Styrene Synthesis Lab Notebook

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06.02.15

- Designed and ordered primers for yeast FDC1 (Dan K, Danny)
- Emailed iGEM HQ to request PAL biobrick (Dan K)
    - PAL part # BBa_K1129003

06.03.15

- Inoculated solid and liquid yeast cultures (Dan K, Danny)

06.04.15

- Ran PCR on yeast colonies with FDC1 primers (Dan K, Danny)
    - Ran on two colonies: Y2 + Y3
    - Temperature gradient: 70°C to 57°C

06.05.15

- Ran gel on PCR products from 6/4 (Dan K, Danny)
    - Y2 was amplified at 67°C
    - Y3 was amplified at 57°C
- Performed PCR cleanup on Y2 + Y3 products (Dan K, Danny)
    - Nanodrop: Y2 7.4 ng/uL, Y3 14.1 ng/uL
- Ran double gradient PCR on additional two yeast cultures (Dan K, Danny)
    - 1st gradient: 59°C to 51.5°C, 4 cycles
    - 2nd gradient: 78°C to 72.5°C, 26 cycles
- Ran gel: no amplification results (Dan K, Danny)

06.08.15

- Ordered UbiX (w/ and w/o overhang), FDC1 (w/o overhang), VF and VR2 primers (Dan K, Danny)
- Prepared Y2 + Y3 PCR products for sequencing (Dan K, Danny)

06.09.15

- Analyzed Y2 + Y3 sequences (Dan K, Danny)
- Performed PCR of Y2 + Y3 to further amplify FDC1 DNA (Dan K, Danny)
    - Gradient: 70°C to 57°C
- Ran gel of re-amplified FDC1 gene (Dan K, Danny)
    - All lanes contained 1.5 kb band, as expected
- Performed PCR cleanup on re-amplified FDC1 (Dan K, Danny)
    - Nanodrop results: Y2 431.4 ng/uL, Y3 361.7 ng/uL

06.10.15

- Digested FDC1 gene and RFP plasmid (Dan K, Tyler)
    - Used EcoRI and PstI-HF restriction enzymes
    - Created digestion mixtures for RFP, Y2, Y3
- Performed PCR cleanup on RFP, Y2, Y3 digest solutions (Dan K, Tyler)
- Ligated Y2 and Y3 FDC1 genes with RFP plasmid (Dan K, Tyler)
    - Used T4 ligase + T4 ligation buffer

06.11.15

- Used PCR to obtain UbiX from E. coli (Dan K, Tyler)
    - Used UbiX primers w/ and w/o overhang
    - Gradient: 70°C to 58.9°C
- Ran gel on PCR products (Dan K, Tyler)
    - All lanes contained 500 bp band, as expected
    - Overhang lanes were darker
    - 2 log ladder
- Ran PCR cleanup on UbiX samples (Dan K, Tyler)
    - Nanodrop: UbiX w/ overhang 344.5 ng/uL, UbiX w/o overhang 90.2 ng/uL
- Transformed FDC1 into E. coli (Dan K, Tyler)
    - Transformed both Y2 and Y3
    - NEB 5-alpha competent E. coli
    - Plated cells; grew overnight
- Order E. coli codon optimized FDC1 gene (Saccharomyces cerevisiae) on IDT (Kosuke)

    - Order number: 11164675
    - Expected ship date: 06.22.15
- Order E. coli codon optimized PAL gene (Anabaena variabilis) on IDT (Kosuke)
    - Order number: 11167256
    - Expected ship date: 06.22.15

06.12.15

- Ran colony PCR on E. coli transformed with FDC1 gene (Dan K, Tyler)
    - Took samples from 12 white colonies
    - Used VF2 and VR primers, Taq1 polymerase
    - Annealing temperature: 52°C
- Ran gel on PCR products (Tyler)
    - Colonies 1, 5, 8 (from Y3 1X plate) had bands at desired length indicating plasmid with FDC1 insert
- Cultured 12 colonies from PCR in LB Chlor (Dan K, Tyler)
- Performed first step of FDC1 miniprep (MX1 buffer) and stored samples in -20 (Kosuke)

06.15.15

- Completed miniprep of FDC1 plasmid (Dan K, Tyler)
    - Nanodrop: colony 1 79.2 ng/uL, colony 5 107.2 ng/uL, colony 8 129.0 ng/uL
- Prepared sequencing samples and sent to Elim (Dan K, Tyler)
    - Ordered sequences for all three colonies: 1, 5, 8
    - VF2 and VR primers
- Digested UbiX and RFP plasmid (Dan K, Tyler)
    - EcoRI-HF and SpeI-HF
- Ligated UbiX with RFP plasmid (Dan K, Tyler)
    - T4 ligase with T4 ligation buffer

06.16.15

- Biobrick FDC1 (Dan K, Tyler)
    - 20 uL of each colony (1, 5, 8)
- Transform UbiX plasmid and TetR promoter into E. coli (Dan K, Tyler)
    - NEB 5-alpha competent E. coli
    - Plated cells; grew overnight

06.17.15

- Ran colony PCR on E. coli transformed with UbiX gene (Dan K)
  - Took samples from 12 white colonies (UbiX 1x plate)
  - Used VF2 and VR primers, Taq1 polymerase
- Ran gel on PCR products (Dan K)
  - All the colonies (from UbiX 1X plate) had bands at 1.7 kb length indicating complications with transformation
- Cultured colonies 2 and 3 from UbiX PCR in LB Chlor overnight (Dan K)
- Cultured one colony from TetR 1x plate in LB Chlor overnight (Dan K)

06.18.15

- Miniprep UbiX colonies 2,3 and TetR culture (Dan K)
  - Nanodrop: UbiX 2 28.1 ng/uL, UbiX 3 102.1 ng/uL, TetR 53.0 ng/uL

06.19.15

- Prepared UbiX samples 2,3 for sequencing (Tyler)
- Inoculated LB Chlor medium with E.coli expressing PAL from iGEM registry (Dan K, Tyler)
- Ordered forward and reverse oligos for TetR/RBS (Dan K, Tyler)

06.22.15

- Miniprep PAL culture (Dan K, Tyler)
  - Nanodrop: PAL 77.0 ng/uL
- Analyzed UbiX samples 2,3 sequences (Dan K, Tyler)
  - Found that despite band size on gel, UbiX was in fact correctly transformed
  - Found that bad foward primer design resulted in loss of XbaI restriction enzyme site and no RBS

06.23.15

- Ordered new forward UbiX primer (Dan K, Tyler)
- Codon optimized PAL and FDC1 gene are delayed shipping to 07.06.15 (Kosuke)
- PCR forward and reverse oligos for TetR and RBS sequence (Dan K, Tyler)
  - 7 cycles at annealing temperature of 62°C
  - 7 cycles at annealing temperature of 58°C

- Ran gel on PCR products (Dan K, Tyler)
    - All four samples had bands around 125 bp indicating annealing of oligos
- Performed PCR cleanup on combined samples (Dan K, Tyler)
    - Nanodrop: TetR/RBS 491.7 ng/µl

06.24.15

- Performed double digestion of TetR/RBS, FDC1 plasmid, PAL plasmid (Dan K, Tyler)
    - Used restriction enzymes EcoRI HF, SpeI HF, XbaI
- Performed ligation of TetR/RBS with FDC1 plasmid and PAL plasmid (Dan K, Tyler)
- Transformed ligated plasmids into NEB 5-alpha competent E. coli (Dan K, Tyler)
- Ran PCR on codon optimized PAL with IDT Amp primers (Dan K, Tyler)
    - Annealing temp is 65°C, 30 cycles
- Ran gel of PCR product (Dan K, Tyler)
    - band at length of 2000 kb as expected
- PCR clean up of codon optimized PAL (Dan K, Tyler)
    - Nanodrop: CO PAL 49.0 ng/µl

06.25.15

- Worked on summary presentation (Dan K, Tyler)
- Confirmed growth of colonies from previous day's inoculation (Dan K, Tyler)
    - Moved plates from 37ºC shaker to refrigerator

06.26.15

- Performed colony PCR on TetR/RBS with FDC1 plasmid and PAL plasmid (Dan K, Tyler)
    - 12 colonies for each sample from 1x plate
    - Ran gel on products to find possible colonies with correct plasmid
        - Colony 8 of FDC1
        - Colony 12 of PAL
- PCR of UbiX plasmids 2 and 3 with new forward primer (Dan K, Tyler)
    - Nanodrop: UbiX 2 116.5 ng/µl, UbiX 3 111.5 ng/µl

06.29.15

- Cultured colony 8 of FDC1 and 12 of PAL in LB Chlor (Dan K, Tyler)
- Ran gel on UbiX F2, F3 (Dan K, Tyler)
    - 1% agarose, 80 volts, 45 minutes, 100 bp ladder
- Digested C.O. PAL, UbiX F2 and RFP plasmid (Dan K, Tyler)
    - EcoRI HF, SpeI HF, cutsmart buffer
- Ligated C.O. PAL and UbiX F2 into RFP plasmid (Dan K, Tyler)

06.30.15

- Miniprep of FDC1 colony 8 and PAL colony 12 (Dan K, Tyler)
  - Nanodrop: FDC1 colony 8 20.5 ng/µl, PAL colony 12 21.3 ng/µl
- Ordered sequencing of FDC1 colony 8 and PAL colony 12 (Dan K, Tyler)
  - VF2 and VR primers, annealing temperature at 57°C
  - Insufficient plasmid DNA for sequencing
- Transformed C.O. PAL and UbiX F2 using LB Chlor plates (Dan K, Tyler)
  - Used 25 µl of NEB 5-alpha instead of 50µl

07.01.15

- Analyzed sequencing results of FDC1 colony 8 and PAL colony 12 (Dan K, Tyler)
  - No TetR/RBS in either sample
- Transformation of part BBa_K525998 (T7 promoter and RBS) (Dan K, Tyler)
  - Plate 1, well 3M
  - Used NEB Turbo Cells
- Colony PCR of UbiX F2 and C.O. PAL transformation (Dan K, Tyler)
  - 12 colonies each
- PCR of C.O. FDC1 (Dan K, Tyler)
  - Nanodrop results: 31.9 ng/µl
  - Gel looked smeared

07.02.15

- Ran gel of colony PCR of UbiX F2 and C.O. PAL (Tyler, Dan X)
  - Picked UbiX F2 colonies 3, 4, 6
  - Picked C.O. PAL colonies 5, 7, 8
  - Inoculated in LB chlor at 37°C overnight
- Re-run PCR of C.O. FDC1 (Dan K)
  - nanodrop results: 36.3 ng/µl
  - Gel had stronger band at 2000 bp as expected
- Picked 3 colonies from T7 promoter with RBS plate (Dan K)
  - Cultured at 37°C for day
- Miniprep of three colonies of T7 Promoter with RBS (Dan K, Tyler)
  - Nanodrop results: 1: 9.5 ng/µl, 2: 8.7 ng/µl 3: 10.0 ng/µl

07.06.15

- Miniprep UbiX F2 and C.O. PAL colonies (Dan K, Dan X)
  - Nanodrop results
    - UbiX F2 3: 30.9 ng/µl, 4: 36.2 ng/µl, 6: 46.3 ng/µl

        - C.O. PAL 5: 41.2 ng/µl, 7: 40.0 ng/µl 8: 37.2 ng/µl
- Order sequencing for UbiX F2 and C.O. PAL results (Dan K, Dan X)
- Grow up two more cultures of T7 promoter with RBS (Dan K, Dan X)
    - Incubate at 37°C overnight
- Digestion and Cleanup of C.O. FDC1 into RFP plasmid (Dan K, Dan X)
    - Used EcoRI HF and SpeI HF
    - Nanodrop: C.O. FDC1 19.5 ng/µl, RFP Plasmid 6.4 ng/µl
- Ligation of C.O. FDC1 into RFP plasmid (Dan K, Dan X)
- Transformation of C.O. FDC1 ligated plasmid and RFP plasmid (Dan K, Dan X)
    - 1x (5 µl) and 10x (50 µl) plate

07.07.15

- Miniprep T7 colonies 4 and 5 (Dan K, Dan X)
    - Nanodrop results: 4: 35.9ng/µl, 5: 47.9 ng/µl
- Colony PCR of C.O. FDC1 (Dan K, Dan X)
    - picked 12 colonies from 10x plate
    - Ran gel of 12 samples with 1kb ladder. All colonies had around correct band length
    - Created 12 liquid cultures. One for each colony.
- Analyzed sequencing data from UbiX F2 and C.O. PAL colonies (Dan K, Dan X)
    - Correct sequences
- Create liquid cultures of UbiX F2 colony 3 and C.O. PAL colony 5 for Cryo-stock (Dan K, Dan X)

07.08.15

- Mini-prepped C.O. FDC1 colonies 1, 4, 8, 12 (Dan K, Dan X)
    - Nanodrop results:
- Ordered sequencing for C.O. FDC1 plasmids 1, 4, 8, 12 and cryo-stock samples for C.O. PAL colony 5 and UbiX F2 colony 3 (Dan K, Dan X)
- Created cryo-stocks for C.O. FDC1 colonies 1, 4, 8, 12 and C.O. PAL colony 5 and UbiX F2 colony 3 (Dan K, Dan X)
    - 30 percent glycerol, stored in -80°C

07.09.15

- Analyzed sequencing data from C.O. FDC1 colonies (Dan K, Dan X)
    - Correct sequences (colony 4 had poor ab file)
- Digested T7/RBS plasmid 5, Y3 FDC1 re-amplified, UbiX F2 2 (Dan K, Dan X)
    - Cut smart buffer, SpeI HF, XbaI, PstI HF
- PCR cleanup of digested products (Dan K, Dan X)
    - Nanodrop results: T7 7.5 ng/µl, FDC1 20.6 ng/µl, UbiX 29.1 ng/µl

- Ligation of T7 with FDC1 and UbiX
- Transformation into NEB 5 alpha competent cells (Dan K, Dan X)
    - plated 1x, 5x, 50x plates
- Ordered primers for iGEM PAL (Dan K, Dan X)

07.10.15

- Colony PCR of T7/RBS/FDC1 and T7/RBS/UbiX (Dan K, Dan X)
    - 18 colonies for each from 1x, 5x, and 10x plates
- Ran all samples on large gel (Dan X)
    - Colony 17 of T7/RBS/FDC1 showed promise
- Grew up colony 17 of T7/RBS/FDC1 (Dan X)
    - 2 ml of LB chlor at 37°C for weekend

07.13.15

- Mini-prepped colony 17 of T7/RBS/FDC1 (Dan K, Tyler, Dan X)
    - Culture was left for whole weekend at 37°C so possible mutations
    - Nanodrop: 35.2 ng/µl
- Created cryo-stocks for colony 17 of T7/RBS/FDC1 (Dan K, Tyler, Dan X)
    - 30 percent glycerol
- Ordered sequencing for colony 17 of T7/RBS/FDC1 (Dan K, Tyler, Dan X)
- Transformation of C.O. FDC1 and C.O. PAL into T7 expressing competent cells (Dan K, Dan X)
    - Used C.O. FDC1 plasmid 1 and C.O. PAL 5 cryo plasmid
    - Plated 1x and 10x
- PCR of iGEM PAL from plasmid with new primers: iGEM PAL F & R (Tyler)
    - Used mini-prepped PAL from 6.22
    - Q5 polymerase with gradient annealing
        - 1) 57.9º
        - 2) 61.9º
        - 3) 67.4º
        - 4) 70.0º
- Ran gel on amplified iGEM PAL (Tyler)
    - Expected bands just above 1500 bp
- PCR clean up (Tyler)
    - Nanodrop results: negative
    - Redo entire process another day
- Grow another liquid culture of colony 17 of T7/RBS/FDC1 picked from plate (Dan K, Tyler, Dan X)

07.14.15

- Analyzed sequencing of colony 17 of T7/RBS/FDC1 (Dan K)
  - correct sequence
- Mini prep new liquid culture of colony 17 of T7/RBS/FDC1 (Dan X)
  - Nanodrop: 25.1 ng/µl
  - Cryo-stock of sample, 30% glycerol
- Picked colonies from T7 expressing E.*coli* plates (Dan K)
  - 5 colonies from each sample into 5 ml LB Chlor
  - Incubate at 37°C overnight
- Autoclave two 100 ml of LB-Chlor in 250 ml flasks (Dan K)

07.15.15

- Inoculate 100 ml LB-Chlor with 5 ml of T7 expressing cultures (Dan K)
  - 37°C for 5 hours
- PCR of iGEM PAL from plasmid with new primers: iGEM PAL F & R (Tyler, Dan X)
  - Used mini-prepped PAL from 6.22
  - Q5 polymerase at 61°C
  - Three samples
- Ran gel on amplified iGEM PAL (Tyler, Dan X)
  - All three samples had expected bands just above 1500 bp
- PCR clean up (Tyler, Dan X)
  - Nanodrop results: 1 70.0 ng/µl, 2 116.7 ng/µl, 3 123.7 ng/µl
- Added 100 µl of IPTG to each 100 ml LB Chlor flask (Dan K)
  - Grow up at 30°C overnight

07.16.15

- Colony PCR Of T7/RBS/UbiX plates (Dan K, Tyler)
  - 20 new colonies
- Ran gel on all samples (Dan K, Tyler)
  - 2-log ladder and 1 kb ladder
  - two gels (12 and 8 lanes)
- Grew up colony 3, 10, 14, 20 of T7/RBS/UbiX and re-grow colonies from T7 expressing E.*coli* plates (Dan K, Tyler)
  - 5 ml of LB chlor at 37°C overnight
- Pelleted cells, resuspended in 5 ml TBS, lysed with super sonicator, pelleted and collected supernatant lysate (Dan K, Tyler)
  - looks like most cells already died and lysed, therefore expecting low protein

- Prepared magnetic beads by washing in TBS buffer three times (Dan K, Tyler)
- Incubated magnetic beads with 1 ml of lysate at room temperature for one hour (Dan K, Tyler)
- Extracted protein and run SDS gel with Mark 12 ladder, lysate and supernatant (Dan K, Tyler)
- Washed gel with methanol/acetic acid solution then soaked in SYPRO dye overnight (Dan K, Tyler)

07.17.15

- Prepared cryo-stocks of T7/RBS/UbiX colonies 3, 10, 14, 20 and T7 expressing codon optimized FDC and PAL (Dan K, Tyler)
    - 30 percent glycerol
- Mini prep T7/RBS/UbiX colonies 3, 10, 14, 20 (Dan K, Tyler)
    - Nanodrop: 3 103.1 ng/µl, 10 67.5 ng/µl, 14 95.5 ng/µl, 20 79.8 ng/µl,
- Ordered sequencing for T7/RBS/UbiX colonies 3, 10, 14, 20 (Dan K, Tyler)
- Washed gel with 10% methanol, 7% acetic acid solution for thirty minutes (Dan K, Tyler)
- Scanned gel (Dan K, Tyler)
    - Bands at expected length for C.O. PAL and FDC
        - Codon optimized PAL (62.77 kilodaltons)
        - Codon optimized FDC (57.31 kilodaltons)

07.20.15

- Analyzed sequencing of colonies of T7/RBS/UbiX (Dan K)
    - correct sequence
- Extracted C.O. PAL and C.O. FDC protein from cell lysate 2 (Dan K)
    - Anti-FLAG magnetic beads
    - 1.5 ml of lysate used
    - 30 µl used for elution
- Ran BCA protein assay kit (Dan K)
    - C.O. FDC 1: 399 µg/ml
    - C.O. FDC 2: 249 µg/ml
    - C.O. PAL 1: 1652 µg/ml
    - C.O. PAL 2: 896 µg/ml
- Digestion of iGEM PAL and T7/RBS plasmid (Tyler, Dan X)
    - PstI HF + XbaI on iGEM PAL
    - PstI HF + SpeI on T7/RBS
- PCR clean up of digestion products (Tyler, Dan X)
    - iGEM PAL #1=17.4 ng/µl, #2=23.3 ng/µl

    - T7/RBS plasmid #1= 1.8 ng/µl, #2=6.3 ng/µl
- Ligation of iGEM PAL into T7/RBS plasmid (Tyler, Dan X)
    - T4 ligase
- Transformation of T7/RBS/iGEM PAL (Tyler)
    - NEB 5-alpha competent E. *coli*
    - 1 x and 10 x plate

07.21.15

- Colony PCR of T7/RBS/iGEM PAL (Tyler, Dan X)
    - 18 colonies with TAQ polymerase
- Run Gel (Dan X)
    - Colonies 2, 8, 12, 18 had expected band length
- Grow cultures of colonies 2, 8, 12, 18 T7/RBS/iGEM PAL (Dan X)
- Ordered materials needed (Dan K)
    - Flavin Mononucleotide
    - Polystyrene
    - Solvent for In Situ removal of Styrene
        - bis(2-ethylhexyl)phthalate (BEHP)
        - n-Dodecane
    - Azobisisobutyronitrile
- Order synthesized UbiX with T7 promoter, RBS and flag tag (Kosuke)
- Run PAL functionality assay in 20µl samples at 25°C for 1 hour (Dan K, Tyler)
    - 1=Tris with PAL and Phe
    - 2=Tris with PAL only
    - 3=Tris with Phe only
    - 4=Tris only
    - 5=Tris with tCA only
- Run nanodrop on functionality assays (Dan K, Tyler, Dan X)
    - tCA detected in rxn 1 showing PAL functionality

07.22.15

- Run miniprep on T7 RBS/iGEM PAL liquid cultures colonies 2,8,12,18 (Dan X)
    - nanodrop: culture 2: 10.7ng/ul, culture 8: 5.8ng/ul, culture 12: 13.9ng/ul, culture 18: 6.5ng/ul
    - concentrations were bad because WN likely had no ethanol
- Created cryo stocks of cultures 2,8,12,18 (Dan K, Dan X)
- Ordered sequencing for colonies 2,8,12,18 (Dan K, Dan X)
- Using cryo-stocks re-make liquid cultures of colonies 2,8,12,18 (Dan K)

07.23.15

- Run miniprep on new T7 RBS/iGEM PAL liquid cultures colonies 2,8,12,18 (Dan K, Dan X)
    - nanodrop: culture 2: 32.7 ng/ul, culture 8: 31.6 ng/ul, culture 12: 58.9 ng/ul, culture 18: 46.3 ng/ul
- Ordered sequencing for new colonies 2,8,12,18 (Dan K, Dan X)
- Extracted C.O. PAL and C.O. FDC protein from cell lysate 1 and 3 (Dan K, Tyler, Dan X)
    - Anti-FLAG magnetic beads
    - 1.5 ml of lysate used
    - 30 µl used for elution
- Ran BCA protein assay kit (Dan K, Tyler, Dan X)
    - C.O. PAL 3 1148.97 µg/ml
    - C.O. PAL 4 1091.76 µg/ml

07.24.15

- Attempted to run PAL assay on Spectrophotometer to generate enzyme constants (Dan K, Tyler, Dan X)
    - tCA could not be measured because plastic plates absorb in the same UV region
    - Calculated expected constants and planned experiment accordingly
- Researched options for generating UV range absorbance spectra (Tyler)
    - quartz plates/cuvettes
    - UV-transparent plastics
- Called patrick from Nanodrop to talk about difference between UV-VIS and Nucleic acid setting (Dan K)

07.27.15

- Ordered primers for site directed mutagenesis of FDC (Dan K)
- Created concentration to absorbance curve for tCA and Phe (Tyler, Dan X)
- Emailed Nanodrop people a diluted tCA reading (Dan K, Dan X)
- Ordered UV transparent spectrophotometer plates (Tyler)

07.28.15

- Analyzed sequencing data for T7 RBS/iGEM PAL colonies 2,8,12,18 (Dan K)
    - Colonies 2 and 12 had the correct sequence
    - Colonies 8 and 18 were missing T7 promoter and RBS
        - weird data, not sure how it was formed
- Set up custom tCA measuring application in Nanodrop (Tyler, Dan X)

- Carried out 4 hour kinetic experiment using Nanodrop (Tyler, Dan X)
    - ~10 min intervals
    - Used standard curve from custom application
- Set up site-directed mutagenesis of FDC and FDC w/ T7 (Dan K)
    - Annealing temperature of 60°C and extension time of 2 mins
- Add 1µl of DpnI to PCR tubes and incubate for 30 mins at 37°C (Dan K)
- PCR clean up (Dan K)
- Transform into E.*coli* competent cells (Dan K)
    - Add 3 µl of product

07.29.15

- Curated data from prior day's experiment (Tyler, Dan X)
    - Would prefer to use 2nd floor spectrophotometer
    - Set up stencil code for generating plots, analyzing data
- Investigated UbiX dependence on dimethylallyl monophosphate (DMAP) (Tyler, Dan K)
    - Conclusion: UbiX will not convert FMN into correct cofactor in the absence of DMAP
- Picked four colonies from site-directed mutagenesis plates for FDC and T7/FDC (Dan K)
    - Grow in 4 ml LB-Chlor

07.30.15

- Mini prepped site-directed mutagenesis colonies (Dan K, Tyler)
    - Nanodrop results:
        - FDC 1= 47.9 ng/µl, 2= 33.3 ng/µl, 3= 64.2 ng/µl, 4= 31.2 ng/µl
        - FDC w/ T7 1= 39.8 ng/µl, 2= 97.7 ng/µl, 3= 78.9 ng/µl, 4= 133.5 ng/µl
- Prepared site-directed mutagenesis colonies for sequencing (Dan K, Tyler)
- Input experimental data to improve model (Dan X)

08.01.15

- Analyzed S.D.M. sequencing data (Dan K)
    - No FDC colonies worked
    - T7/FDC colonies 1 and 2 had mutations at the correct base
    - T7/FDC colonies 3 and 4 had incorrect sequencing (only forward)
        - re-order sequencing for these two colonies next week?
- Re-run site-directed mutagenesis of FDC (Tyler, Dan X)
    - Set up PCR of FDC 5 plasmid with new primers
        - Annealing temperature of 60°C and extension time of 2 mins

- Add 1µl of DpnI to PCR tubes and incubate for 30 mins at 37°C
- PCR clean up
- Transform into E.*coli* competent cells
  - Add 3 µl of product

08.03.15

- Prepared 4 ml LB chlor liquid cultures from site-directed mutagenesis of FDC (Dan X)
  - 37°C overnight
- Registered biobricks for submission (BBa_K1692000 to BBa_K1692006) (Dan K)
- Create new standards for tCA and Phe (Tyler)
  - 600 µm, 360 µm, 216 µm, 130 µm, 78 µm, 47 µm, 28 µm, 17 µm
- Run spectrophotometer with two columns of tCA standards and three columns of reactions (Tyler)

08.04.15

- Mini prepped site-directed mutagenesis colonies (Dan K, Dan X)
  - Nanodrop results:
    - FDC w/ T7 1= 63.3 ng/µl, 2= 94.3 ng/µl, 3= 94.7 ng/µl, 4= 121.5 ng/µl
- Prepared site-directed mutagenesis colonies for sequencing (Dan X)
- Analyzed spectrophotometer data in R (Tyler)
- Prepare DNA for biobricks (Dan K)
  - PanK, T7/FDC 2 (7.30, 97.7 ng/µl), C.O. FDC 1 (7.08, 54.1 ng/µl), iGEM PAL with T7 (7.23, 58.9 ng/µl), C.O. PAL 5 cryo (7.08, 36.6 ng/µl), UbiX 3 (7.06, 30.9 ng/µl), UbiX with T7 3 (7.17, 103.1 ng/µl)
- PCR of C.O. PAL 5 cryo with VF2 and VR for sequencing (Tyler, Dan X)
  - PCR clean up
    - Nanodrop results: 104.2 ng/µL

08.05.15

- Analyze sequencing data for S.D.M FDC (Dan K)
  - No colonies with correct mutated base pair
- Order/prepare sequencing for PCR of C.O. PAL 5 cryo (Dan K)
- Sent in 7 biobricks (Dan K)
  - USPS tracking number: 9405503699300116463989
- Analyzed spectrophotometer data in R (Tyler)

08.06.15

- Analyze sequencing data for PCR of C.O. PAL 5 cryo (Dan K)
  - Successful

- Re-run S.D.M of FDC with 4 samples on gradient (Dan K, Tyler)
    - 62.2°C, 60.5°C, 58.4°C, 56.7°C
    - 37 min incubation at 37°C with DnpI
    - Transformation with 3.5 µl at 10x
- Register for MALDI MS (Dan X)
- Re-run first PAL activity assay for MALDI ms analysis (Dan X)
    - 15 µl 10 mM Phe, 5 µl of PAL enzyme 1
- Analyzed spectrophotometer data in R (Tyler)

08.06.15

- Gamma-gamma dimethylallyl monophosphate arrived in mail (Dan K, Tyler, Dan X)
- Found and performed protocol for AIBN polymerization of styrene (Dan K, Tyler, Dan X)
    - 10 ml of styrene, 55 mg AIBN, 60°C for one hour
    - precipitate in 200 ml methanol
    - Tried filtering, but filter dissolved
- Grew 6 LB chlor cultures of S.D.M. FDC (Dan K)

08.09.15

- Re-run protocol for AIBN polymerization of styrene (Dan K)
    - 10 ml of styrene, 60 mg AIBN, 60°C for one hour
    - precipitate in 100 ml methanol
    - Pellet, wash in methanol, dry
- Dissolve dried sample of polystyrene from 08.06.15 in acetone and create sheet (Dan K)
    - Try to shrink with oven, but ignited

08.10.15

- Miniprep S.D.M. FDC colonies (Dan X)
    - Nanodrop results: 1/1 49.4 ng/µl, 1/2 37.4 ng/µl, 2/1 33.7 ng/µl, 2/2 37.3 ng/µl, 4/1 29.1 ng/µl, 4/2 40.6 ng/µl
- Order and prepare sequencing for S.D.M. FDC colonies (Tyler)
- PCR of synthesized UbiX with Flag Tag (Dan K)
    - Annealing temp=65°C, elongation time 25 seconds
- PCR clean up of UbiX with Flag Tag (C.O. UbiX) (Tyler)
    - Nanodrop results: 51.5 ng/µl
- Digestion of CO UbiX and GFP plasmid (Tyler)
    - XbaI and PstI-HF

- PCR clean up of digested products (Dan X)
    - C.O. Ubix 15.1 ng/µl, GFP 27.3 ng/µl
- Ligation with T4 ligase (Dan K)
- Transformation 1x, 5x, 10x (Tyler)
- Processed polystyrene from balls and styrofoam for sheets (Forrest, Dan K)
    - Used acetone to dissolve polystyrene
    - Hot water bath for stretching

08.11.15

- Analyzed sequencing data for S.D.M FDC round three (Dan K)
    - No correct sequences
- PCR of S.D.M FDC from S.D.M. T7 FDC plasmid (Dan X, Dan K)
- PCR clean up (Dan X)
    - Nanodrop: 95.4 ng/µl
- Order and prepare sequencing for first S.D.M FDC PCR (Dan K)
- PCR of S.D.M. FDC PCR using primers with overhang (Tyler)Annealing temperature at 57°C
- PCR clean up (Dan X)
    - Nanodrop: 140.7 ng/µl
- Examined plates from previous day (Tyler)
    - Selected 12 white colonies for colony PCR
- Performed and ran colony PCR of C.O. UbiX (Tyler)
- Ran gel of colony PCR (Dan X, Dan K)
    - Looks like all colonies except 4, 11, 12 with correct band length
- Grow liquid cultures of C.O. UbiX colonies 3, 5, 8, 10 (Dan K)
- Processed polystyrene from balls and styrofoam for sheets (Forrest, Dan K)
    - Noticed that cloudy polystyrene is caused by air bubbles
    - Clear polystyrene is better for stretching and folding
- Digestion of CO UbiX and GFP plasmid (Tyler)
    - XbaI and PstI-HF
- PCR clean up of digested products (Tyler, Dan K)
    - SDM FDC 21.9 ng/µl, GFP 13.5 ng/µl
- Ligation with T4 ligase (Tyler)
- Transformation 1x, 5x, 10x (Tyler)

08.12.15

- Miniprep/cryostock C.O. UbiX colonies and prepared cryo stocks (Dan X)
    - Nanodrop results: 3: 79.1 ng/µl, 5: 44.5 ng/µl, 8: 44.9 ng/µl, 10: 75.1 ng/µl,
- Ordered and prepared sequencing of C.O. UbiX colonies (Dan X, Dan K)
- Analyzed sequencing of linear S.D.M. FDC (Dan K)
    - correct sequence
- Performed and ran colony PCR of S.D.M FDC (Tyler, Dan X)

- Ran gel of colony PCR (Dan X, Dan K)
    - Looks colonies 1, 4, 9, 12 with correct band length
- Grow liquid cultures of S.D.M. FDC colonies 1, 4, 9, 12 (Dan X)
- Transformed all four colonies of C.O. UbiX into T7-expressing E.*coli* (Tyler)
- Analysed MALDI-MS data and decided to rerun experiment with controls (Dan K, Tyler, Dan X)
- Prepare mixture of 100 µl of 10 mM Phe with 4.35 µl of PAL 3 protein (Dan K)

08.13.15

- Miniprep/cryostock S.D.M. FDC colonies 1, 4, 9, 12 and prepared cryo stocks (Dan X)
    - Nanodrop results: 1: 47.8 ng/µl, 4: 38.5 ng/µl, 9: 25.0 ng/µl, 12: 61.6 ng/µl
- Ordered and prepared sequencing of S.D.M. FDC colonies (Dan X, Dan K)
- Analyzed sequencing of C.O. UbiX (Dan K)
    - Colonies 8 and 10 had correct sequence
    - Colony 3 had missing base pair
    - Colony 5 had a point mutation
- Selected 5 colonies each from plates 8 and 10 of CO UbiX for growth in liquid medium (Tyler)
- Performed colony PCR on the ten colonies that were selected for liquid cultures of UbiX (Tyler)
- Used 10 µl of magnetic beads to remove PAL from 100 µl sample for MALDI MS (Dan K)
    - 1 hour incubation at room temperature
- Dropped off three samples at Stanford for MALDI MS (Dan K, Tyler, Dan X)
    - 10 mM Phe
    - 10 mM tCA
    - Experiment with Phe and PAL

08.14.15

- Autoclave 100 mL of lb in 250 mL flask (Tyler)
- Create Cryo stock of T7-expressing C.O. UbiX culture (Tyler, Dan X)
- Inoculate 100 mL warm lb chlor with 4.5 mL (use 0.5 mL for cryostock), 37° for 5 hours, add 100 uL IPTG into lb chlor flask, grow at 30° overnight (Tyler)
- Run gel for colony PCR UbiX (Dan X)

08.15.15

- Pellet cells, store at -80° until Monday (Tyler)

08.17.15

- Re-suspend in 5 mL TBS, lyse with sonicator, pellet junk, collect lysate and freeze
- Sonicate cells and store cell lysate in -80 fridge (Dan X)
- UbiX/FDC/PAL extraction using Anti-FLAG tag protocol (Tyler)
    - Anti-FLAG magnetic beads
    - 1.5 ml of lysate used
    - 30 µl used for elution
- protein bca assay: (Dan X)
    - UbiX: 406.333 µg/ml
    - PAL: 400.682 µg/ml
    - FDC: 377.273 µg/ml
    - Proteins are in filter tubes labeled UbiX 2, PAL 2, FDC 2

08.18.15

- Prepare PAL, FDC, UbiX samples for SDS PAGE analysis (Tyler)
- Ran SDS PAGE (Tyler, Dan X)
    - 150 V, ~ 35 min
- Processed gel and analyzed results (Tyler, Dan X)
    - UbiX and PAL present
    - No sign of FDC

08.19.15

- Purify additional FDC and UbiX protein (Tyler, Dan X)
    - Two additional 1.5 mL lysate samples
    - BCA:
        - FDC1: 633.9 ug/ml
        - FDC2: 713.4 ug/ml
        - UbiX: out of range ~2000+ ug/ml
        - UbiX dot: 1915.7 ug/ml
- Prepare FDC, UbiX samples for SDS PAGE analysis (Tyler, Dan X)
    - Increased volume of lysate
    - Used 1st wash instead of 2nd wash

08.20.15

- Analyzed SDS gel (Tyler, Dan X)
    - FDC from earlier in the week did not show up again so it was disposed of
    - Newly purified FDC had band at expected position
    - PAL and UbiX also had bands at expected positions
- Sent email to AmSty (Tyler)
    - asked about economic impact, utility to industry, polymerization techniques

- plan in vitro experiments (Tyler, Dan X)

08.21.15

- digestion ligation transformation of second FDC PCR product (previous sdm didn't work) (Dan X)
- PCR clean up: RFP: 22.7 ng/ul, sdm FDC: 33.0 ng/ul
- gave ligated product to CRATER to increase efficiency of transformation (Dan X)

08.24.15

- colony PCR of sdm FDC, all bands were below 500 so none could be FDC (Dan X)
- SDS page gel preparation, left in sypro ruby overnight (Tyler)
- Prepare combinations tCA, Phe, Styrene, FMN (10mM stocks, 1mM in each well) (Dan X)

08.25.15

- Ran combinations tCA, Phe, Styrene, FMN in spectrophotometer (Dan X)
    - All concentrations at 1 mM
- Scanned gel (Dan K, Tyler, Dan X)
    - Bands at expected length for C.O. PAL, FDC and UbiX
        - Codon optimized PAL (62.77 kilodaltons)
        - Codon optimized FDC (57.31 kilodaltons)
        - Codon optimized UbiX (21.75 kilodaltons)

08.26.15

- Prepared in-vitro test of UbiX and FDC (full spectrum every 30 minutes) (Dan K, Tyler, Dan X)
    - UbiX, FMN, DMAP, Mn 2+, Tris
    - UbiX, FMN, DMAP, tCA, Mn 2+, Tris
    - UbiX, FMN, DMAP, FDC, Mn 2+, Tris
    - UbiX FMN, DMAP, FDC, tCA, Mn 2+, Tris
- Took absorbance spectra of each reaction every 30 minutes (Tyler)
    - No discernable change in absorbance profiles over 3 hours
- FDC (old) flag tag extraction (Dan X)
    - BCA protein assay
        - FDC3: 337.3 ug/ml
        - FDC4: 393.3 ug/ml
- PCR of S.D.M. FDC using primers with overhang (Dan K)
    - Nanodrop: 96.9 ng/µl

08.27.15

- Digestion, ligation, transformation of S.D.M. FDC PCR product (Dan X)
  - Enzyme cleanup: RFP: 13.2 ng/µl, sdm FDC: 33.0 ng/µl
  - gave ligated product to CRATER to increase efficiency of transformation
  - Transformed 25 µl, 50 µl and 100 µl
- Ordered 8 primers for Gibson assembly of super Styrene Plasmid (Dan K)
- Ran continuous FDC/UbiX spectrophotometric assay to detect drop in tCA (Tyler)
  - Overnight (≈19 hours)
  - Results inconclusive, but possible suggestion that tCA concentration decreases over time

08.28.15

- Colony PCR of S.D.M. FDC with CRATER (Dan K)
  - No colonies with correct band length of 1.7 kb
- Grow liquid cultures of all colonies on shaker at room temperature (Dan X)
- Analyze spectrophotometric data (Tyler)
- Talk to labs downstairs about MS and microscopy (Dan K, Tyler)
  - Jesica will help with HPLC and GC-MS
  - NMR Mark for polystyrene analysis
  - Joey Varelas for microscopy

08.31.15

- Ran colony gel for SDM FDC (Tyler)
  - None of the 12 colonies contained the desired insert
  - 2nd PCR with overhang amplified nonspecifically
- Miniprep of colonies 1 and 2 for sequencing (Dan X)
  - Nanodrop results: FDC-1: 16.3 ng/ul, FDC-2: 16.0 ng/ul
- Prepared Q5 reaction mixes for Gibson PCR (Tyler)
- PCR of RFP, FDC, UbiX, PAL for Gibson Assembly (Tyler)
- PCR cleanup of RFP, FDC, UbiX, PAL for Gibson Assembly (Tyler)
  - Nanodrop results:
    - RFP = 81.7 ng/µl
    - PAL = 123.7 ng/µl
    - FDC = 91.4 ng/µl
    - UbiX = 75.6 ng/µl

09.1.15

- Ordered and prepared sequencing for mini prepped colonies 1,2 (Dan K)
- Ran gel of RFP, FDC, UbiX, PAL (Gibson Assembly) and S.D.M. FDC (Tyler)
  - Ran all or half of DNA for gel extraction

- Performed gel extraction on RFP, FDC, UbiX, PAL (Gibson Assembly) and S.D.M. FDC (Tyler)
    - RFP = 19.1 ng/µl
    - PAL = 15.5 ng/µl
    - FDC = 7.3 ng/µl
    - UbiX = 20.9 ng/µl
    - S.D.M. FDC = 9.5 ng/µl
- PCR amplification of gel extraction (Tyler)
- Ran gel of PCR products (Tyler)
    - Cluttered bands
- PCR clean up (Dan K)
    - RFP = 57.4 ng/µl
    - PAL = 63.0 ng/µl
    - FDC = 51.9 ng/µl
    - UbiX = 40.0 ng/µl
    - S.D.M. FDC = 99.9 ng/µl
- Made 4 ml LB-Chlor of UbiX F2 cryo stock for in-situ solvent experiment (Dan K)

09.2.15

- Analyzed sequencing data for S.D.M. fail (Dan K)
    - No useful information
- Ran gel with all the PCR amplified gel extract and gel extract (by mistake) (Tyler)
    - Not very useful, except that gel extract had correct band lengths
- Gibson assembly of gel extracted products from 9.2 (Dan K)
    - RFP = 1 µl
    - PAL = 1.5 µl
    - FDC = 3 µl
    - UbiX = 0.5 µl
- Transformed gibson assembled plasmid (Dan K, Tyler)
    - 4 µl plasmid, 50 µl of cells, 3 plates (20 µl, 100 µl, 200 µl)
- Created standards for Phe, tCA, Sty, and all three at three concentrations (Tyler)
    - 1mM, 0.1 mM, 0.01 mM
- Performed in-situ solvent removal experiment (Dan K)
    - 24 tubes of LB-chlor with no solvent (600 µl of milli-Q)
        - Three groups of styrene concentration 0, 75, 150, 300, 600, 1200, 2400, 4800 (mg/L)
    - 24 tubes of LB-chlor with solvent (600 µl of dodecane)
        - Three groups of styrene concentration 0, 75, 150, 300, 600, 1200, 2400, 4800 (mg/L)
    - All tubes inoculated with 1 µl of UbiX F2 liquid culture
    - 16 hours of incubation

09.3.15

- Measured OD-600 of 150 µl from each sample of in-situ experiment (Dan K, Tyler)
  - Found curves as expected
- Talked to Asif downstairs about HPLC, unlikely to happen (Tyler)
- Performed colony PCR of Gibson assembly (Dan K, Tyler)
  - 18 colonies
  - weird RFP colonies and large white colonies (bacillus?)
- Made in-vitro tests for FDC/UbiX and PAL/FDC/UbiX with and without dodecane (Dan K, Tyler)
  - 12 100 µl tubes (3 groups of four)
- Ran gel of colony PCR (Dan K)
  - Three bands at correct length (colonies 5, 3, 11)
- Made liquid cultures of colonies 5, 3, 11, 14 (Dan K)

09.4.15

- Mini-prepped four tubes of Combo plasmid (Kirsten)
  - Note: These plasmids and all following reactions will be stored in the "Styrene-Post Brown" Box for convenience

09.9.15

- PCR out Gibson assembly insert using VF2 and VR (4 plasmids, 2 tubes for each plasmid) (Kirsten)
- PCR Clean up (Kirsten)

09.10.15

- Nanodropped PCR from 9/9 (Kirsten)
  - 3A-109.1 ng/µl -
  - 3B-112.9 ng/µl
  - 5A-86.0 ng/µl
  - 5B-83.3 ng/µl
  - 11A- 84.9 ng/µl
  - 11B-97.4 ng/µl
  - 14A-85.6 ng/µl
  - 14B-81.2 ng/µl
- Sent for sequencing using VF2 and VR primers- tomorrow will determine which plasmids have the insert and will send those for more complete sequencing with the five internal primers (Kirsten)
  - Note that A's were sequenced using VF2 and B's were sequenced using VR

09.11.15

- PCR Combo plasmids that have gene with internal primers and send for sequencing by 2PM (Kirsten)

09.12.15

- PCR SDM FDC, clean up, run all on gel, gel extract (Kirsten)
  - Concentration post gel extraction: 15.9 ng/µl
- Colony PCR SDM FDC transformations (Kirsten)
- Transform 3A and 5A combo plasmid in t7 lysY Competent *E. coli*

09.13.15

- Take Combo Plasmid (3A and 5A) plates out from 37ºC incubator and start cell cultures (Kosuke)

09.14.15

- Digest SDM FDC with EcoRI and SpeI (as well as RFP plasmid if we don't have any left), ligate, and transform into Top Ten Competent Cells (plate all 250 µl on LB Chlor) (Kirsten)

09.15.15

- No colonies-redo ligation and transformation into Neb5 Electrocompetent cells and plate on LB chlor (Kirsten)
- Make cryogenic stocks of 3A and 5A combo plasmid (Kirsten)
- Completed protein purification assay/Western blot for combo plasmid (Kirsten and Kosuke)

09.15.15

- Start styrene production assay
  - Grow 3A and 5A in LB chlor until reach OD 600 of 0.6
  - 4 tubes each for both 3A and 5A combo plasmid:
    - 3 ml LB chlor
    - 3 ml LB chlor + IPTG
    - 3 ml LB chlor + 600 µl dodecane + IPTG
    - 3 ml LB chlor + 600 µl dodecane
  - Measure OD 600 every hour for 8 hours
- Pick colonies from SDM FDC transformation and miniprep→ send in for sequencing by 2PM (Kirsten)

- Dry out DNA samples for 3A and SDM FDC and send in for BioBricks tomorrow (Thursday) (Kirsten)
- Make Biobrick pages (Kirsten and Daniel K.)

09.15.15

- Send in Biobricks (Kirsten)

09.16.15

- Send samples in for GCMS (Kirsten)