Pho Lab Notebook

Thursday, February 6th, 2020

- Prepared for wet lab workstream
- Made LB agar for plates
- Plain LB (250ml: 8.75g LB and 3.75g BactoAgar)
  (500ml: 17.5g LB and 7.5g BactoAgar)

Friday, February 7th, 2020

- Digested pSB1K3 (w/ RFP) w/ iGEM protocol
  - Used linearized plasmid backbone protocol (one w/ enzyme mastermix...DpnI)
  - Gel confirmation - correct

Monday, February 10th, 2020

- Transformed YFP (well 5B) from 2019 plate 2 distribution kit

Tuesday, February 11th, 2020

- Allowed Transformation to grow another day
- 3 tiny colonies present

Wednesday, February 12th, 2020

- Inoculated colonies 1 - 2 from the YFP transformation

Thursday, February 13th, 2020

- Miniprepped the inoculation of YFP transformation colonies 1 - 3
  - Col 1: 1.96; 25.8ng/ul
  - Col 2: 1.98; 52.2ng/ul
  - Col 3: 2.04; 50.2ng/ul
- Allowed 3 extra plates grow over the weekend at room temperature

Wednesday, February 19th, 2020

- Inoculated 8 colonies from the 3 extra YFP plates for miniprepping tomorrow
- The incubator was turned off over the weekend so it may have taken a little while to initially start growing

Thursday, February 20th, 2020

- Miniprepped colonies from the additional plates of YFP/1C3

Friday, February 21st, 2020

- Sequencing col 2 and col 3
- Made VF2 and VR stocks

Monday, February 24th, 2020
- Digested minipreps of col 2 and col 3 of YFP/1C3
- Ran gel confirmation

Tuesday, February 25th, 2020
- Ligation of col 2 and col 3 with YFP insert with pSB1K3 vector
- Troubleshooting snapgene insert for biosensor

Wednesday, February 26th, 2020
- Transform YFP/1K3 ligation of col 2 and col 3

Thursday, February 27th, 2020
- Plates put into fridge so that we can colony pcr the next day
- Colonies not the same size

Friday, February 28th, 2020
- Colony PCR
- Gel confirmation
- Col 3,4,5, and 6 are correct

Monday, March 2nd, 2020
- Inoculate colony 3,4,5, and 6

Tuesday, March 3rd, 2020
- Miniprep the 4 inoculations
- Colony 4 had best purity and concentration
- Sent colony 4 for sequencing

Wednesday, March 4th, 2020
- Attempted digestion but didn't have enough ng of DNA (miniprep)
- So we just decided to digest with what we have and inoculate more colonies that were the same size as colony 4

Quarantine

Tuesday, August 11, 2020

- Recorded inventory
- Made plain LB
- Poured carb, cam, kan plates

Monday, August 17, 2020

- Hydrated 3 parts from 2019 Distribution Kit with 10 uL dH_2O
    - Plate 6 4A
    - Plate 5 23O
    - Plate 5 23P
- All 3 are the same part: BBa_J04450 (RFP in pSB1C3, induced by IPTG)

Tuesday, August 18, 2020

- Transformed all 3 hydrations from yesterday, and pUC19
- Plated transformed hydrations on cam plates, puc on carb plate
- Spread IPTG on warm plates w/ glass beads prior to plating

Wednesday, August 19, 2020

- Lawn growth on pUC19 (carb) plate
    - Carb may no longer be effective
- Plated pUC19 on cam, kan plates to check if cam, kan antibiotics still effective
- No growth of other plated transformations; let grow for another day

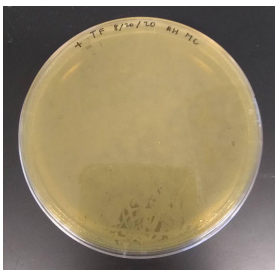
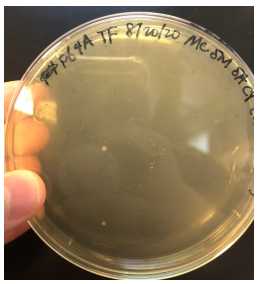
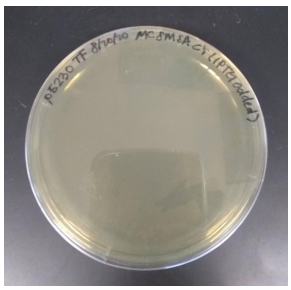
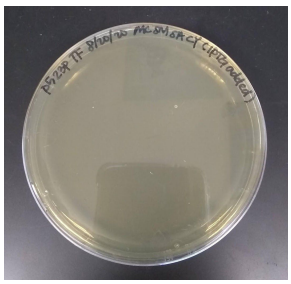
Thursday, August 20, 2020

- No growth of pUC19 on cam or kan plates - cam, kan still effective
- Replated pUC19 on carb
- Still no growth of 3 transformations; pelleted all 3 transformation solutions and replated with entire pellet

Friday, August 21, 2020

- pUC19 has lawn growth
- P6 4A has 2 red colonies (expressed RFP!)
- No growth on P5 23O or P5 23P
- Let all transformations sit out and grow over weekend

pUC19 (lawn growth)	P6 4A (2 red colonies)	P5 23O (no growth)	P5 23P (no growth)

Monday, August 24, 2020

- P5 23O from 8/20 plating has mold; bleached and discarded
- No observable additional growth of 3 transformed parts; incubated overnight

Tuesday, August 25, 2020

- Incubator was off, and no additional growth
- P5 23P from 8/18 plating has mold; bleached and discarded
- Inoculated colony 1, colony 2 from P6 4A

Wednesday, August 26, 2020

- Miniprepped colony 1, colony 2 from P6 4A
    - Used BioRad kit (usually use Omega kit)

	Concentration	Purity
Colony 1	88.2 ng/ul	1.88
Colony 2	75.9 ng/ul	1.95

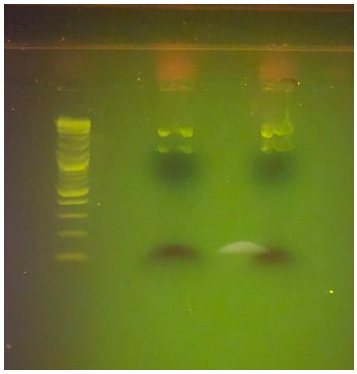
Thursday, August 27, 2020

- Restriction digest of miniprepped colony 1 and colony 2 from yesterday
- Colony 1
    - DNA 11.34 ul
    - E-HF 1 ul
    - P-HF 1 ul
    - Cutsmart buffer 5 ul
    - H_2O 31.66 ul
- Colony 2
    - DNA 13.18 ul
    - E-HF 1 ul

    - P-HF 1 ul
    - Cutsmart buffer 5 ul
    - H_2O 29.82 ul

Friday, August 28, 2020

- Ran 2% blue TBE gel of colony 1, colony 2 digest from yesterday
    - 0.4 g agarose, 20 ml TBE, 2 ul 10,000x SYBR Safe



Tuesday, September 1, 2020

- Hydrated other part from Pho order
    - 1000 ng → add 10 ul H_2O → 100 ng/ul concentration

Wednesday, September 2, 2020

- Restriction digest of yesterday's hydrated part
    - DNA 5 ul (500 ng DNA total)
    - Cutsmart buffer 5 ul
    - E-HF 1 ul
    - P-HF 1 ul
    - H_2O 38 ul
- Ran 1.5% blue TBE gel of digested part
    - 6 ul ladder
    - 5 ul DNA + 1 ul loading dye

Thursday, September 3, 2020

- Hydrated Pho part
- Restriction digest of Pho part

Friday, September 4, 2020

- Ligation of Pho part in pSB1C3
- pSB1C3 from Colony 1

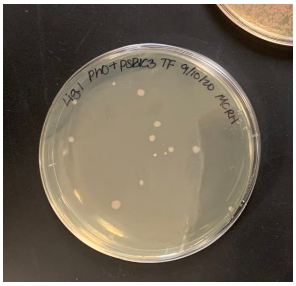
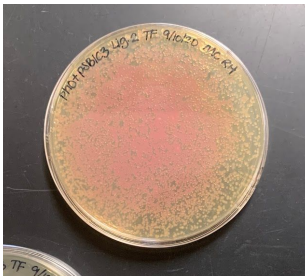
    - Pho insert 1.5 ul
    - pSB1C3 vector 0.57 ul
    - T4 Ligase buffer 2 ul
    - T4 DNA Ligase 1 ul
    - Water 14.93 ul
- pSB1C3 from Colony 2
    - Pho insert 1.5 ul
    - pSB1C3 vector 0.66 ul
    - T4 Ligase buffer 2 ul
    - T4 DNA Ligase 1 ul
    - Water 14.93 ul

Thursday, September 10, 2020

- Transformation of Pho insert in pSB1C3 ligations (Colony 1, Colony 2)
- Pelleted cells before plating
    - pUC19 plated on carb plate
    - Colony 1, Colony 2 ligations plated on cam plates

Friday, September 11, 2020

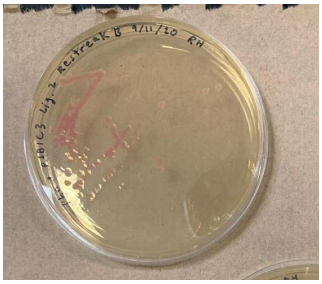
- Results from transformation

pUC19	Ligation 1 Pho + pSB1C3	Ligation 2 Pho + pSB1C3
		

- pUC19 did not have lawn growth → new plates with new CARB antibiotics worked
- Ligation 1 had colonies that all expressed RFP (religation of RFP coding device to backbone)
- Ligation 2 had almost all colonies that expressed RFP, with a few small colonies that were cream-colored
- Restreaked 3 non-red colonies from Ligation 2 plate, let grow on top of incubator over weekend

Monday, September 14, 2020

- Plates from restreaked 3 colonies of Ligation 2 Pho + pSB1C3

Colony 1 (A)	Colony 2 (B)	Colony 3 (C)
		

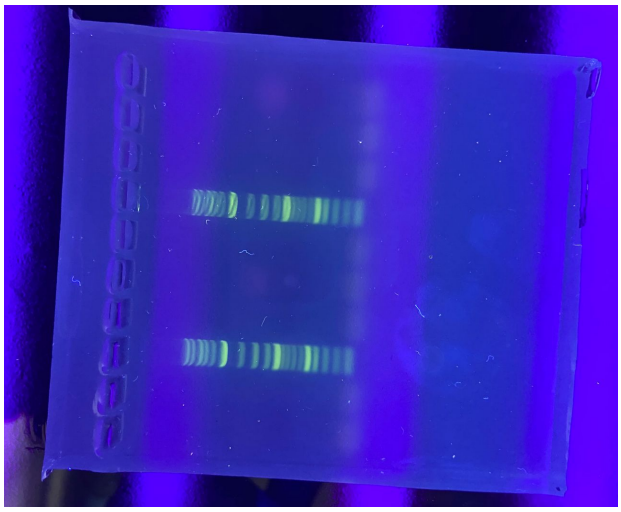
- Restreaked Colony 1 (A) has a small white colony → possible successful ligation of Pho + pSB1C3; might also express RFP after future growth
- Restreaked Colony 2 (B) has pinkish red colonies → RFP coding device re-ligated to backbone
- Restreaked Colony 3 (C) has white colonies → probably successful ligation of Pho + pSB1C3; do colony PCR tomorrow to confirm

Tuesday, September 15, 2020

- Colony PCR of 6 white colonies from Plate C, 2 red colonies from Plate B

Wednesday, September 16, 2020

- Gel of yesterday's colony PCR



- Faint bands in lane 1, lane 6 that correspond with where Pho part should be
- Inoculated those two colonies (Colony 1, Colony 6 from Plate C)

Thursday, September 17, 2020

- Miniprep of Colony 1, Colony 6

	Concentration	Purity
Colony 1	133.7 ng/ul	1.84
Colony 6	76.1 ng/ul	1.92

- Sent both minipreps out for sequencing
    - Col 1 → BKM822
    - Col 6 → BKM826
- Inoculated Colony 1, Colony 6 from Plate C for glycerol stocks

Friday, September 18, 2020
- Created glycerol stocks of Colony 1, Colony 6

Monday, September 28, 2020
- Inoculated from glycerol stocks into liquid cultures

Tuesday, September 29, 2020
- Inoculated from liquid cultures into flasks

Wednesday, September 30, 2020
- Tested biosensor cells with varying concentrations
- Measured with spectrophotometer and Fluoro-Q
- Inconclusive data
- Inoculated from glycerol stocks into liquid cultures

Thursday, October 1, 2020
- Inoculated from liquid cultures into flasks

Friday, October 2, 2020
- Tested biosensor cells with varying phosphate concentrations
- Measured with spectrophotometer and Fluoro-Q
- No observable GFP expression

Monday, October 5, 2020
- Inoculated from glycerol stocks into liquid cultures

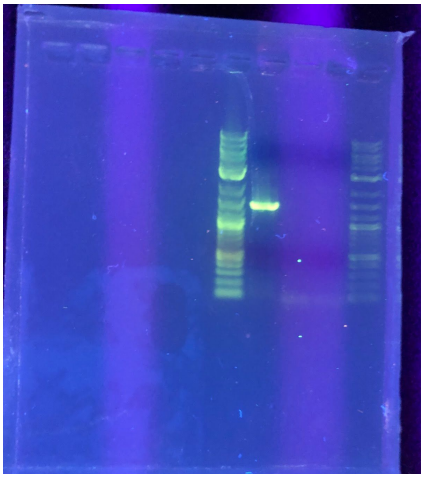
Tuesday, October 6, 2020

- Inoculated from liquid cultures into flasks
- Tested biosensor cells with varying phosphate concentrations
- Measured with spectrophotometer, Fluoro-Q, and plate reader
- Cell growth, but no GFP expression

Wednesday, October 7, 2020

- Miniprep liquid cultures of Colony 1 & Colony 6 glycerol stock cells
    - Col 1: concentration: 92.3 / purity: 1.85
    - Col 6: concentration: 109.5 / purity: 1.75

Thursday, October 8, 2020

- Run successful PCR on Pho insert
    - 

Friday, October 9, 2020

- Run PCR on pSB1C3 backbone
    - Primer melting temperature 68 degrees Celsius
    - Fail

Monday, October 12, 2020

- Run another PCR on pSB1C3 backbone
    - Fail

Tuesday, October 13, 2020

- Colony PCR on 4 colonies with pSB1C3 backbone

Thursday, October 15, 2020

- Gel on colony PCR

- Band on Colony 3
- PCR cleanup on insert
- Gibson assembly insert and pSB1C3 backbone
- Transformation

Friday, October 16, 2020
- Let plates continue growing over weekend

Monday, October 19, 2020
- Inoculated from plate into liquid culture

Tuesday, October 20, 2020
- Miniprep
- Made glycerol stock

Wednesday, October 21, 2020
- Make MOPS media

Thursday, October 22, 2020
- Inoculate biosensor cells into MOPS media & LB for testing on 10/25

Friday, October 24, 2020
- Compare biosensor cells in MOPS with and without phosphate
    - Cells with phosphate → 863 (Arbitrary Units of GFP / OD_{600})
    - Cells without phosphate → 325 (Arbitrary Units of GFP / OD_{600})

Saturday, October 25, 2020
- Testing of biosensor cells with plate reader & Fluoro-Q
    - Successful!
    - Built characterization curve