Week of June 3rd

MONDAY, 6/4/2018

People present: Anna, Stefanie, Soohyun
Time: 12:30PM-6PM (Lab: 2PM-6PM -- 4 hours)
Location: Keck 201 and ABL 126
Goals: Create competent cells and miniprep

Competent Cells

TSS Buffer

Materials

- 5g PEG 8000
- 1.5 mL 1M $MgCl_2$ (or 0.30 g $MgCl_2 * 6H_2O$)
- 2.5 mL DMSO
- Add LB to 50 mL

Procedure

- Combined 5g PEG 8000, 0.30g MgCl2, and 2.5mL DSMO
    - Added 0.03mL of MgCl2, instead of 0.3mL
- Added 50mL of LB to the mixture
- Mixed with magnetic stir bar and stir plate (medium speed for approx. 1 minute)
- Tested pH - should be 6.5
- Adjusted pH, if pH is not 6.5 -- add acid/base
    - Used 0.1 M HCl, 0.5 M HCl, and 10 M NaOH to titrate
    - Added too much 0.1M HCl. Tried to balance with 10M NaOH, but added too much. Added more 0.5M HCl, and got the right concentration
        - Possibly produced a lot of salt in the process -- may affect the cells
- Used syringe to suction up the solution
- Pushed through filter; collected in tube

Competent Cells

Materials

- DH5a cells in liquid culture (Obtained from Anna)

Procedure

- Centrifuged liquid cultures (4 degrees C) for 10 minutes at 2500 rpm
- Discarded supernatant (LB), leaving cell pellet
- Resuspended cell pellet in 1.6 mL TSS buffer
- Distributed competent cell solution to (32) 1.5 mL tubes to make 50 uL aliquots
- Moved tubes to Keck for flash freezing and storage at 8 degrees C

DNA Miniprep

Materials

- Cells (Obtained from Anna)
- Miniprep kit

Procedure

- Followed QIAGEN protocol found in mini prep kit

- Pelleted cells by centrifuging for 5 minutes at top speed
- Resuspended bacteria in 250uL of buffer P1
- Transferred to microcentrifuge tube
- Added 250uL of buffer P2 to tube and inverted tube 6 times

- Added 350uL of buffer N3 to tube and inverted 6 times
- Centrifuged for 10 minutes at top speed
- Applied supernatant (result from previous step) to the spin column
- Centrifuged for 60 seconds; discarded flow-through
- Added 750uL of Buffer PE to the spin column
    - Forgot to add ethanol to Buffer PE
- Centrifuged for 60 seconds; discarded flow-through
- Transferred the spin column to a new micro centrifuge tube
- Centrifuged for 2 minutes; discard flow-through
- Placed the spin column in a new micro centrifuge tube
- Added 50uL of water to the spin column
- Let stand for 4 minutes
- Centrifuged for 30 seconds

Liquid Culture

Materials

- Plate with cultures (Obtained from Anna)
- 4mL LB
- 4uL Antibiotic- chloramphenicol

Procedure

- Added 4mL of LB and 4uL of chloramphenicol to test tube
- Used toothpick to pick up a single colony from plate
- Placed in test tube with LB

TUESDAY, 6/5/2018

People present: Stefanie, Soohyun
Time: 10:30PM-12PM and 1-1:30PM and 3-5PM (4 hours)
Location: Keck 201 and ABL 126
Goals: Remake TSS Buffer and prep DNA

DNA Miniprep

Materials

- Cells (Obtained from Anna)
- Miniprep kit

Procedure

- Pelleted cells by centrifuging for 5 minutes at top speed
- Resuspended bacteria in 250uL of buffer P1
- Transferred to microcentrifuge tube
- Added 250uL of buffer P2 to tube and inverted tube 6 times
- Added 350uL of buffer N3 to tube and inverted 6 times
- Centrifuged for 10 minutes at top speed
- Applied supernatant (result from previous step) to the spin column
- Centrifuged for 60 seconds; discarded flow-through
- Added 750uL of Buffer PE to the spin column
- Centrifuged for 60 seconds; discarded flow-through
- Transferred the spin column to a new micro centrifuge tube
- Centrifuged for 2 minutes; discarded flow-through
- Placed the spin column in a new micro centrifuge tube
- Added 50uL of water to the spin column

- Let stand for 4 minutes
- Centrifuged for 30 seconds

TSS Buffer

Materials

- 5g PEG 8000
- 1.5 mL 1M $MgCl_2$ (or 0.30 g $MgCl_2 * 6H_2O$)
- 2.5 mL DMSO
- Add LB to 50 mL

Procedure

- Combined 5g PEG 8000, 0.30g MgCl2, and 2.5mL DSMO
- Added 50mL of LB to the mixture
- Mixed with magnetic stir bar and stir plate (medium speed for approx. 1 minute)
- Tested pH - should be 6.5
- Adjusted pH, if pH is not 6.5 -- add acid/base
    - Used 0.5M HCl to titrate
- Used syringe to suction up the solution
- Pushed through filter; collected in tube

Soohyun made competent cells. Refer to her Notebook for details; refer to Monday 6/4 for general procedure.

We checked the OD of our liquid cultures from yesterday

- 1mL of LB in a corvette for the blank
- 1mL of culture in a corvette to measure -- one for each
- Measured using the OD machine in Keck 201

--> Chose the liquid culture with OD ~ 0.25 for transformations

Heat Shock

Materials

- Refer to Soohyun's Notebook for details

Procedure

- Soohyun began the process -- Refer to her Notebook for details
    - Followed bootcamp protocol
- Plated on plates made in bootcamp
    - Protocol calls for warm/RT plates
    - We used chilled plates that were left in the incubator for a few minutes -- not exactly room temperature
    - Some plates were mushy; we had difficulty plating the bacteria without digging into the agar. We were able to plate some of the bacteria successfully on other plates
- Incubated plates overnight

--> Overgrowth found on the 2 of the 4 plates. 2 plates had no growth. No red colonies observed.

PCR: amplifying pRSF backbone

WEDNESDAY, 6/6/2018

People present: Stefanie, Soohyun
Time: 10:30AM-12:45PM and 1:45-4:45PM (5 hours)
Location: Keck 201 and ABL 126
Goals: Make new plates

Agar Plates

Materials

- LB Agar
- DI Water
- Antibiotic- Chloramphenicol

Procedure

- Measured 20g of LB Agar (it's premixed -- we don't have to follow bootcamp protocol)
- Added DI water to make 500mL
- Mixed using magnetic stir bar and stir plate at high speed until all powder was dissolved
- Autoclave in bin for 20 minute cycle
    - Labeled new plates while waiting: 6/6 iGEM LB agar Chl^{34}
- Placed flask in water bath (50 degrees C) while we went out to lunch
- Added antibiotic
- Poured agar plates using aseptic technique
- Left to solidify overnight

Antibiotic Comparison

Materials

- Competent cells we plated on Tuesday 6/5

Procedure

- (Anna prepped a plate with chlorophenicol and streaked half of the plate with her own bacteria)
- Collected bacteria colony (single) from our plates from yesterday (Tue 6/5) and streaked the other half of the plate
    - Goal: See if our original plates had enough antibiotic, or if the plate was contaminated with antibiotic resistant bacteria

THURSDAY, 6/7/2018

People present: Anna, Stefanie, Soohyun
Time: 11AM-2:30PM (3.5 hours)
Location: ABL 126
Goals: Golden Gate for our project

Soohyun stored the new plates we made yesterday in 4 degree fridge: 6/6 iGEM agar plates Chl^{34}

Soohyun checked the antibiotic comparison plate we streaked yesterday --> no colonies observed

- Conclusion: the antibiotic added to our plates from bootcamp was not effective (not enough, or didn't work well)

Soohyun aliqotted 1mL tubes of DNA-grade water in the hood and added water to H.W. samples
Anna centrifuged the samples
Soohyun mixed samples using a pipette

NanoDrop & Wetlab Calculator

Materials

- NanoDrop Lite in ABL 125
- H.W. samples
- Kimwipes

Procedure

- Pipetted a drop of water onto the NanoDrop for the blank
- Pipetted a drop of each H.W. sample and measured the path length and labelled the tubes -- cleaned Nanodrop between each measurement

- Entered path length values into Wetlab Calculator
- Looked up plasmid length on benchling and entered into Wetlab Calculator
- Obtained volumes of other materials needed for Golden Gate

Golden Gate Assembly

Materials

- Bsal
- DNA grade water
- H.W. DNA
- Buffer
- Ligase

Procedure

- (Followed bootcamp protocol)
- Assembled reaction in PCR tubes on ice
- Closed tubes, tapped on the counter several times
- Placed in the thermocycle
- Programmed thermocycle: iGEM3

Thermocycle Program: iGEM3

	A	B	C
1	Lid temp: 105		
2	Step	Temperature (C)	Time (min)
3	1	37.0	10
4	2	37.0	1.5
5	3	16.0	3
6	4	Go to 2	Rep 25
7	5	37.0	5
8	6	50.0	5
9	7	80.0	10
10	8	12.0	Inf.

- Left in thermocycler in ABL 126 to be kept at 12C until we can transform them

Table1

	A	B	C	D	E
1	**Reagents**	**Amounts**		**Thermocycler Temp**	**Time**
2	Template (pRSF Duet)	0.2 uL		98° C	3:00
3	F primer (iG001)	2 uL	**25X**	**98° C**	**00:10**
4	R primer (iG002)	2 uL		**60° C**	**00:30**
5	dNTPs	2 uL		**72° C**	**2:00**
6	5X HF Phusion Buffer	10 uL		72° C	10:00
7	Phusion	0.5 uL		4° C	hold
8	dH2O	33.3 uL			
9	**Total**	**50 uL**			

1. Mixed all reagents together in PCR tube; mixed by flicking. Phusion added last.
2. Put in thermocycler; program detailed in above table

PCR: amplifying TetR from UBER plasmid

Table2

	A	B	C	D	E
1	**Reagents**	**Amounts**		**Thermocycler Temp**	**Time**
2	Template (MK1283 UBER)	0.2 uL		98° C	3:00
3	F primer (D47)	2 uL	**25X**	**98° C**	**00:10**
4	R primer (iG006)	2 uL		**62° C**	**00:30**
5	dNTPs	2 uL		**72° C**	**00:30**
6	5X HF Phusion Buffer	10 uL		72° C	10:00
7	Phusion	0.5 uL		4° C	hold
8	dH2O	33.3 uL			
9	**Total**	**50 uL**			

1. Mixed all reagents together in PCR tube; mixed by flicking. Phusion added last.
2. Put in thermocycler; program detailed in above table

PCR: Amplifying T7 RNAP from UBER plasmid

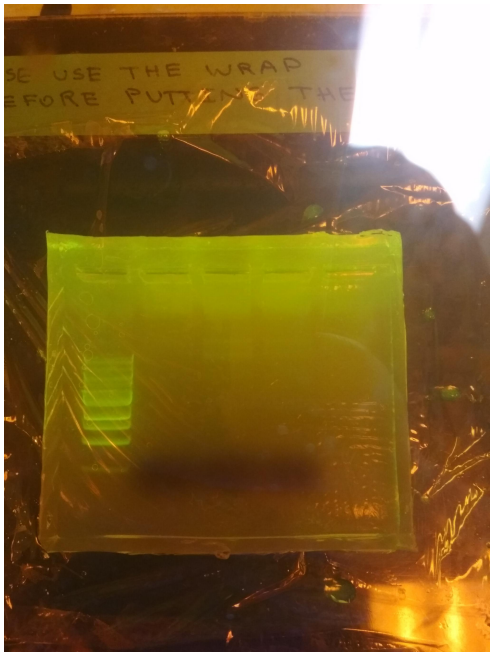
Table3

	A	B	C	D	E
1	**Reagents**	**Amounts**		**Thermocycler Temp**	**Time**
2	Template (MK1283 UBER)	0.2 uL		98° C	00:30
3	F primer (iG005)	2 uL	**25X**	**98° C**	**00:10**
4	R primer (D50)	2 uL		**65° C**	**00:30**
5	dNTPs	2 uL		**72° C**	**1:30**
6	5X HF Phusion Buffer	10 uL		72° C	10:00
7	Phusion	0.5 uL		4° C	hold
8	dH2O	33.3 uL			
9	**Total**	**50 uL**		**50 uL**	**Total**

1. Mixed all reagents together in PCR tube; mixed by flicking. Phusion added last.
2. Put in thermocycler; program detailed in above table

Ran samples on a gel. No expected bands were observed.

IMG_20180605_193346.jpg



Repeated pcr

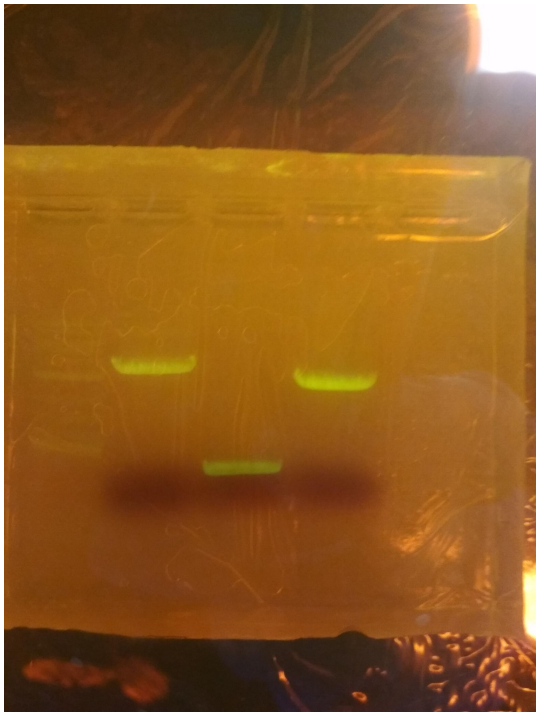
TetR: 65 C - 0:45
pRSF: 61C - 3:10
T7: 68C - 2:00

increased the elongation time and tried different annealing temperature

FRIDAY, 6/8/2018

1. Ran new samples on gel; expected bands were observed.

IMG_20180606_121601.jpg



1. Cut out bands from gel and purified DNA w/ Zymo kit according to instructions.
   [pRSF] = 197.0 ng/uL
   [TetR] = 221.8 ng/uL
   [T7] = 253.3 ng/uL

Golden Gate Assembly mKate + pRSFDuet-1

Golden Gate assembly

	A	B
1		
2	**Reagent**	**Volume (µL)**
3	insert	0.8
4	backbone	0.2
5		
6		
7	BsaI	0.5
8	T4 DNA Ligase	0.5
9	T4 Buffer	1.5
10	10X BSA	1.5
11	H20	9.79

Thermocyclinig conditions

	A	B	C	D
1	BsaI Golden Gate, Long, ≥6 parts: 142.5 min / 2:23 Short, ≤5 parts: 97.5 min / 1:38			
2		Step	Temp	Time
3		Initial Digestion (opt.)	37°C	10 min
4	Repeat25× / 15×	Digestion	37°C	1.5 min
5		Annealing & Ligation	16°C	3 min
6		Digestion & Ligase Inact.	50°C	10 min
7		Inactivation	80°C	10 min
8		Storage	12°C	∞

Making universal media for bacterial strains:

Table7

	A	B
1	**Reagent**	**Amount**
2	Tryptone	10 g
3	Yeast Extract	5 g
4	NaCl	10 g
5	Ascorbic Acid	0.5 g
6	Sodium Acetate	1.5 g
7	10 N NaOH	100 uL
8	ATCC Vitamin Supplement	10 mL
9	ATCC Mineral Supplement	10 mL
10	dH2O	To 1 L

1. Autoclaved reagents 2-7, 10
2. Cooled media to room temp, then added reagents 8-9.

SATURDAY, 6/9/2018

Golden Gate assembly of HW samples

Table5

	A	B	C
1	insert	amounts of insert	water
2	HW 21117	**0.69**	9.9
3	HW 36850	**0.53**	10.06
4	HW 35539	**0.48**	10.11
5	HW 29572	**0.46**	10.13
6	HW 37769	**0.75**	9.84
7	HW 31422	**0.75**	9.84
8	HW 36709	**0.58**	10.01

Table4

	A	B	C	D
1				
2	**Reagent**	**Volume (µL)**		
3	insert			
4	backbone pSPB430	0.41		
5				9.4
6				
7	Esp3I	0.5	Add last!	
8	T4 DNA Ligase	0.5		
9	T4 Buffer	1.5		
10	10X BSA	1.5		
11	H2O			

Miniprepping mKate Goldengate product:

1. Miniprepped mKate in pRSF backbone according to Qiagen Miniprep kit instructions; eluted with 50 uL of water
2. labeled DNA "mKate" and put in iGEM Primer box in -20C fridge.
    a. [mKate] = 44.3 ng/uL

Sequencing pRSFDuet-1-mKate:

Table6

	A	B
1	**Reagents**	**Amount**
2	DNA (mKate)	11.3 uL
3	Primer (C53)	2.5 uL
4	H2O	1.2 uL
5	**Total**	**15 uL**

1. Put all reagents in PCR tube.
2. Sent out for sequencing

Week of June 11

MONDAY, 6/11/2018

People: Stefanie and Soohyun
Time: 10:30AM-3:30PM and 4:30-5:00PM (5.5 hours)
Location: ABL 126 and Keck 201
Objectives: made liquid cultures of (5) successful HW sequences, second attempt at assembling HW 31422 and 29572

Golden Gate
*Aliquotted T4 (20 uL each). Stored T4 buffer in -20 deg fridge in ABL 126, green container

Golden Gate Assembly of 29572 and 31422

- Backbone = pSPB430
- Left in thermocycler at 12 deg C overnight

Make Liquid Cultures

Goal: Make liquid cultures for the transformed cells Anna made over the weekend

Materials

- Colony from transformed plates (obtained from Anna)
  - H.W. 36709
  - H.W. 21177
  - H.W. 35539
  - H.W. 36850
  - H.W. 37769
- 4mL LB
- Test Tubes
- 4uL Antibiotic: Chl^{34}

Procedure

- Obtained plates from Silberg lab 4C fridge
- Label test tubes: iGEM H.W. #####
- Protocol calls for incubation of 12-18 hours... postpone until later
- Stored plates back in Silberg lab 4C fridge

Golden Gate Assembly

Goal: Repeat the 2 failed assemblies we performed last week of the H.W. DNA so we will have all 7 DNA sequences transformed and ready for whenever we are ready to begin our project.

Materials

- DNA insert
  - 0.75uL H.W. 31422 + 9.84 uL DNA grade water
  - 0.46uL H.W. 29572 + 10.13 uL DNA grade water
- 0.41uL Backbone
- 0.5uL Esp31**
- 0.5uL T_4 DNA ligase**
- 1.5uL T_4 Buffer
- 1.5uL 10X BSA

Procedure

- Labeled PCR tubes: H.W. 29572 and H.W. 31422
- Added respective amounts of water to tubes

- Obtained T_4 buffer from Silberg lab -20C fridge and ice from Keck
    - Labeled 5 microcentrifuge tubes T_4 buffer
    - Let T_4 buffer stock defrost on ice
    - Vortexed for about 2 seconds
    - Aliquoted 20uL into each tube
    - Stored stock and 4 aliquots in -20C fridge in ABL 126
- Added to both tubes:
    - 1.5uL 10X BSA
    - 1.5uL T $_4$Buffer
- Add 0.46uL H.W. 29572 to one tube
        - H.W. 31422 still frozen -- waited to thaw
- Waiting for response on what backbone to use -- placed stocks in 4C fridge ABL 126; PCR tubes left at room temperature

Interlab

Goal: Begin calibrating the plate reader so that we can accurately measure our data, and so we know how to use the plate reader

NOTE: Used plate reader in Keck 201, closest to the computer

Calibration 1

Materials

- 1mL LUDOX CL-X (from provided kit)
- diH_2O
- 96 well plate, clear with clear flat bottom

Procedure

NOTE: Reference Interlab Study 2018 Notebook for Plate reader protocol

- Added 100uL LUDOX to wells A1, B1, C1, D1
- Added 100uL diH_2O into wells A2, B2, C2, D2
- Measured the absorbance
- Imported data into the excel sheet provided by iGEM
- Discarded plate in solid biohazard wastebin

Calibration 2

Materials

- 300uL silica beads
- diH_2O
- 96 well plate, clear with clear flat bottom

Procedure

- Made the microsphere solution
    - Vortexed silica beads for ~35 seconds
    - Pipetted 96uL microspheres into a micro centrifuge tube
    - Added 904uL diH2O to the tube
    - Vortexed for ~30 seconds
- Added 100uL diH_2O into wells A2, B2, C2, D2...A12, B12, C12, D12 (across rows)
- Vortexed microsphere solution for ~10 seconds
- **Added 200uL of microsphere solution to A1**
- Transferred 100uL from A1 to A2
- Mixed by pipetting up and down 3x
- Changed pipette tip
- Transferred 100uL from A2 to A3
- Repeated the process of transferring and mixing through A11
- Mixed A11; transferred 100uL to WASTE (not A12)
- Repeated steps (starting from bold bullet point) for Row B, C, D

    - NOTE: We believe an error in pipetting occurred in rows C and D. When 100uL was transferred from C2 to C3 the amount left in the wells looked wrong. C2 had a larger volume than other column 2 wells, and when 100uL was taken from C3 the volume left in C3 was much less than other column 3 wells. We continued pipetting through the rest of the row. The same occurrence happened in row D between wells 1 and 2.
        - We believe the seal on the pipette tip wasn't secure. When we observed the pipette we noticed that it would extract the right amount of liquid and then release some, resulting in a smaller volume transfer than was expected.
    - NOTE: 100uL from D11 was transferred to D12
        - We repeated the procedure above in rows E and F as replacement for rows C and D
    - NOTE: new microsphere solution was made for row F
- Remixed each well within 10 minutes of measuring absorbance
    - Started from most dilute (column 11) and moved to least dilute (column 1) using same pipette tip
    - Changed pipette tips for each row
- Measured absorbance
- Discarded plate in solid biohazard wastebin

Golden Gate Assembly Cont.

Procedure

- Response: Use PSB 430 as backbone
- Retrieved stocks from ABL 126 4C fridge and waited for them to thaw
- Added to other tube (the one that did not get DNA earlier) 0.75uL H.W. 31422
- Added to both tubes 0.41uL PSB 430 backbone
- Added to both tubes:
    - 0.5uL Esp31
    - 0.5uL T_4 DNA ligase
- Flicked tubes a few times to mix
- Centrifuged for a few seconds
- Placed tubes in thermocycle through program: iGEM3 (Reference Week of June 3rd: Thursday 6/7 for details)
- Left in thermocycle until we are ready to transform them

Summary: We had some issues with different components of the assemblies. I'm not sure if the issues will affect the assemblies, but hopefully they will turn okay.

Make Liquid Cultures Cont.

Procedure

- Obtained plates from Silberg lab 4C fridge -- moved to ABL 126
- Added to test tubes:
    - 4mL of LB
    - 4uL of chloramphenicol
- Used toothpick to pick up a single colony from plate
- Placed in test tube
- Soohyun stored the cultures in a shaking incubator in Keck

Summary: The procedure was straightforward and we didn't run into any issues. For our first culture we hadn't found the toothpicks yet and decided to use a pipette tip. Soohyun pipetted up and down to remove the colony, but we thought it might be best just to leave the tip in the culture, similar to a toothpick. Aside from that, the task went smoothly.

Daily Summary

- Today was confusing with several things going on at the same time and multiple questions being answered at different times, but we accomplished almost everything we set out to do.

- We began calibrations of the plate reader for our interlab study. We performed 2 (we had some difficulty with the 2nd), and have 1 more calibration to do tomorrow before we can start taking measurements. We also made the 2 golden gate assemblies, but I have my doubts about their successful. Lastly, we made liquid cultures to continue pushing forward in our own project.

TUESDAY, 6/12/2018

People present: Soohyun, Stefanie, Anna, Jordan
Time: 10AM-12PM and 2:45-5:45PM (5 hours)
Location: ABL 126, Keck 201, Keck 301
Goals: Transform and plate golden gate assemblies, DNA miniprep the liquid cultures --> nano drop --> send off for sequencing, ~~finish calibrations for interlab~~, transform DNA for interlab

DNA Miniprep Liquid Cultures

Goal: Extract DNA from cells, nanodrop to see how much we have, and send off for sequencing to see if the H.W. sequences were inserted the way we wanted.

Materials

- Liquid cultures of H.W. sequences from yesterday (6/11)
- Qiagen QIAprep Spin Miniprep kit

Procedure

- Pelleted cells by centrifuging for 5 minutes at 4,000rpm
- Resuspended bacteria in 250uL of buffer P1 by pipetting up and down
- Transfered to microcentrifuge tube
- Added 250uL of buffer P2 to tube and invert tube 4 times
- Added 350uL of buffer N3 to tube and insert 4 times
- Centrifuged for 10 minutes at 13,200rpm
- Applied supernatant (liquid result from previous step -- don't get the white stuff) to the spin column
- Centrifuged for 60 seconds at 13,200rpm; discard flow-through
- Centrifuged for 60 seconds at 13,200rpm; discard flow-through
- Added 750uL of Buffer PE to the spin column
- Centrifuged for 60 seconds at 13,200rpm; discard flow-through
- Transferred the spin column to a new micro centrifuge tube
- Centrifuged for 2 minutes at 13,200rpm; discard flow-through
- Placed the spin column in a new micro centrifuge tube
- Added 50uL of buffer EB to the spin column
- Let stand for 4 minutes
    - NOTE: stood for ~10 minutes
- Centrifuged for 30 seconds
- Left in -20C fridge in ABL 126 for safe keeping until we get back from lunch and the IBB Debate (~2 hours)

--> **Nanodrop- Keck 301**

- Took 1uL of _____ and measured:
    - diH2O
    - H.W. 35539 = 280.0 ng/uL
    - H.W. 36850 = 186.0 ng/uL
    - H.W. 36709 = 173.1 ng/uL
    - H.W. 21177 = 138.6 ng/uL
    - H.W. 37769 = 172.2 ng/uL

--> Gave to Albert to send off for sequencing

Summary: The process went smoothly. We didn't run into any issues (very happy!) and the entire process didn't take too long. I think we're making progress and becoming more confident in DNA mini prepping.

Interlab Cont.

Rehydrating and Electrotransforming the DNA

Goal: Transform the DNA provided by iGEM into our competent cells so we can begin the interlab study tomorrow.

Materials

- DNA provided by iGEM
- diH_2O
- DH5a cells (made by Anna)
- SOB

Procedure

- Pierced foil with a pipette tip that contains 10uL diH_2O
- Pipetted up and down
- Transferred DNA to 2mL microcentrifuge tube
- Obtained DH5a cells from iGEM box in Keck 201 -40C fridge
- Waited for cells to thaw, kept on ice. Kept cuvettes on ice.
- Added 1uL of DNA to cells
    - Worked with cells by holding them
- Flicked to mix, recollected at bottom
- Pipetted 50uL cells into cuvette, put back on ice
- **Electrotransformed** the cells
    - Wiped off cuvette
    - Took top off and placed in electrotransform machine (make sure the cuvette is in between the 2 metal pieces)
    - Pipetted 1mL of SOB (basically more enriched LB) from stock
        - SOB kept on ice
    - Pulsed the cuvette (want to get a measurement of 5.4 (higher is ok, lower isn't))
    - Took cuvette out of electrotansfrom machine and added the SOB
    - Pipetted up and down a few times
    - Moved to falcon tube
- Placed cultures in 37C shaker to recover for 1 hour
- Obtained plates from ABL 126 and placed in 37C incubator in Keck 201 to warm up
- Took cultures from shaker; obtained 50uL of culture and plated them
    - Used beads to spread bacteria around plate
- Stored in 37C incubator in Keck 201

Summary: Jordan and Anna observed us throughout the process. It was very helpful being shown exactly what to do. It provided a level of confidence in what we were doing. We didn't run into any issues.

Daily Summary

- Today was productive. We were able to get through several processes with no apparent issues arising.
- We prepped the DNA from the H.W. sequences that were successfully assembled; they are now ready to be sent for sequencing. We also began the interlab study by rehydrating the DNA and transforming them into our competent cells. Now we are ready to begin the interlab study as soon as we perform the 3rd calibration.

Making MG1655 glycerol stocks:

Streaked out MG1655 onto plain LB plate. Put in 37C incubator @ 4:18 pm.

WEDNESDAY, 6/13/2018

People present: Stefanie, Soohyun, Anna

Time: 11AM-1PM and 2-3:45PM (3.75 hours)

Location: ABL 126 and Keck 201

Goals: ~~Perform calibration 3, make liquid cultures for the plates (competent cells for interlab) we made yesterday (6/12)~~, redo transformations of the 2 failed H.W. sequences, make more LB agar plates

Results: None of the transformations performed yesterday worked --> nothing grew on our plates

LB Agar Plates

Goal: Make more plates so that we can redo our transformations (we only have 6 plates left from our last set)

Materials

- 40g LB Agar powder
- DI Water
- Antibiotic- Chloramphenicol

  $34mg/mL*v_1=25ug/mL*1000mL$

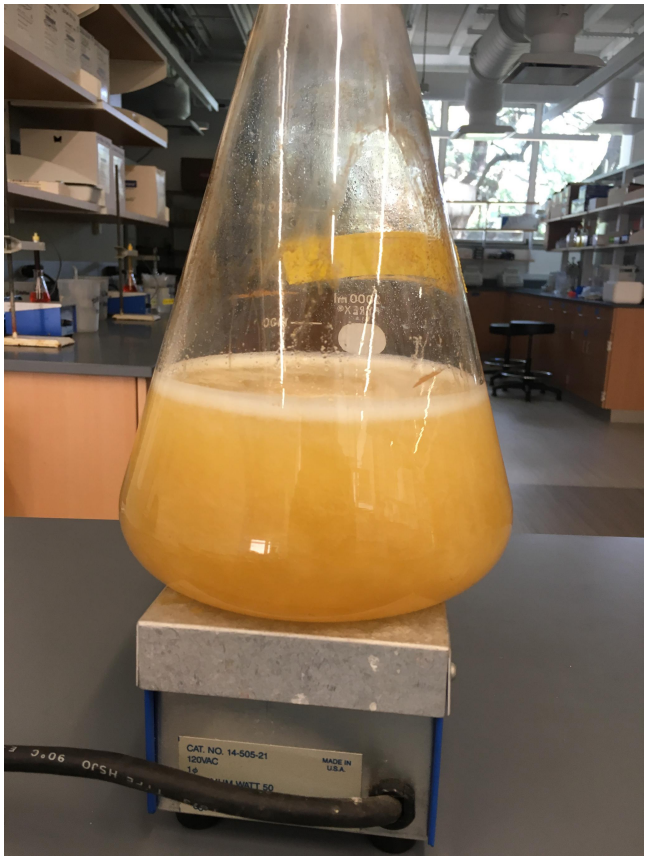
  $v_1=(0.025mg/mL*1000mL) / 34mg/mL$

  $v_1=0.735mL$

Procedure

- Measured and added 40.02g of LB Agar powder to 2L flask
- Added DI water to make 1L
- Mixed using magnetic stir bar and stir plate at high speed (speed setting 5-6) until all powder was dissolved
  - NOTE: Powder was not fully dissolved

Image from iOS.jpg



- Autoclaved in bin for 20 minute cycle
  - Labeled new plates while waiting: 6/13 iGEM LB agar Chl[34]

Left to do other things while LB was in the autoclave

Interlab Cont.

Replated Transformations from yesterday (6/12)

Materials

- Plates from yesterday (6/12)
- Left over transformed cells from yesterday (6/12)
- Beads

Procedure

Performed by Stefanie

- Obtained the plates from yesterday from the 37C incubator in Keck 201
- In fume hood, poured the entire liquid culture onto the plate from yesterday (6/12)
    - Reused plates because nothing had grown on said plates
- Used beads to spread the culture around the plate
- Removed beads
- Placed plates back in 37C incubator in Keck 201

LB Agar Plates Cont.

Procedure

- Dr. C removed the LB from the autoclave and placed it in the 50C water bath
- Placed LB on stir plate and let cool for ~10 minutes
- Added 750uL chl34
- Let stir for ~1 minutes
- Used aseptic technique to pour plates
    - Wiped workspace with ethanol
    - Lit bunsen burner
    - Pour plates near flame, running top of flask through the flame between pours
- Left plates in ABL 126 to solidify overnight

Summary: I really hope these plates work better than the last ones did. We changed the amount of antibiotic we put in the LB agar, hopefully it will work better. We didn't run into any issues, but the process did take a good amount of time. On the bright side, we were able to make a lot of plates.

Interlab Cont.

Redid Transformations from yesterday (6/12)

Materials

- DH5a competent cell aliquots
- Aliquoted DNA provided by iGEM
- Cuvettes
- SOB

Procedure

- Labelled cuvettes and tubes
- Obtained cells from -20C fridge in Keck 201
- Performed each transformation separately, repeated the steps for each:
    - Added 1uL DNA to competent cells
        - Worked with cells while keeping them in ice for as long as possible, holding them as little as possible
    - Flicked to mix
    - Pipetted 50uL out of cells
    - Transferred into cuvette
    - Flicked to spread cells
    - Transformed cells

    - Added 1000uL SOB to cuvette
        - SOB not kept on ice
    - Pipetted up and down to mix
    - transferred cells to a falcon tube
- Placed tubes in shaker at 37C to incubate for 1 hour

Prep for Sending DNA for Sequencing

Materials

- 500ng DNA -- calculate from concentrations obtained from nanodrop
- 2.5uL primer
- Water (add up to 15uL)

Sequencing DNA Prep

	A	B	C	D	E
1	Sequence	DNA (uL)	Primer (uL)	H2O- calculated (uL)	H2O- added (uL)
2	H.W. 36709	2.8885037551	2.5	9.6114962449	9.6
3	H.W. 35539	1.7857142857	2.5	10.7142857143	10.7
4	H.W. 36850	2.688172043	2.5	9.811827957	9.8
5	H.W. 21177	3.6075036075	2.5	8.8924963925	8.9

Procedure

- Label connected PCR tubes (1,2,3,4...) on top and side
    - Note: *Add largest amounts first*
- Added water to tubes
- Added DNA
- Added Primer
- Made sure all PCR tubes were closed tightly
- Placed confirmation page in package with PCR tubes
- Placed package in the box outside of Keck 201

Note: Do before 5pm M-Th, before 4PM on F
Summary: Anna took us through the process step by step. I now feel informed and will potentially be able to do this on my own when needed. We didn't run into any issues; the process was straightforward and didn't take too long.

Soohyun removed the tubes from our transformations today from shaker, plated them using beads to spread them, and placed them in 37C incubator overnight.
Summary: Hopefully these transformations work. We talked with all of the grad students and no one can think of a reason that the transformations yesterday didn't work. So we're hoping today's went well. The process seemed more efficient doing them one by one, but it was more difficult doing the procedure the way that Shyam asked vs. Jordan. All in all, it went well, hopefully the results reflect that.

Daily Summary

- Today was slightly disheartening to find out most of the work we did yesterday didn't have good results. But, we redid a lot of the work and made more plates, so we're prepping for the future.

- We made new LB agar plates (a lot of them), hopefully they are good and we won't need to take the time to make more anytime soon. We also re-plated our transformations yesterday, tomorrow we will see if that helps. (If so then the concentration was too low yesterday and that is why nothing grew.) On the other hand, we performed another set of transformations -- hopefully those went well and we will see growths tomorrow. The third calibration is left to tomorrow, again.

Making MG1655 glycerol stocks:
Took MG1655 out of 37C incubator @ 10:19 am.

Sent HW 21177, 36709, 35539, and 36850 for sequencing

Table1

	A	B	C	D	E
1	Sequence	DNA	Primer	H2O	
2	36709	2.8885037551	2.5	9.6114962449	9.6
3	35539	1.7857142857	2.5	10.7142857143	10.7
4	36850	2.688172043	2.5	9.811827957	9.8
5	21177	3.6075036075	2.5	8.8924963925	8.9

OD Curves:

1. Streaked Vmax, B. subtilis, C. glutamicum, nissle1917, DH10B, P. putida, S. oneidensis, and S. melitoti onto plain LB plates.
2. Streaked L. lactis onto plain m17 plate
3. Vmax, B. subtilis, C. glutamicum, nissle1917, DH10B, L. lactis went in 37C incubator
4. P. putida, S. oneidensis, and S. melitoti went into 30C incubator

THURSDAY, 6/14/2018

People present: Stefanie, Soohyun, Anna
Time: 10:30AM-12:45PM and 1:15-5:15PM (6.25 hours)
Location: Keck 201 and ABL 126
Goals: Perform calibration 3, make liquid cultures from yesterday's plates (6/13), DNA miniprep Anna's cultures, Golden Gate mKate

Results: All of our plates have colonies!!!

Interlab Cont.

Calibration 3

Materials

- Fluorescein provided by iGEM
- 10x PBS
    - Need: 10mL 1x PBS
- 96 well plate

Procedure

- PBS Dilution
    - Added 1mL 10x PBS and 9mL water to a falcon tube

    - Mixed by shaking
- Centrifuged fluroscene for 30 seconds at 3,000rpm
- Added 1mL 1x PBS to fluorescein
- Pipetted up and down to mix until no particles were visible
- Made dilution of 10x fluorescein
    - Added 100uL of 10x fluorescein and 900uL 1x PBS to microcentrifuge tube
- Performed serial dilutions
    - Added 100uL of 1x PBS into wells A2-A12 (repeat from B,C,D)
    - **Added 200uL of 1x fluorescein to A1**
    - Transferred 100uL from A1 to A2
    - Mixed by pipetting up and down 3x
    - Changed pipette tip
    - Transferred 100uL from A2 to A3
    - Repeated the process of transferring and mixing through A11
    - Mixed A11; transferred 100uL to WASTE (not A12)
    - Repeated steps (starting from bold bullet point) for Row B, C, D
        - NOTE: the 1st set of serial dilutions was performed with 10x fluorescein, we redid the serial dilutions a 2nd time with the 1x fluorescein
- Measured fluorescence
- Discarded into the biohazard wastebin

Summary: All of the calibrations are done! Going through the process was difficult, but it helped us to practice several techniques and learn how to use the plate reader. Calibration 1 went smoothly, calibration 2 and 3 ran into some issues due to our mistakes. Aside from that, the calibrations went smoothly, it just took several days.

DNA Miniprep

Goal: Miniprep the DNA from the 2 failed H.W. sequences, PRSF, and mKate sequences so that we can have them ready for our project when we finish the interlab study.

Materials

- H.W., PRSF, and mKate sequences
- Qiagen QIAprep Spin Miniprep kit

Procedure

- Pelleted cells by centrifuging for 5 minutes at 4,000rpm
    - NOTE: For future preps - transfer cultures to microcentrifuge tubes and centrifuge for 30 seconds twice, at top speed --> still pellets DNA but does so faster
- Resuspended bacteria in 250uL of buffer P1 by pipetting up and down
- Transfered to microcentrifuge tube
- Added 250uL of buffer P2 to tube and inverted tube 6 times
- Added 350uL of buffer N3 to tube and inverted 6 times
- Centrifuged for 10 minutes at 13,200rpm
- Applied supernatant (liquid result from previous step) to the spin column
- Centrifuged for 60 seconds at 13,200rpm; discarded flow-through
- Centrifuged for 60 seconds at 13,200rpm; discarded flow-through
- Added 750uL of Buffer PE to the spin column
- Centrifuged for 60 seconds at 13,200rpm; discarded flow-through
- Transferred the spin column to a new micro centrifuge tube
- Centrifuged for 1 minute at 13,200rpm; discard flow-through
- Placed the spin column in a new micro centrifuge tube
- Added 50uL of buffer EB to the spin column
- Let stand for 4 minutes
- Centrifuged for 30 seconds

--> **Nanodrop- ABL 128**

- Took 1uL of _____ and measured:
  - diH2O
  - H.W. 31422 = 153.7 ng/uL
  - H.W. 29572 = 112.6 ng/uL
  - mKate A6= 112.1 ng/uL
  - PRSF dull = 154.4 ng/uL

--> Took to Keck 201, gave to Anna

Summary: We didn't encounter any issues. The process was straight forward. We prepped the DNA (Dr. Beason told us a way to do a few steps more easily), and nanodropped it.

Interlab Cont.

Make Liquid Cultures

Goal: Make liquid cultures of the plates with the DNA from iGEM for the interlab study so that we can dilute and use them tomorrow.

Materials

- Plates with cultures from iGEM DNA and H.W. sequences
- LB
- Chloramphenicol

Procedure

- Obtained plates from Keck 201
- Added to test tubes (2 sets of 8):
  - 4mL of LB
  - 4uL of chloramphenicol
- Used toothpick to pick up a single colony from plate
- Placed in test tube
- Placed in 37C shaker in Keck 201

Summary: The process was very easy. We didn't encounter any issues.

Golden Gate Assembly

Goal: Assemble the mKate sequence with the PRSF backbone so that we can later assemble it with the H.W. sequence to pair the two.

Materials

- 0.8uL backbone (PRSF)
- 0.36uL mKate
- 1.5uL 10xBSA
- 1.5uL T4 ligase buffer
- 0.5uL Esp31**
- 0.5uL t4 ligase**
- 9.84 DNA grade water

Procedure- Performed by Stefanie

- Labeled PCR tube: mKate
- Added ____ to tube:
  - 9.84uL water
  - 1.5uL 10X BSA
  - 1.5uL T_4 Buffer
  - 0.8 uL PRSF
  - 0.36uL mKAte
  - 0.5uL Esp31

    - 0.5uL T4 ligase
- Flicked tube a few times to mix
- Placed tube in thermocycle through program: iGEM3 (Reference Week of June 3rd: Thursday 6/7 for details)
- Left in thermocycle until we are ready to transform them

Summary: I performed the assembly by myself. It was difficult finding everything that I needed and I question whether I did things correctly. I calculated the amounts of reagents I needed using the Wetlab calculator, but I'm not entirely sure I placed the values in the correct places. Also, I had trouble when pipetting the DNA into the PCR tube. At first the pipette wasn't picking anything up, and then it was, but I couldn't tell if it was the right amount.

Stefanie took plates with colonies from Keck 201 to ABL 126 and parafilmed them. Stored them in 4C fridge in ABL 126.

OD Curves:

1. Took out all plates from yesterday at 10:20 am
2. Picked one colony from all strains and cultured in 2 mL of universal medium (LB + ascorbic acid + acetate + vitamin + mineral supplement)
3. Vmax, B. subtilis, C. glutamicum, nissle1917, DH10B, L. lactis, and MG1655 went into shaking 37C incubator
4. P. putida, S. oneidensis, and S. melitoti went into shaking 30C incubator.

Sent out HW 29572 and 31422 for sequencing

FRIDAY, 6/15/2018

People present: Soohyun, Stefanie
Time: 11AM-2:30PM and 7:30-9PM (5 hours)
Location: Keck 201, ABL 126
Goals: Finish Interlab

Result: mKate assembly didn't work -- used the wrong primer, should have used BsaI rather than Esp31

Soohyun took the liquid cultures from the 37C shaker in Keck 201 and placed them in the 4C fridge in Keck 201.

Interlab Cont.

Cell Measurements

Materials

- LB
- 16 12mL falcon tubes
- 16 50mL falcon tubes
- 32 1.5 microcentrifuge tubes
- Foil
- Overnight liquid cultures
- Cuvettes
- 2 96 well plates

Procedure

- Made 1:10 dilutions of our overnight cultures
    - Labeled falcon tubes:
        - (-) 1 and (-) 2
            - Meaning: negative control colony 1 and negative control colony 2
        - (+) 1 and (+) 2
        - TD1 1 and TD1 2...
    - Added 4.5mL LB to 16 falcon tubes

    - Added 500uL of each liquid culture to its respective falcon tube
- Measured Abs600 of each dilution
    - 1mL of LB for blank
    - Added 1mL of each falcon tube to a cuvette and measured
        - (Data below in table: Columns A, B)
- Diluted cultures again to get an Abs600 of 0.02
    - Calculations: Ex. Negative 2

      $c_1 v_1 = c_2 v_2$

      .5147 * x = 0.02 * 12mL

      x = 0.466mL = 466.29uL

      --> Conclusion: Add 466uL of Negative 2 diluted culture to 11.534mL of LB to create a culture of 0.02 Abs600 with a final volume of 12mL.
    - Other calculations: Reference the table below

Abs600 of Diluted Cultures & Calculations for 2nd Dilution

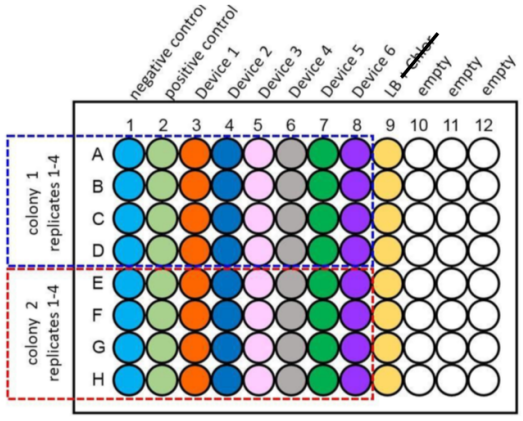
	A	B	C	D	E	F	G	H
1	**Sample**	**OD**		**Volume for OD= 0.02 (mL)**	**Volume for OD= 0.02 (uL)**		**Approximate V for OD= 0.02 (uL)**	**Volume (uL) LB for 12mL final V**
2	Negative 1	0.5605		0.4281891169	428.1891168599		428	11572
3	Negative 2	0.5147		0.4662910433	466.29104332...		466	11534
4	Positive 1	0.5135		0.4673807205	467.38072054...		467	11533
5	Positive 2	0.5289		0.4537719796	453.77197958...		454	11546
6	TD1 1	0.6405		0.37470726	374.70725995...		375	11625
7	TD1 2	0.4438		0.540784137	540.78413699...		541	11459
8	TD2 1	0.5574		0.4305705059	430.57050592...		431	11569
9	TD2 2	0.4790		0.5010438413	501.0438413361		501	11499
10	TD3 1	0.5705		0.4206836109	420.68361086...		421	11579
11	TD3 2	0.5205		0.4610951009	461.09510086...		461	11539
12	TD4 1	0.5720		0.4195804196	419.58041958...		420	11580
13	TD4 2	0.3756		0.6389776358	638.97763578...		639	11361
14	TD5 1	0.6703		0.3580486349	358.04863493...		358	11642
15	TD5 2	0.5422		0.4426410918	442.641091848		443	11557
16	TD6 1	0.4945		0.485338726	485.33872598...		485	11515
17	TD6 2	0.5229		0.4589787722	458.9787722318		459	11541

    - Labelled 50mL falcon tubes in the same manner as before (reference 1st bullet of this procedure)
    - Added *respective amounts* of LB to each falcon tube
        - NOTE: Respective amounts mentioned above can be found in columns H of table above
            - NOTE: Odd numbers were rounded up to next even number
                - Ex. 11533 --> 1154; 11569 --> 11570
    - Added *respective amount*s of the diluted cultures to each falcon tube
        - NOTE: Respective amounts mentioned above can be found in columns G of table above
            - NOTE: Odd numbers were rounded up to next even number
                - Ex. 467 --> 468; 375 --> 376
- Aliquoted hour 0 samples

- Labeled microcentrifuge tubes:
  - (-) 1 0 and (-) 1 6 and (-) 2 0 and (-) 2 6... (Repeat for all other samples -- 32 tubes)
    - Meaning: negative control colony 1 0 hours and negative control colony 1 6 hours and negative control colony 2 0 hours and negative control colony 2 6 hours
- Transferred 500uL from all samples to respective 0 hour tubes
- Placed tubes on ice

- Stored remainder of cultures in ABL 126 shaker at 37C and 220rpm for 6 hours
  - Covered tops with foil to allow cells air
- Moved to Keck 201
- Aliquoted hour 0 samples
  - Transferred 100uL of each culture to respective wells of 96 well plate according to iGEM protocol (reference picture below)

Screen Shot 2018-06-15 at 6.43.50 PM.png



- Measured absorbance and fluorescence of plate using Keck 201 plate reader. Used settings that provided the best results in our calibrations
- Obtained hour 6 samples
- Moved to Keck 201
- Aliquoted hour 6 samples
  - Transferred 100uL of each culture to respective wells of 96 well plate according to iGEM protocol (reference picture above)
- Measured absorbance and fluorescence of plate using Keck 201 plate reader. Used settings that provided the best results in our calibrations

Summary: The interlab took several days longer than it should have. The calibrations were much harder than the measurements themselves were. We ran into several issues with the calibrations, most being error on our part. We didn't run into any issues with the cell measurements. The only iffy part were the measurements from hour 6. No trend arose.

OD Curves:

1. Took out culture tubes from incubators at 10:00 am
2. Everything except S. oneidensis and C. glutamicum grew.
3. Tried picking and culturing colonies again (see yesterday's protocol for OD curves), but with plain LB medium instead.
   a. one colony from all strains was picked and cultured in 2 mL of plain LB
   b. All strains except P. putida, S. oneidensis, and S. melitoti went into 37C shaking incubator; those three went into 30C shaking incubator.

Goldengate assembly of pRSFDuet-1-mKate:

Table2

	A	B
1	**Reagent**	**Amount (uL)**
2	Insert (pSPB460 mKate)	0.4
3	Backbone (pRSF PCR)	0.3
4	BsaI	0.5
5	T4 DNA ligase	0.5
6	T4 buffer	1.5
7	10X BSA	1.5
8	H2O	10.3
9	**Total**	**15**

1. Mixed all reagents together in PCR tube labeled mKate GG.
2. Put in thermocycler; protocol as follows:

End ligation.png

End-On-Ligation Golden Gate Assembly

	A	B	C	D
1		Step	Temp	Time
2		Initial Digestion (opt.)	37°C	10 min
3	Repeat25×	Digestion	37°C	1.5 min
4		Annealing & Ligation	16°C	3 min
5		Storage	16°C	∞

3. Zymo'd goldengate assembly product and eluted with 10 uL dH2O
4. Transformed into XL1 with 2 uL of purified assembly product via electroporation; cells recovered in 1 mL SOC (SOB + 10 uL/mL 1M MgSO4 + 10 uL/mL MgCl2 + 20 uL/mL 1M glucose) @ 37C, shaking. Cells put in shaking 37C at 4:53 pm, taken out at 5:53.
5. Plated 200 uL of cells, undiluted, onto Kan50 plate.
6. Plate went in 37C incubator at 6:00 pm

Week of June 17

MONDAY, 6/18/2018

People present: Soohyun, Stefanie, Anna
Time: 10:30AM-12:30PM and 2:30-7:45 (7.25 hours)
Location: ABL 126, Keck 201
Goals: Miniprep mKate and 2 remaining H.W. sequences & send for sequencing, cotransform mKate into E. coli M61655 with pTARA/UBER plasmid, make plates for our cotransformations, plate co-transformations

OD Curves:

1. Took out all bacterial strains at 11:15 am
2. Everything grew in LB except for C. glutamicum, S. melitoti, and S. oneidensis
3. Tried again:
    a. Streaked all strains onto plain LB plates
    b. S. oneidensis, S. melitoti, and P. putida were put in 30C incubator at 6:25 pm
    c. The rest of the strains were put in 37C incubator at 6:25 pm

Making more media to test growth of strains:

1. Test growth with:
    a. LB + TB salts + vitamins + minerals + ascorbate
    b. LB + TB salts + vitamins + minerals
2. Recipes:

Table1

	A	B
1	**2X LB**	
2	**Reagent**	**Amount**
3	Tryptone	5 g
4	Yeast Extract	2.5 g
5	NaCl	5 g
6	10N NaOH	50 uL
7	H2O	To 250 mL

Table2

	A	B
1	**100X ascorbate**	
2	**Reagent**	**Amount**
3	Ascorbic acid	2.5 g
4	dH2O	to 100 mL

Table3

	A	B
1	**100X MgSO4**	
2	**Reagent**	**Amount**
3	MgSO4	1.1 g
4	dH2O	To 50 mL

Table4

	A	B
1	**Basic Medium**	
2	**Reagent**	**Amount**
3	2X LB	250 mL
4	100X vitamins	5 mL
5	100X minerals	5 mL
6	100X MgSO4	5 mL
7	5X TB salts	50 mL
8	dH2O	185 mL

Table5

	A	B
1	**Basic Medium + Ascorbate**	
2	**Reagent**	**Amount**
3	Base Medium	100 mL
4	100X Ascorbate stock	11 mL

TUESDAY, 6/19/2018

OD Curves:

1. Took out all strains at 10:30 am
    a. Observations:
        I. L. lactis colonies look a lot more rounded on the LB plate compared to the M17 plate
        II. C. glutamicum glycerol stock is definitely contaminated
        III. S. melitoti doesn't look right; colonies are bigger than they should be (previous plate that Anna grew had much smaller colonies after long growth period)
2. To do: make new glycerol stocks of C. glutamicum and S. melitoti
3. Picked a colony of each strain into 2 mL Basic Medium + 0.5% glucose and 2 mL Basic Medium + glucose + ascorbate; 2 colonies ea. of S. meliloti were picked into basic medium adn basic medium + ascorbate

4. Strains went into the appropriate incubator at 5:05 pm

Note: Basic medium was made with 5X m9 salts; hopefully, this will not affect anything

Making improved UBER w/o NLS:

1. Primer annealing:

Table6

	A	B
1	Reagent	Amount
2	iG003 primer	1 uL
3	iG004 primer	1 uL
4	dH2O	3 uL
5	ITA Mastermix	15 uL
6	Total	20 uL

2. Zymo purified ITA product; eluted with 10 uL dH2O; tube labeled "iG003 + iG004"

DNA Miniprepping mKate and H.W. Sequences

Goal: Extract DNA from cells, nanodrop to see how much we have, and send off for sequencing to double check if the mKate and H.W. sequences were inserted the way we wanted.

Materials

- Liquid cultures of mKate and H.W. sequences from last week
- Qiagen QIAprep Spin Miniprep kit

Procedure

- Transferred part of cultures to microcentrifuge tubes and centrifuged for 30 seconds at 13,200rpm. Repeated twice.
- Resuspended bacteria in 250uL of buffer P1 by pipetting up and down
- Added 250uL of buffer P2 to tube and inverted tube 4 times
- Added 350uL of buffer N3 to tube and inverted 4 times
- Centrifuged for 10 minutes at 13,200rpm
- Applied supernatant (liquid result from previous step -- don't get the white stuff) to the spin column
- Centrifuged for 60 seconds at 13,200rpm; discarded flow-through
- Centrifuged for 60 seconds at 13,200rpm; discarded flow-through
- Added 750uL of Buffer PE to the spin column
- Centrifuged for 60 seconds at 13,200rpm; discarded flow-through
- Transferred the spin column to a new micro centrifuge tube
- Centrifuged for 2 minutes at 13,200rpm; discarded flow-through
- Placed the spin column in a new micro centrifuge tube
- Added 50uL of buffer EB to the spin column
- Let stand for 4 minutes
- Centrifuged for 30 seconds

--> **Nanodrop- Keck 301**

- Took 1uL of _____ and measured:
  - mKate = 479 ng/uL
  - H.W. A = 114 ng/uL
  - H.W. B = 384.7 ng/uL

Summary: The process was straightforward. As we perform more and more mini preps the process gets easier and more efficient. This time we did what Dr. Beason told us and it sped up the process even further. Centrifuging the cultures in microcentrifuge tubes allowed us to pellet the cells in a minute vs. 10 minutes. We had a realization that we need to be more careful. When closing the microcentrifuge tubes after filling them to capacity, some liquid splashed back. Lesson learned.

Prep DNA for Sequencing

Goal: Send DNA for sequencing to make sure our mKate and H.W. sequences are constructed correctly.

Materials

- 500ng DNA -- calculate from concentrations obtained from nanodrop
- 2.5uL primer
- Water (add up to 15uL)

Sequencing DNA Prep

	A	B	C	D	E	F	G
1		**Calculations**			**Actual**		
2	Sequence	DNA (uL)	Primer (uL)	H2O (uL)	H2O (uL)	DNA (uL)	Primer (uL)
3	H.W. A	4.3859649123	2.5	8.1140350877	8.11	4.38	2.5
4	H.W. B	1.2997140629	2.5	11.2002859371	11.2	1.29	2.5
5	mKate	1.0438413361	2.5	11.4561586639	11.45	1.04	2.5

Procedure

- Labeled connected PCR tubes (1,2,3,4...) on top and side
- Added water to tubes
- Added DNA
- Added Primer
- Made sure all PCR tubes were closed tightly
- Placed order
    - NOTE: Realized we used the wrong primer for H.W. sequences --> we redid the preparation for H.W. and mKate sequence
- Printed and placed confirmation page in package with PCR tubes
- Placed package in the box outside of Keck 201

Summary: This was the first time we did this by ourselves. It wasn't too hard, but we did run into some issues as mentioned above. Aside from that hiccup, it was easy and didn't take too long. I need to get comfortable with the online part (ordering).

Co-Transform mKate/pTARA/UBER

Goal: Transform both plasmids into the competent cells.

Materials

- MG1655 competent cell aliquots
- mKate, UBER, and pTARA sequences
    - 0.4uL mKate + 0.4uL pTARA
    - 0.4uL mKate + 0.4uL UBER
    - 0.5uL UBER
- Cuvettes
- SOB

Procedure

- Labelled cuvettes and tubes

- Obtained cells from -20C fridge in Keck 201
- Performed each transformation separately, repeated the steps for each:
    - Added respective amount of DNA to competent cells
        - Worked with cells while keeping them in ice for as long as possible, holding them as little as possible
    - Flicked to mix
    - Pipetted 50uL/all of cells out
    - Transferred into cuvette
    - Flicked to spread cells
    - Transformed cells
    - Added 1000uL SOB to cuvette
        - SOB not kept on ice
    - Pipetted up and down to mix
    - transferred cells to a falcon tube
- Placed tubes in shaker at 37C to incubate for 1 hour
    - NOTE: Remained in shaker for several hours longer, waiting for our plates to be finished

Summary: The process was the same as other transformations, we just added both sequences to the cells. I like electrotransformation more than heat shock. Furthermore I like doing the transformations one by one rather than altogether. It seems to go by faster each time we do transformations, but we're still getting the hang of it. It seems like 5.6 seems to be our most common reading.

Make Plates for Co-Transformations

Goal: Make plates for the co-transformations we performed earlier today. (The transformations need a different kind of antibiotic)

Materials

We want 250mL LB

- 10g LB Agar powder
- DI Water
- Antibiotic:
    - 250uL KanR/Amp/Chl

Procedure

- Measured and added 10.00g of LB Agar powder to 1L flask -- 2 times (2 flasks)
- Added DI water to make 250mL
- Mixed using magnetic stir bar and stir plate at medium speed (speed setting 3) until all powder was dissolved
- Autoclaved in bin for 20 minute cycle
    - Labeled new plates while waiting:
        - 6/18 iGEM LB agar KanR + Chl34
        - 6/18 iGEM LB agar KanR + Amp
- Placed LB on stir plate and let cool for ~10 minutes
- Added:
    - 250uL KanR + 250uL Chl34
    - 250uL KanR + 250uL Amp
- Let stir for ~1 minutes
- Used aseptic technique to pour plates
    - Wiped workspace with ethanol
    - Lit bunsen burner
    - Pour plates near flame, running top of flask through the flame between pours
- Left to solidify for ~ 1 hour
- Placed plates in sleeve and left in 4C fridge in ABL 126; Took 2 plates to Keck 201

Summary: It was easy to make the plates today. This was the first time we have made LB agar plates with an antibiotic other than Chl34; the process was the same, which was reassuring and made it simple. To my surprise, the plates solidified rather quickly, but that worked to our advantage so we could plate our transformations.

Plate Co-Transformations

Materials

- Co-transformed liquid cultures
- 1 KanR + Chl34 plate
- 1 KanR + Amp plate
- 1 Amp plate

Procedure

- Obtained cultures from 37C shaker in Keck 201
- Pipetted 50uL onto respective plate
    - mKate+pTARA on KanR+Chl
    - mkate+UBER on KanR+Amp
    - UBER on Amp
- Used beads to spread cultures on plate
- Removed beads
- Placed plates in 37C incubator in Keck 201

Summary: Very simple and quick. I prefer using the beads in comparison to streaking. That said, I prefer it because I'm not that great at streaking, so I could always use more practice with streaking.

WEDNESDAY, 6/20/2018

People present: Soohyun, Stefanie
Time: 10:45AM-1PM and 2:30-5PM (4.75 hours)
Location: ABL 126, Keck 201
Goals: Work on a presentation of what we did in Interlab, analyze the data we got and made visual representations, perform a PCR of Shewanella and E. coli (prep primers beforehand)

OD Curves:

1. Took out all cultures at 10:30 am
2. None of the S. melitoti cultures grew; everything else grew in at least one of the mediums
    a. Vmax: grew in both basic medium + basic medium + ascorbate
    b. B. subtilis: both
    c. **C. glutamicum:** grew in medium + ascorbate
    d. nissle1917: both
    e. mg1655: both
    f. DH10B: both
    g. L. lactis: both
    h. P. putida: both
    i. **S. oneidensis:** grew in medium w/o ascorbate
3. Streaked C. glutamicum culture out onto plain LB plate to test for contamination
4. Made new glycerol stock of C. glutamicum
    a. 300 uL of C. glutamicum culture + 300 uL of 50% sterile glycerol
5. Picked a colony from from S. meliloti plate and streaked onto plain LB plate

Making improved UBER w/o NLS:

1. Goldengate assembly of all parts:

Table7

	A	B	C	D	E
1	**Reagent**	**Amount (uL)**		**Temp**	**Time**
2	Insert (iG003+iG004)	0.28 (40 fmol)		37C	10:00
3	Insert (UBER gBlock)	0.42 (40 fmol)	25X	37C	1:30
4	Backbone (pSPB430)	0.45 (20 fmol)		16C	3:00
5	BsmBI	0.5		16C	hold
6	T4 DNA ligase	0.5			
7	T4 buffer	1.5			
8	10X BSA	1.5			
9	H2O	9.9			
10	**Total**	**15**			

2. Zymo purfied goldengate product; eluted with 10 uL dH2O
3. GG product is labeled "UBER + pSPB430"

PCR

- In PCR tubes, combined
  - 10 uL Q5 buffer
  - 20 ng DNA (0.3)
  - 2.5 uL of each primer
  - 0.5 uL Q5 polymerase --> add last!
  - 1 uL dNTPs
  - up to 50 uL H_2O

PCR DNA and H2O amounts

	A	B	C
1		DNA	Water
2	E. coli	0.1805054152	33.3194945848
3	Shewanella	0.1831501832	33.3168498168

- Placed samples in PCR in Keck
  - Set annealing temperature to average of samples
  - Set extension time to longest extension time

PCR cycle

	A	B	C
1		Temperature (C)	Time
2	Initial Denaturation	98	30 sec
3	Denaturation	98	10 sec
4	Annealing	65	30 sec
5	Extension	72	4 min, 6 sec
6	Repeat 34 times		
7	Final Extension	72	2 min
8	Hold	12	

THURSDAY, 6/21/2018

People present: Soohyun, Stefanie, Katherine, Anna
Time: 10:30AM-1:15PM (2.75 hours)
Location: ABL 126
Goals: Perform gel electrophoresis of our 2 PCR's from yesterday (6/20) and miniprep the UBER liquid culture. Begin the mKate time course.

OD Curves:

1. Took out C. glutamicum and S. meliloti plates at 11:10 am.
2. C. glutamicum looks ok (small colonies)
3. S. meliloti didn't grow, probably because I put it in the 37C incubator instead of the 30C incubator.
    a. threw away plate
    b. Picked another colony of S. meliloti from old plate and streaked onto plain LB plate; put in 30C incubator at 6:37 pm

Making improved UBER w/o NLS:

1. Transformed XL1 with 0.5 uL of GG product; electroporated and let rest in SOB in 37C shaking incubator; start @ 5:26 pm, end @ 6:26 pm
2. Also transformed XL1 w/ pBBR478 and 480; same protocol as with GG product
3. Plated 50 uL undiltued of each transformant on LB Chl34 plates
4. Plates went in 37C incubator at 6:37 pm

Gel Electrophoresis
Procedure
Desired 40mL gel -- sample staining process

- Measured 0.28g agarose powder
    Calculations: 40mL * 0.007 = 0.28g
- Measured 40mL 1x TBE in 50mL graduated cylinder
- Centered and leveled gel tray using (bubble thing)
- Mixed agarose and TBE in flask
- Microwaved and observed for 30 seconds + 5 seconds + 5 seconds, swirled in between
- Let cool for ~1 minute
- Poured gel into caster

- Waited 15 minutes for gel to set

**REDO*- talked to Anna and she wants us to stain gel, not the samples

Desired 50 mL gel -- gel pre-staining process

- Measured .35g agarose powder
  Calculations: 50mL * 0.007 = 0.35g
- Measured 50mL 1x TBE
- Mixed agarose and TBE
- Added 5uL of SYBR Safe stain (in 0.5x TBE)
- Microwaved for 30 seconds + 5 seconds + 5 seconds, swirled in between
- Let cool for ~1 minute
- Recentered and leveled the gel tray
- Poured gel into caster
- Waited 15 minutes for gel to set
- Filled electrophoresis chamber with diH2O (followed online protocol)
  - NOTE: NOT OK -- chamber should be filled with what was used to make the gel (TBE)
- Moved gel in holder to the tray
- Removed holder --> gel ripped in 2 at the wells
- Removed gel from water and placed gel in flask
- Microwaved 30 seconds (gel had remelted)
- Repoured gel into caster, waited ~20 minutes and checked (Gel was not set)

Summary: This was very difficult. We weren't sure what to do and couldn't seem to find the right thing to do until we asked the advisors directly. We looked through the bootcamp protocol (not clear/specific), through online protocols, and through websites, but we didn't find anything. We redid the gel multiple times, and according to Katherine it still didn't work even when the gel was done correctly. We will have to redo it, and hopefully we will have a better grasp of it next time.

Second attempt:
Remade gel and ran for ~50 min at 100 V and then _____ min at 120 V. Saw dye front move, but could not visualize anything under blue light. We will repeat the gel after doing PCR and transformations again.

Miniprep UBER

Miniprepped UBER culture to stock more plasmid. Stored in Keck at -20 degrees C.

Orthogonal transcription fluorescence measurements

1. Measure OD of all cultures

image.png

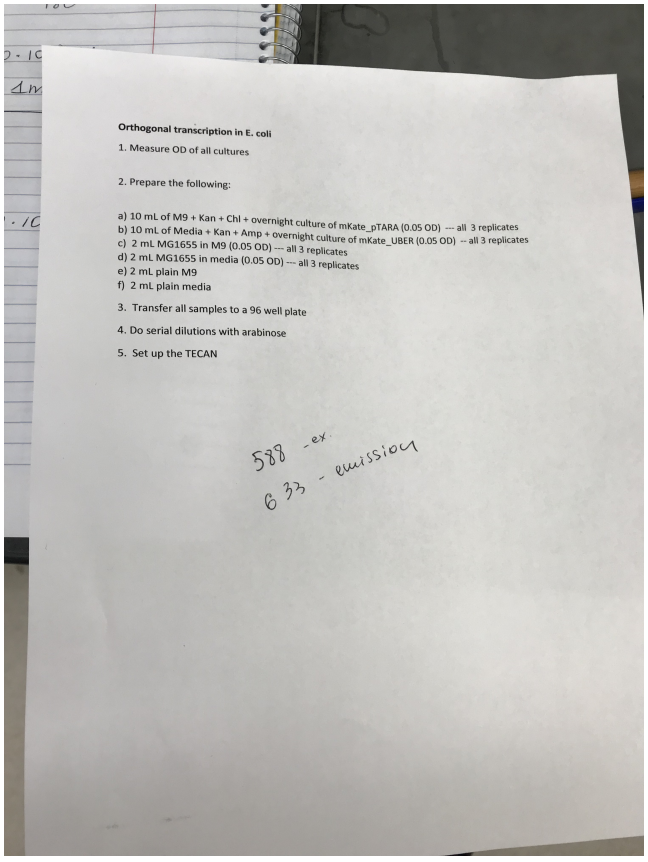


Table8

	A	B	C	D	E	F
1	P1	0.713	7.13	70.12623	volume 10 mL,	dilute to 0.05
2	P2	0.83	8.3	60.24096		
3	P3	0.94	9.4	53.19149		
4	U1	0.52	5.2	19.23077		
5	U2	0.46	4.6	21.73913		
6	U3	0.59	5.9	16.94915		
7	M1	0.093	0.93	107.5269		
8	M2	0.08	0.4	250		added twice
9	M3	0.11	1.1	90.90909		
10	B					
11		using old M9				

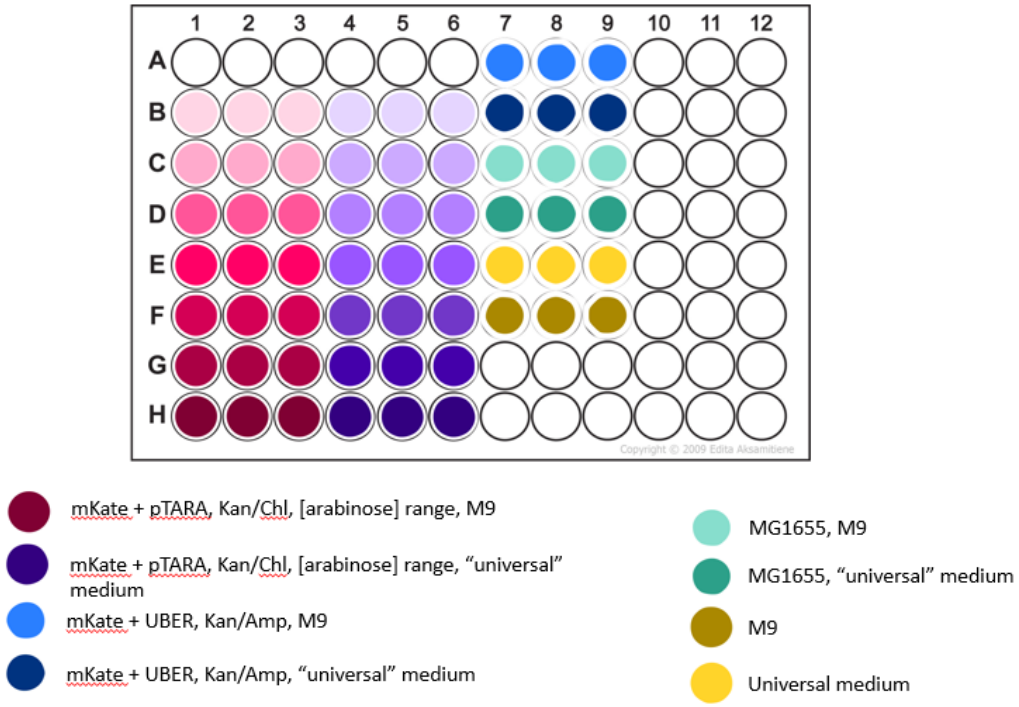
2. Prepare the following:

a) 10 mL of M9 + Kan + Chl + overnight culture of mKate_pTARA (0.05 OD) --- all 3 replicates

b) 10 mL of Media + Kan + Amp + overnight culture of mKate_UBER (0.05 OD) -- all 3 replicates

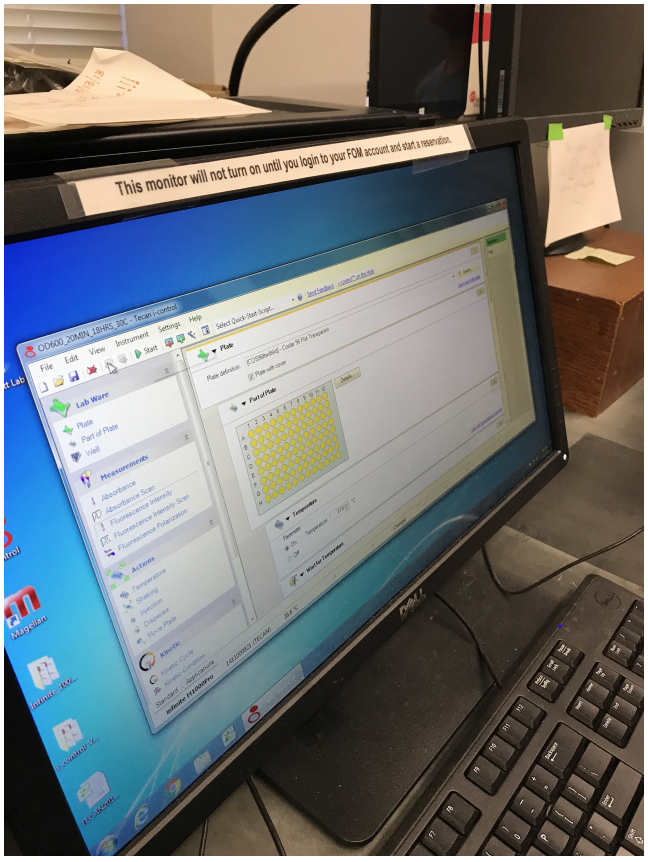
c) 2 mL MG1655 in M9 (0.05 OD) --- all 3 replicates
d) 2 mL MG1655 in media (0.05 OD) --- all 3 replicates
e) 2 mL plain M9
f) 2 mL plain media
3. Transfer all samples to a 96 well plate
4. Do serial dilutions with arabinose

plate layout.png



concentrations of arabinose: 10 mM for 1H-6H, 1 mM for 1G-6G etc.

image.png



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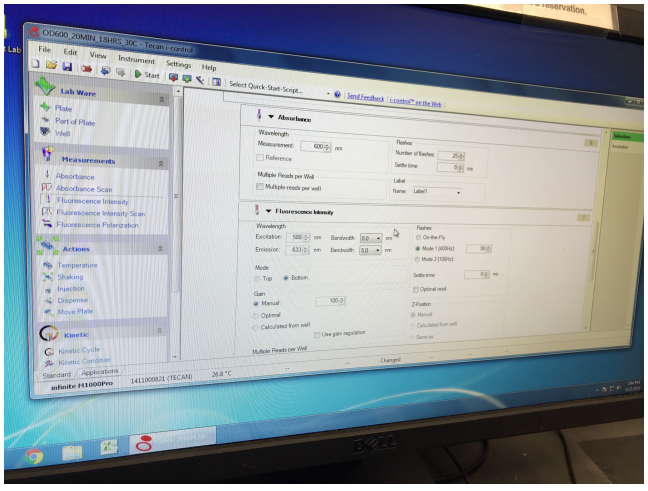

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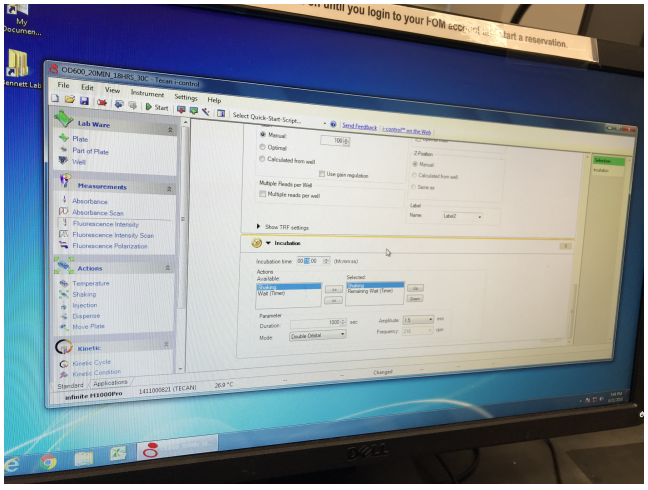


These are the settings with which we analyzed the samples on the TECAN pro machine (note we used 20 min cycles, not 19 min as pictured).

FRIDAY, 6/22/2018

People present: Soohyun, Stefanie, Katherine, Anna
Time: 10:30AM-1PM and 2:15-8PM (8.25 hours)
Location: ABL 126
Goals: Redo interlab and our PCRs, make LB broth for making new competent cells.

Results: Overnight cultures look turbid!!! Our PCRs failed.

Took plates with Shyam's parts and UBER plasmid out of the incubator. Colonies observed on all plates.
Put the plated into 4C frige

Redo PCR from Wednesday (6/20)
Primer Calculations

- Figured out length of amplicons -- benchling
  - Shewanella: 8,196 bp
  - E. coli: 6,212 bp
- Calculated elongation time (30s/kb)
  - Shewanella: ((8,196 bp / 1000 bp) * 30 seconds) / 60 seconds = 4.1 minutes = 4 minutes 6 seconds
  - E. coli: ((6,212 bp / 1000bp) * 30 seconds) / 60 seconds = 3.1 minutes = 3 minutes 6 seconds
- Went to a NEB Tm calculator website (https://tmcalculator.neb.com/#!/main)
  - Chose Q5 polymerase
  - Inserted forward and reverse primer sequences -- found on benchling
  - Found Tm (anneal temperature)
    - Shewanella: 64C
    - E. coli: 66C
- Found primers for each strain
  - Shewanella: iG011 and iG012
  - E. coli: iG013 and iG014

PCR
Materials

- Shewanella genome
- E. coli genome
- primers: iG011, iG012, iG013, iG014
- Nuclease free water
- dNTP
- Q5 buffer
- Q5 polymerase

Procedure

- Labeled tubes: E. coli and Shewanella
- Added to tubes:
    - 33.33uL H2O
    - 0.2uL respective DNA
    - 2.5 of forward primer and backward primer (2.5 ea.)
        - Shewanella: iG011 and iG012
        - E. coli: iG013 and iG014
    - 1uL dNTPs
    - 10uL Q5 buffer
    - 0.5uL Q5 polymerase
- Placed in MasterCycler in ABL 126 under iGEMPRC3 program

Table9

	A	B	C	D	E
1	P1	0.404	0.594059	594	11406
2	P2	0.404	0.594059	594	11406
3	N1	0.399	0.601504	602	11398
4	N2	0.405	0.592593	593	11407
5	TD1-1	0.397	0.604534	605	11395
6	TD1-2	0.365	0.657534	658	11342
7	TD2-1	0.393	0.610687	611	11389
8	TD2-2	0.4	0.6	600	11400
9	TD3-1	0.396	0.606061	606	11394
10	TD3-2	0.409	0.586797	587	11413
11	TD4-1	0.372	0.645161	645	11355
12	TD4-2	0.364	0.659341	659	11341
13	TD5-1	0.402	0.597015	597	11403
14	TD5-2	0.405	0.592593	593	11407
15	TD6-1	0.42	0.571429	571	11429
16	TD6-2	0.419	0.572792	573	11427

LB Broth Preparation

Goal: Prepare LB broth for making new competent cells.

Materials

- LB Broth (Miller's) Premix

- diH2O

Procedure

- Measured 25g of Premix
- Added Premix to bottle
- Filled bottle to 1L with diH2O
- Used stir bar and stir plate to mix until all powder has dissolved
- Covered in foil and added autoclave tape
    - NOTE: Don't cover in foil -- When making LB broth cover it with the lid, loosely on
    - NOTE: Don't leave stir bar in -- Swirling the bottle after is sufficient
- Transferred bottles to Keck 2nd floor
- Removed foil and stir bars; Covered with lids
- Placed in autoclave for 20 minute cycle

Summary: This was the first time we have made LB broth since bootcamp. The process was essentially the same, minus a few things. The process was difficult, but that was on account of ABL being closed and us having to accommodate that.

Making a New Ladder for DNA Electrophoresis

Goal: The ladder used for our PCR Wednesday (6/20) was of unknown origin and it didn't work. So we want to make a new one, that way we know it is correct.

Materials

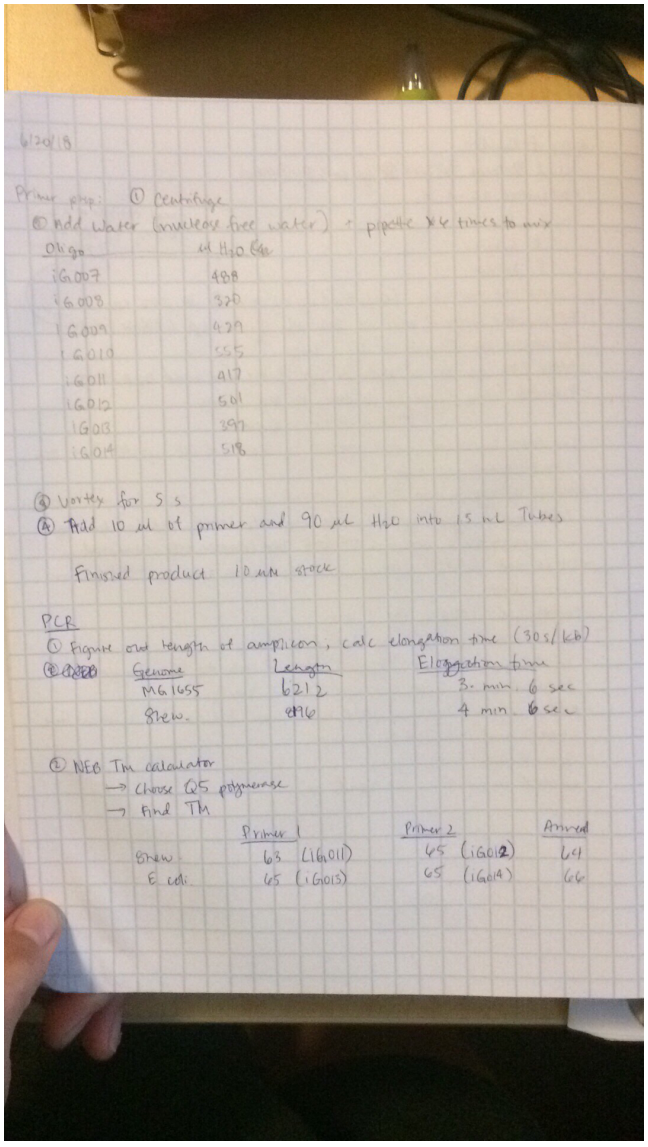
- 1 kb ladder (no dye added)
- Nuclease free water
- Purple loading dye

Procedure

- Located 1kb ladder, NF water, and purple loading dye
- Added 80uL NF water, 20uL 1kb ladder
- Waited for dye to reach room temperature
- Added 20uL of purple loading dye
- Stored in 4C fridge in ABL 126

Summary: Very straight forward and easy, didn't take much time.

image.png



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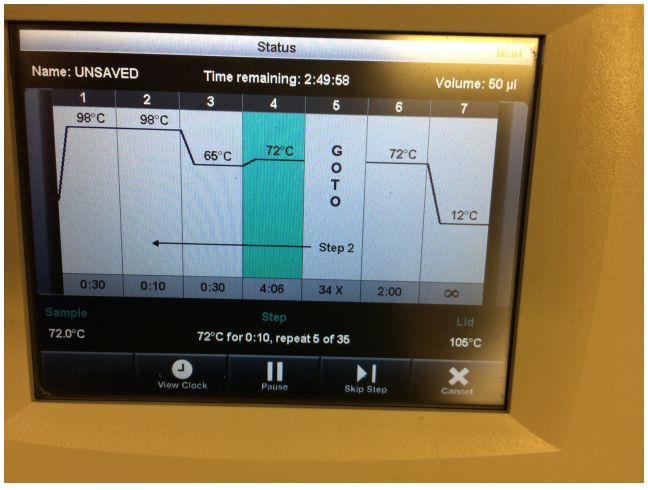
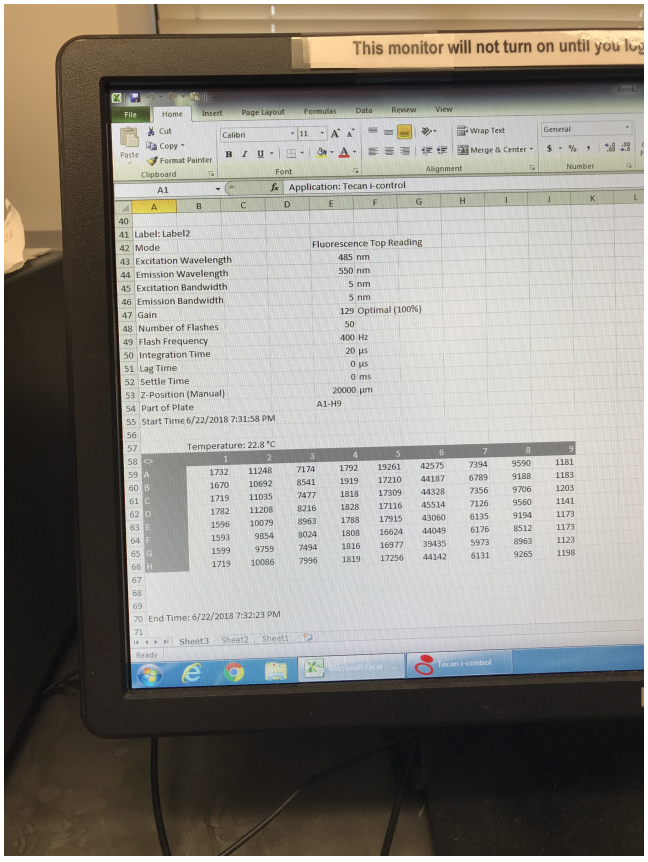


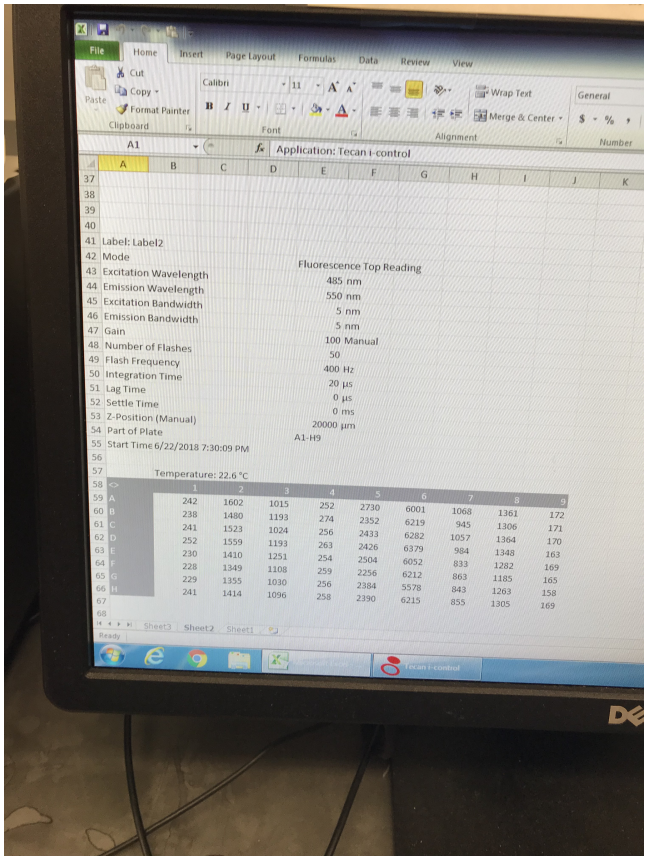
This shows the full PCR cycles with both times and temperatures

image.png



This is the data when the gain is optimized

image.png



This is the data without optimizing the gain

Week of June 24

SUNDAY, 6/24/2018

People: Anna

OD Curves:

1. Took out S. meliloti at 10:00 am
    a. They grow really slowly
2. Picked 2 colonies and grew in 2 mL base media w/ and w/o ascorbate (one colony per type of medium)
    a. in 30C shaking incubator @ 12:08 pm

PCR for E. coli and Shewanella (ribosomal operon)

No bands were observed on the gel.

Repeat the pcr:

Table1

	A	B	C
1		**E. coli**	**Shewanella**
2	primer fwd	2.5	2.5
3	primer rev	2.5	2.5
4	Q5 buffer	10	10
5	dNTPs	1	1
6	DNA	0.3	0.3
7	Q5 polymerase	0.5	0.5
8	water	33.2	33.2

MONDAY, 6/25/2018

People present: Soohyun, Katherine, Anna
Started wetlab 11:00am(presentation to students at 9:30 this morning)
Left lab for lunch at 1:00 pm, this was the end of the day
Goals: - DNA miniprep
- Golden gate assembly for TetR and T7
- DNA electrophoresis for another batch of the E. Coli and the Shewarenella
    -Preparing the gel, using a prestained ladder, staining the E. Coli and the Shewarenella

OD Curves:

1. Checked on S. meliloti at 10:10 am
    a. No growth seen yet
2. Will leave in 30C shaking incubator to see if it will still grow

PCR of unmodified UBER from pPM47-tetR-T7.1:

Table2

	A	B	C	D	E
1	**Reagent**	**Amount (uL)**		**Thermocycler Temp**	**Time**
2	Template (pPM47-tetR-T7.1)	0.2		98C	0:30
3	F primer (D47)	2	25X	98C	0:10
4	R primer (D50)	2		61.5C	0:30
5	5X HF buffer	10		72C	3:20
6	dNTPs	2		72C	10:00
7	Phusion polymerase	0.5		4C	hold
8	water	33.3			

1. Put all reagents in PCR tube; put tube in thermocycler w/ program specified in table.
2. Ran PCR on 0.8% agarose gel; 90V; 50 min run
    a. No bands observed
3. Try again with Q5 polymerase:

Table3

	A	B	C	D	E
1	**Reagent**	**Amount (uL)**		**Thermocycler Temp**	**Time**
2	Template (pPM47-tetR-T7.1)	0.2		98C	0:30
3	F primer (D47)	2	25X	98C	0:10
4	R primer (D50)	2		61.5C	0:30
5	5X Q5 buffer	10		72C	2:30
6	dNTPs	2		72C	10:00
7	Q5 polymerase	0.5		4C	hold
8	water	33.3			

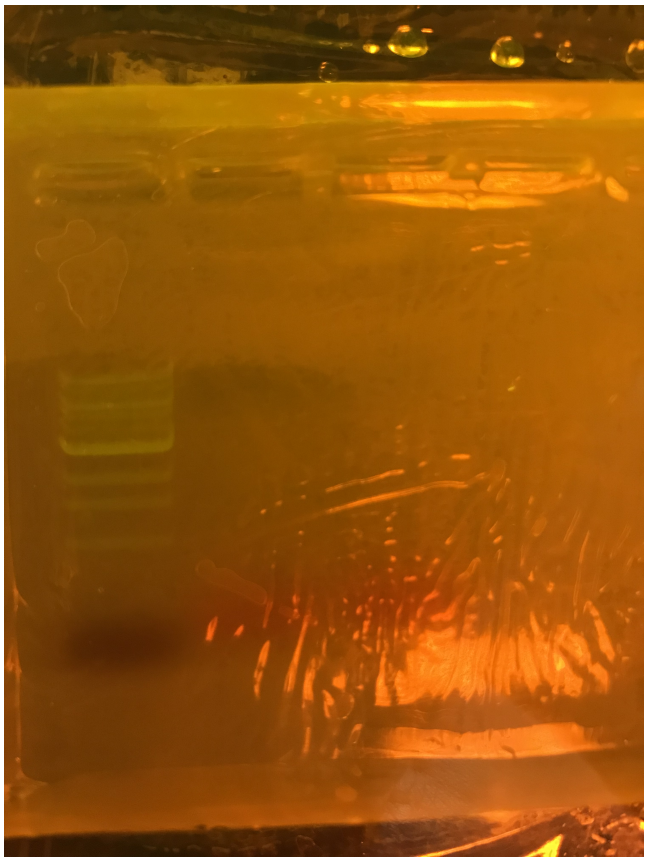
Making improved UBER w/o NLS:
Sent out UBER in part plasmid assembly from 6/20 for sequencing

DNA Electrophoresis (attempt 2)

Prepared another gel, using 5 uL of DNA stain inside.

The gel electrophoresis did not work again- (using Anna's preprepared gel which we reheated and then prepared in a small tray). Left the gel running for approximately 15 minutes at around 120 volts. Pipetted 15 uL of the preparred DNA ladder so that it is more visible (10 worked, but was hard to see). Using TAE as the medium in the tray instead of the TBE.

image.png



Golden Gate Assembly

Golden gate assembly of the TetR and the T7, used Shayam's Excel sheet to deterimine the amount to add- added 0.2 uL of TetR and T7 RNA in a tube respectively. Also added 0.2 uL of the 430 pSPB into both vials and then followed the instructions from the wetlab bootcamp for Golden gate assembly.

Started the Thermocylcer with the first setting as listed on the wetlab bootcamp

Used EB31 as the backbone, and followed the golden gate protocals for that per the wetlab bootcamp.

Table5

	A	B	C
1	**Label**	***LengthDNA [bp]***	***ρDNA [ng/µL]***
2	TetR	734	77.8
3	T7 RNA	2877	253.3
4	pSPB430	1877	221.8

TUESDAY, 6/26/2018

People present: Soohyun, Katherine, Anna
Goals:-Preparing and running 8 different PCRs for the E. Coli to see which one works the best
- Running these PCRs in a gel electrophoresis
- Preparing PCR for 16415 and 31399
- Electroporation and incubation of 423, 479,449, 447, 616, and 639
- Running two TECANS for P1-M2 overnight

Came into lab at 10:30, grabbed lunch around 12:20 and returned to lab at 1:00. Grabbed coffee around 3:50 and then returned at 4:10 to grab our PCRs and prepare the gels. Left lab just after 7:00

PCR E. Coli, 16415, 31399

These values multiplied by 8 were added in creating the samples for E. Coli
80 uL Q5 buffer
160 ng DNA (~~2.4~~ 7.33 uL) -
20 uL each primer -
4 uL Q5 polymerase (last)
8 uL dNTPs -
Up to 400 uL nf H20
266.4 uL H2O -

Table6

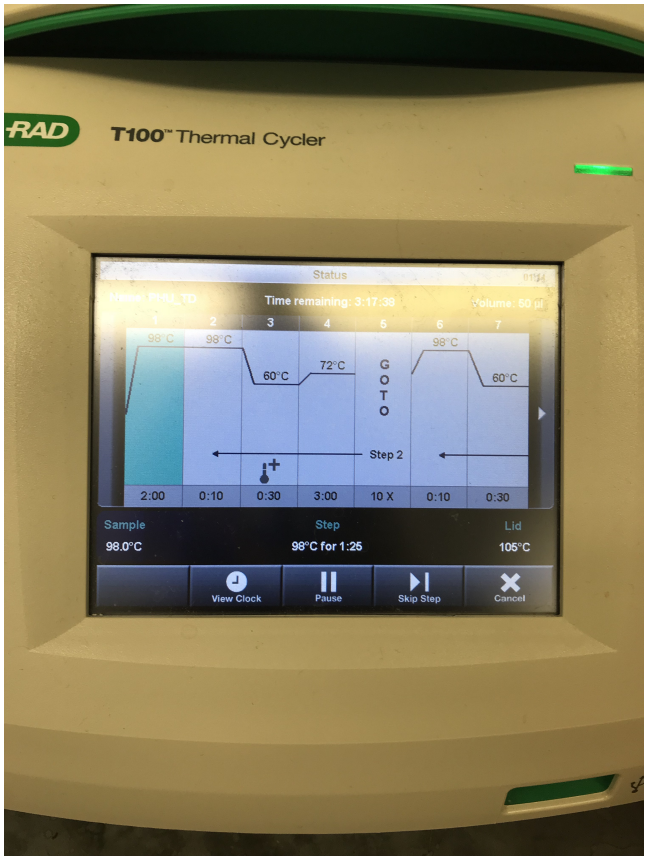
	A	B	C
1		DNA	Water
2	E. coli	0.1805054152	33.3194945848
3	Shewanella	0.1831501832	33.3168498168

We used the following touchdown producdures for E. Coli:

- Temperature gradient
  - Use gradient block to test range of temperature to determine ideal annealing temperature
  - Range: 70-62
    - Do 4 different temperatures: 62, 65, 67, 69

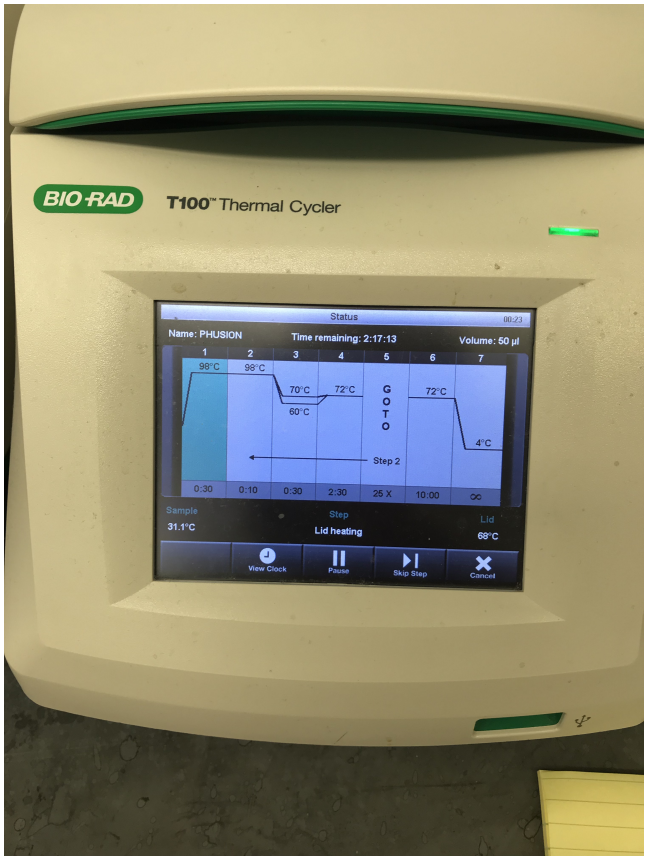
        - 69.3, 66.1, 63.8, 60.7
    - Place tubes with identical samples from back to front of the PCR machine
    - Run gel afterwards to see which temperature produced the optimal band
- Elongation times
    - Increase elongation time to 4 min 30 sec. Test other times
        - Do 3 different times (do at 65C)30 s/kb (4 min 6 sec)40 s/kb (5 min 24 sec)50 s/kb (6 min 48 sec)

image.png



Touchdown protocol for the E. Coli

image.png



Gradient standard protocol for the E. Coli

The following table was used in creating samples of the two Harris Wang parts

Table4

	A	B	C
1		**16415 (primers 7 and 8) --9**	**31399 (primers 9 and 10) --10**
2	primer fwd	2.5	2.5
3	primer rev	2.5	2.5
4	HF buffer	10	10
5	dNTPs	1	1
6	DNA	0.5	0.5
7	phusion	0.5	0.5
8	water	33	33

Flavodoxins PCR thermocycling conditions

	A	B	C	D	E	F	G	H
1	**Step #**	1	2	3	4	5	6	7
2	**Temp**	98C	98C	60 C	72C	GO TO Step 2	72C	4C
3	**Time**	0:30	0:10	0:30	0:07	35X	5:00	hold

PCR of unmodified UBER from pPM47-tetR-T7.1:

1. Ran PCR product on 0.8% agarose gel; 90V; start @ 12:58 pm, end 1:23 pm
2. No band at 4 kb bright, fuzzy band below 0.1 kb
3. Nevermind! Shyam has the UBER part we need.

OD Curves:

1. S. meliloti are not growing
2. Try growing in medium with 0.5% glucose
    a. in @ 6:10 pm

WEDNESDAY, 6/27/2018

People Present: Soohyun, Katherine, Anna
Goals: - Running the PCRs for the Harris Wang parts

Started lab at 10:50 am, ended lab pretty early, around 1:00 pm

Harris Wang PCRs

Letting the ladder only reach about halfway, making ~50 mL of gel and adding 5 uL of gel stain, then added 10 uL to of dye to the samples and used 15 uL of the DNA ladder. Let's hope I can finally get a PCR electrophoresis to work. Left ladder, middle 16etc and right is the 32etc

First gel of 1% agarose ripped, used 2% agarose because the length of these parts was very small.

1. Run pcrs for Harris Wang sequences on a gel. Stop the gel when the dye is about ½ through since the amplicons are small and we don't want them to run off the gel
    a. Excise the bands from the gel if it worked
    b. Gel purify using Zymo kit

- Gel purification:
    - Incubated at 37 for 10 min. Gel would not dissolve with 300 uL of ABD. Added more ABD and incubated some more. Moved to 42 degree for 5 min. Gel dissolved
    - In total: Added 600 uL of ABD, 300 uL of water
    - Accidentally added 30 uL of water into 16415 instead of 15 uL

1. Repeat the same thing with PCRs as yesterday, but use more template DNA: 100 ng instead of 20 (recommended by Shyam), and primers 13 and 14 for *E. coli* genome

- We're almost out of dNTPs
- PCRs have been left in the marines; will run in gel tomorrow

1. Transform the assembly of UBER part plasmid I made yesterday (talk to me for the explanation of what exactly this is)
2. Make liquid cultures for the transformations you did yesterday and repeat any transformations that failed (hopefully none)

- 447 was difficult to find a single colony. Picked off a small one near the edge but saw no reside on the pipette tip. Hoping for the best

OD Curves:

1. Took out S. meliloti at 2:16 pm
2. No growth!!!!!
3. Took colony from each strain (from plates made on 6/18) and cultured in 2 mL base medium + 0.5% glucose (+ascorbate for C. glutamicum).
    a. in appropriate shaking incubator at 5:35 pm

THURSDAY, 6/28/2018

People: Soohyun, Katherine, Anna
Goals:

1. Do PCRs for ribosomal operon of Shewanella: make two reactionsCheck which primers to use
    a. Standard protocol (don't forget to check the size of the product and Tm)
    b. Touchdown (don't forget to adjust times and temperatures if needed)
2. Purify the rest of the genomes. Albert has liquid cultures for them (pick up the kit in Keck)
3. Go to "Orthogonal translation" folder and open the file with MG1655 rrnB - see what primers it has to amplify the operon (iG013 and iG014). Design primers to amplify operons for all other bacteria (primers should bind to annotated genes on either side of the operon)
4. Run PCRs on a gel and gel purify the products

Entered lab around 1 pm and stayed until 5 pm

Ran the PCRs for E. Coli once again, this time they worked and we were able to cut out the streaks and purify the gel. The touchdown worked the best for the E. Coli, we also checked the DNA concentration for the Harris wang elements

OD Curves:

1. Took out all cultures at 10:30 am
    a. Shewanella did not grow
2. Tecan reserved for Tuesday; put growth curves off until then.

FRIDAY, 6/29/2018

People Present: Soohyun, Katherine, Anna
Goals: No wetlab today, worked on project description and attended meeting to present to Dr. Beason and Dr. Silberg