cells

2018.09.24 (Mon)

- Assemble and transform paptamers into TOP10
- Inoculation of sat's transformation
- Safety experiment
- Search pSB1C3
- Send for Sequencing
    - FC2* ready in fridge
- Extraction and concentration of dyes (done by Liyana's team)
- Try dyeing pieces of fabrics (done by Liyana's team)
- Changing of F3'H rbs34 to default RBS
- Use new antibiotics for all
- Grow WT cells in LB with antibiotics

2018.09.25 (Tues)

- Biosynthesis 1.8:
    - Refreshing starts at 11:40am
    1. pBAD-FNS in Brep-F3'H competent cell (K+C);
    2. WT (nil)
    3. Brep-FNS & Brep-F3'H cotransform (K+C) inoculated from tube 1 (both tube 1&2 are missing J23100 promoter for EL222 on Brep-F3'H, tube 2 is better*)
        1. With Blue light
    4. Brep-F3'H-pTet-FNS (K)
    5. Brep-FNS in Brep-F3'H competent cell (K+C) haven't sequence
    - Protocol, Luteolin*
        - paper: Strain Improvement of Recombinant E coli for Production of Plant Flavonoids
        - Overnight culture in LB, 37, 300rpm

        - Refresh in 50ml LB with Antibiotics: starting OD 0.1, ending OD 0.6 (~2.5hr);
            - * Brep-FNS & Brep-F3'H Co-transformed (with Blue Light) was refreshed at 15:05
        - Induction, more than Max Induction at 30 degree, 3hr; START 15:05
            - 1. pBAD-FNS in Brep-F3'H competent cell (K+C); 500ul 20% Ara
            - 6. Brep-F3'H-pTet-FNS (K); 50ul 200uM ATC
        - Centrifuge to collect cells (5000rpm, 6min), culture medium fresh M9 with Antibiotics at OD 1.8-2.0
            - 20ml resuspended in M9*-> 40ml of OD2.00
                - 7.85: 10+30
                - 5.07: 16+24
                - 4.39: 18+22
                - 4.42: 18+22
                - 4.64: 17+23
        - Addition of Substrate (40ul 0.2mM Naringenin) & Inducer
        - Continue Incubation at 30 degree, 36hr; START 09/25 19:30, END 09/27 ???

- Transform Brep-rbsD-F3'H into BL21*
    - LB+K plate
- Pick colonies for all the paptamers
- Check sterilisation protocol plates
- SDS PAGE of cell pellets (Luteolin 1.7)
    - 15ul Ladder, 30ul Protein samples
    - Loading sequence: Ladder, C, space, Brep-F3'H, pBAD-FNS, Brep-FNS, Brep-F3'H&Brep-FNS, Brep-F3'H&Brep-FNS-CPR
    - 120V, 60min
    - Gel photo to be taken on 09/26

2018.09.26 (Weds)

- Carry-on Luteolin 1.8
    - Refresh ON Brep-FNS & Brep-F3'H (missing J23100 promoter for EL222) cotransform (K+C) to OD 0.60
    - *with Light repression during Growth and induction phase (although it doesn't have to be induced)
    - @17:30: Add Naringenin as normally
- Miniprep for paptamers, send for seq (Nanda)
    - abnormal RFP observance???
    - only 1 out of 10 tubes is not red
- Pick colonies for Brep-rbsD-F3'H into BL21*
- Overlap PCR and transformation for laptamers
    - Gel ran
- Construction: addition of EL222 into Brep-FNS & 2 packets of Primers received (refer to photo in 26th Sep)
    - EL222-Brep-dRBS-FNS
        - Frag1 : 1.7k
        - Frag2: 1.2k

        - Frag3: 1.0k
    - Brep-dRBS-FNS-EL222
        - Frag4: 1.0k
        - Frag6: 2.0k
        - Frag5: 1.0k
    - Gel loading sequencing: 1, 2, 3, 4, 5, 6, and Jingyun's No.2
    - assembled & transformed, plated on C plates
- Overlap pcr for laptamers

2018.09.27 (Thurs)

- Harvest Luteolin 1.8 (38hr @11AM)
    - Filtered
- Amplification of pSB1C3
    - Running PCR, Gel is casted & kept in drawer
    - Run Gel - bands for Amplified is stronger than bands from iGEM stock
    - Amplified for more psb1c3 (modified protocol for higher yield: 19ug-> 30ug/ul)
        - dNTP: 2uL
        - Template (dated 20/8): 2uL
        - Primers, suffix and prefix: 1uL each
        - Rxn buffer: 10uL
        - Q5: 0.5uL
        - ddH2O: 33.5uL
        - Melting temp: the lower of the 2 primers + 3degC
        - Annealing time: 1min
        - 29 cycles
- Plasmid extraction & Sent for Seq
    - Brep-rbsD-F3'H in BL21* colony 1 & 2
- Sent for Seq
    - Brep-F3'H for J23100 (24/7 plasmid; F3'H Seq primer R)
    - Brep-FNS-CPR 3 for FRONT mutations (CPR PCR Primer F)
- Re-innoculate Brep-FNS-CPR 2
    - to get more plasmid
- Gel Extraction of Nanda's aptamer*
- Pick colonies
    - EL222-Brep-dRBS-FNS
    - Brep-dRBS-FNS-EL222
- Check Naringenin growth experiment
    - export data* & plot
- Write in Biosynthesis protocol*
- Make agar plates for NTU using new antibiotics (Gen+K)

2018.09.28 (Fri)

- Harvest Luteolin 1.8 (38hr @11AM)
    - Blue-light repressed Brep-FNS & Brep-F3'H (missing J23100 promoter for EL222) co-transform (K+C)
        - Blue light in Daytime, White light in Nighttime

    - Decontamination test
    - Make LB K/C agar plates
- Plasmid extraction
    - EL222-Brep-dRBS-FNS
    - Brep-dRBS-FNS-EL222
- Sequencing result check
    - FAILED: Brep-F3'H, missing J23100 (24/7 plasmid; F3'H Seq primer R)
    - FAILED: Brep-FNS-CPR 3 for FRONT missing bp (CPR PCR Primer F)
- Run gel and extraction for more pSB1C3
- 5.30pm iGEM Presentation meeting
- Transform Aptamers into TOP 10
- Check Promoter in front EL222, could it be another promoter
    - TATA Box, -10, -35

2018.09.30 (Sun)

- NTU Control experiment
    - inoculate 3-5 NTU's SgRNA-Kan* colonies in
        - LB+50ug/uL Kan+Gen
        - LB+Gen
        - *1 colony dip twice into each broth
    - inoculate WT bw25141 into
        - LB+50ug/uL Kan , to check for Kan resistance
- Paptamers and Laptamers*
    - pick colony if got, 6 plates in total
    - minute colonies observed
- Make LB agar plates without antibiotics