Week of August 6

MONDAY, 8/6/2018

Testing Broad Host Origins

1. Made Kan50 plates, universal medium (250 mL's worth)
    a. autoclaved 2.5 g tryptone, 1.25 g yeast extract, 2.5 g NaCl, 3.75 g agar, 25 uL 10 N NaOH, 25 mL 10X TB salts, and dH2O to 240 mLs
    b. After cooling agar until gloved hand can comfortably touch flask walls, added 2.5 mL ea. of ATCC vitamin and mineral supplements, 2.5 mL MgSO4, 6.25 mL 20% glucose
    c. poured into petri dishes

People present: Stefanie, Albert
Time: 11:45AM-2:30PM and 3:00-7:15PM (7 hours)
Location: ABL126, Keck 201
Goals: Run gel of 644 and 645 (they were switched when sequenced, so we need to figure out which is which), sequence constructs, clean and transform H.W. assemblies into MG1655 cells, plate transformations on Kan50 plates, make liquid cultures for cloning Shewanella and Corynebacterium tomorrow

Run Gel of 644 & 645

Goal: Run gel to determine which sample is which, based on band locations
Materials

- DNA: 644, 645
- Loading dye
- GelGreen
    - NOTE: From now on we will be using GelGreen instead of SyberSafe)
- 0.8% agarose gel
- DNA ladder

Procedure

- Microwaved 0.8% agarose
- Poured ~50mL aliquot of gel
- Added 5uL of GelGreen stain to aliquot, shook to mix
- Poured gel into caster and added well mold
    - Used mold to get out bubbles in the gel
- Waited ~30 minutes (12:25-1PM)

Nanodrop

Goal: Determine the concentrations of the DNA for sequencing
Materials

- DNA (listed below)
- nfH2O

Procedure

- Took 1uL nfH2O and nanodropped as a blank
- Wiped nano drop with kimwipe
- Took 1uL of _____ and measured:
    - piG209 = 115.5 ng/uL
    - piG204 = 112.7 ng/uL
    - pBBR1 mob- = 90.1 ng/uL
    - piG207 = 90.1 ng/uL

- piG208 = 166.6 ng/uL

Summary: Some of the concentrations changed from what was written before (the previous concentrations were smeared and hard to read, so I redid them). Simple, easy process

Run Gel of 644 & 645 Cont.

Procedure

- Determined the volume of the samples
  - About 20-25uL for both
- Added 4.17 uL loading buffer to each sample
  - NOTE: It's a 6x concentration loading buffer, we want a total of 1x concentration loading buffer
  - Calculations:
    c1v1=c2v2
    6x * v1 = 1x * 25uL
    4.17uL
- Mixed samples using pipette tip (pipetted up and down)

Made New 1kb ladder stock

- Located 1kb ladder, NF water, and purple loading dye
- Added 80uL NF water, 20uL 1kb ladder, 20 uL purple loading dye

Run Gel cont.

- Placed gel into the chamber
- Added more 1x TAE to the chamber, up to fill line
  - NOTE: looked clean, didn't need new TAE
- Inserted samples into gel
- Covered the chamber
- Turned on machine (123 volts)
- Left to run -- 1:37PM

Table1

	A	B	C
1	Well 1	Well 2	Well 3
2	DNA Ladder	644	645

Sequencing Constructs

Goal: sequence constructs to make sure they are correct; send 208 with iG054 in order to confirm the presence of RSF1010 origin

Materials

- nfwater
- DNA:
  - 204
  - 207
  - 208
  - 209
  - pBBR1 mob-
- Primers:
  - B17
  - iG054
  - C80

Procedure

- Labeled tubes: 1, 2, 3, 4, 5, 6
- Added respective amounts of water, DNA to tubes, primer

Sample Calculations: 204

500ng DNA / 112.7 ng/uL = 4.436 uL DNA

Table2

	A	B	C	D	E	F	G	H	I
1	**Sample #**	**Sample**	**Sample Length**	**Primer**	**[DNA] (ng/uL)**	**DNA Amount (uL)**	**Primer Amount (uL)**	**Water Amount (uL)**	**Total (uL)**
2	1	204	6001-8000	B17	112.7	4.44	2.5	8.06	15
3	2	207	6001-8000	B17	90.1	5.55	2.5	6.95	15
4	3	208	6001-8000	B17	166.6	3.00	2.5	9.5	15
5	4	209	6001-8000	B17	115.5	4.33	2.5	8.17	15
6	5	pBBR1 mob-	2001-4000	C80	90.1	5.55	2.5	6.95	15
7	6	208	6001-8000	iG054	166.6	3.00	2.5	9.5	15

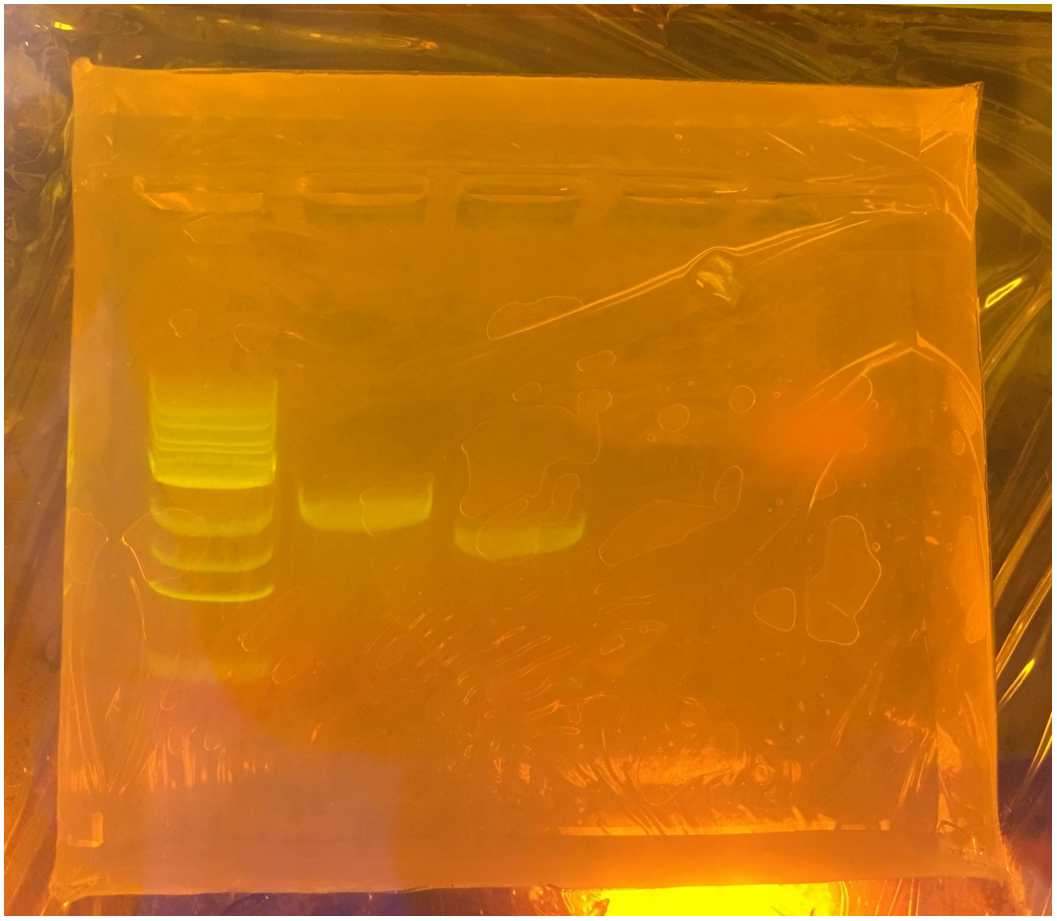
- Printed out order confirmation
- Placed in bag along with samples
- Left outside Keck 201

Summary: The process was normal and simple. No issues. (Had issues finding the length of pBBR1 mob-, but Shyam said the length doesn't really matter. You can put whatever in the length box and it doesn't change what they do.

Run Gel 644 & 645 Cont.

- Stopped power source (2:06)
- Removed gel and placed on UV light

IMG_8463.jpg



- Labeled wrap on gel: iGEM 8/6 644 & 645
- Left in 4C fridge Keck 201

Results: 654 is actually 644, and 644 is wrong.

Summary: Ran into an issue when removing the cover of the electrophoresis chamber. The positive node came out of the chamber along with the cover. I tried to screw it back in, but it wouldn't. I asked Jordan and another grad student and they said to leave it alone. Other than that, I didn't encounter any issues.

Clean H.W. Assemblies

Goal: Clean the assemblies so that they can be transformed and plated later

Materials

- 9 H.W. assemblies from ABL 126 4C fridge

Sample Identities

	A	B
1	**Sample**	**ID**
2	2	piG202
3	3	piG203
4	4	piG204
5	6	piG206
6	7	piG207
7	8	piG208
8	9	piG209
9	10	piG210
10	11	piG211

- Zymo DNA Clean & Concentration Kit

Procedure

- Determined volume in tubes: 12-15uL
  - NOTE:Assumed 15uL for all
- Added 30 uL of DNA Binding Buffer (2x volume)
- Vortexed for ~5 seconds
- Transferred volume to spin column
- Centrifuged for 30 seconds at 13,000rpm, and discarded flow through
- Added 200uL DNA Wash Buffer to ea. column
- Centrifuged column for 30 seconds at 13,000rpm. Repeated wash step
- Added 15 uL of nfH2O to column
- Incubated at room temperature for 1 minute
  - NOTE: stod for ~3 minutes
- Transferred spin column to microcentrifuge tube
- Centrifuged for 30 seconds at 13,000rpm
  - NOTE: a broken cap fell into the centrifuge that I couldn't retrieve

Summary: No issues concerning the process. That said, after centrifuging the open tubes for the last step, a lot of the caps broke (despite my previously successful method). One of the broken caps fell into the gap in the centrifuge. I'm not sure if this is an issue, but I put a piece of tap over the top just in case.

Transform H.W. Assemblies

Goal: We are transforming the assemblies so that we can verify the sequences

Materials

- Cleaned DNA
- Electrocompetent cells: 9 MG1655 aliquots
- Cuvettes
- Falcon Tubes

Procedure

- Obtained MG1655 cells from boxes in Keck 201 -80C fridge
- Waited for cells to thaw, kept on ice. Kept cuvettes on ice
- Labeled tubes: 8/6 iGEM ##
- Added 4uL of DNA to cells*

- Flicked to mix*
- Pipetted 50uL out of cells and transferred into cuvette*
- Flicked to spread cells*
    - NOTE: After flicking to spread the cells, I noticed 2 drops of cells on the side of cuvette for #4; 1 drop for #10; 3 drops for #11 (May affect the successfulness of the transformations)
- Transformed cells*
- Added 1000uL LB to cuvette*
    - LB not kept on ice
- Pipetted up and down to mix*
- Transferred cells to a falcon tube*
- Repeated starred (*) steps for ea. transformation
- Placed tubes in shaker at 37C to incubate for 1 hour (5:55PM)

Summary: I had some difficulty working quickly; I hope it doesn't affect the cells. Aside from that there weren't any problems. All the transformations had good readings (5.6-5.7). I was concerned because there was only 1 set of EC MG1655 cells, and the other 8 aliquots were MG1655, but were not distinguished as EC. But I didn't have any arcs, so I assume they worked just fine.

Cloning Shewanella & Corynebacterium (Making Competent Cells) Pt. 1

Making Liquid Cultures

Goal: Making liquid cultures as part 1 of 2 for making competent cells of each strain. I made liquid cultures tonight, and we will do the actual cloning tomorrow.

Materials

- S. oneidensis and C. glutimicum plates
- Toothpicks
- "base medium"
- Flacon tubes

Procedure

- Labeled tubes:
    - 8/6 Shew. iGEM
    - 8/6 Coryne. iGEM
- Added 5 mL of base medium to both tubes
    - NOTE: Shew. got ~5.25mL
- Used a toothpick to pick up a single colony from both plates and placed into respective tubes
- Put liquid cultures into shakers at ~6:25PM
    - Shew. = 30C
    - Coryne. = 37C
- Parafilmed plates and put into Keck 201 4C fridge on Anna's shelf (fridge all the way to the right that normally holds PCRs)

Summary: Very simple process. I'm always concerned when I have to pick up colonies from a plate because I worry I didn't get enough bacteria, or I whipped it off accidentally. We shall see; I used David's idea to just touch the bacteria, rather than try to scoop it up.

Plate Transformed H.W. Assemblies

Goal: Plate the transformed assemblies so that we can then pick a colonies, do minipreps, and sequence them later this week

Materials

- Liquid cultures of transformed H.W. assemblies
- Kan50 plates

Procedure

Worked in biosafety cabinet

- Obtained liquid cultures from shaker and plates from incubator (warmed them up)
- Pipetted out 100uL from the culture

- Pipetted onto plate
- Used beads to spread culture around plate
- Removed beads
- Placed plates in Keck 201 37C incubator

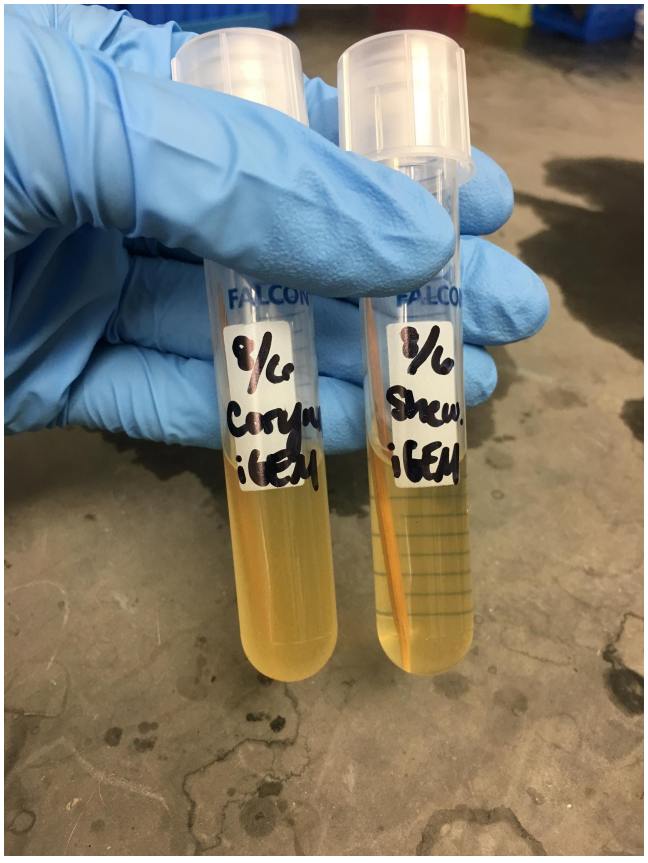
Summary: Simply and easy. I still prefer using beads over streaking. No issues encountered.

TUESDAY, 8/7/2018

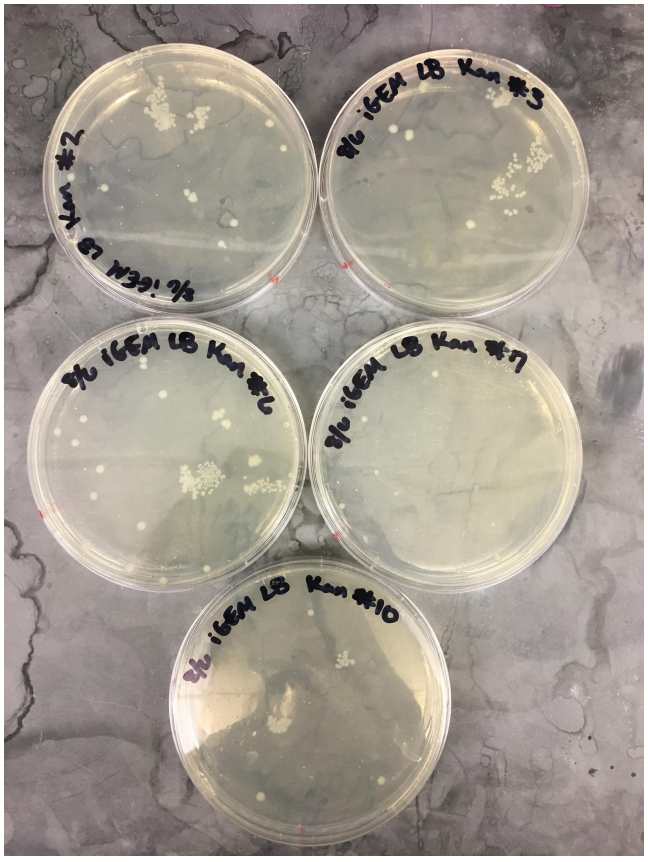
People present: Stefanie, Albert
Time: 11AM-1:15PM and 2-6:45PM (7 hours)
Location: Keck 201
Goals: Begin making competent cells of Corynebacterium and Shewanella, Work on verifying H.W. sequences

Plates and liquid cultures removed from incubators at 10:55AM
Results: C. glutamicum looks turbid, S. oneidensis does not. All plates, except for #8 and #11, have at least 1 colony. Only 3 plates have red colonies (4, 6, and 9).

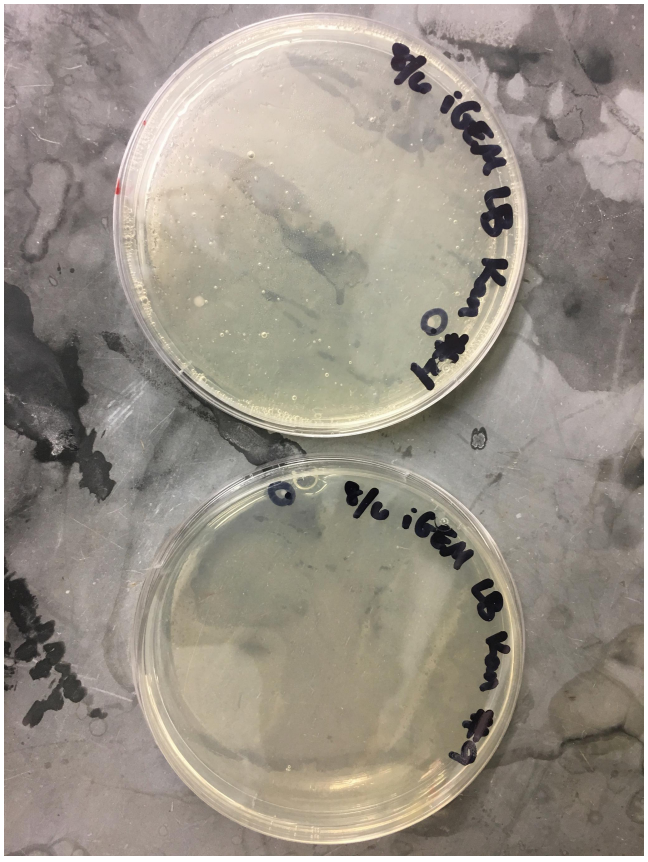
IMG_8465.jpg



IMG_8466.jpg



IMG_8467.jpg



Measure OD600 of C. glutamicum and S. oneidensis

Goal: Determine absorbance of the cultures to see if they are in our desired range

Materials

- Liquid cultures
- Base medium
- Cuvettes

Procedure

Used spectrophotometer in Keck 201

- Filled cuvette with 200uL of base medium for blank, measured
- Filled cuvettes with 200uL of ea. culture and measured

Abs600 Measurements

	A	B
1	**Sample**	**OD Reading**
2	Blank	ZERO (0.1351)
3	C. glut.	1.8339
4	S. one.	0.3142

- Talked to Jordan
  - We want between a measurement between .4 and .6

- So we need to dilute c glut. and let s one. grow more
  - Shewanella put back into 30C shaker at 11:45AM

Summary: Easy process. No issues.

Competent Cells Cont.

Materials

- Glycine
- di-water
- glycerol
- LB

Procedure

- Measured 25.00g of Glycine
- Added 25mL of 20% glucose to 1L of LB
- Dissolved glycine in water
- Filtered while adding (all it took to dissolve the glycine) water with dissolved glycine

Made 20% Glycerol

Goal: We have 10% glycerol, but the protocol calls for 15% glycerol. So by making 20% glycerol and adding half and half of the total volume, we will make 15% glycol.

- Added 100mL of glycerol to a 1L flask
- Added 400mL of di-water
- Covered flask with foil and autoclave tape
- Placed in autoclave

Re-checked Shew. OD600

Used spectrophotometer in Keck 201

Removed culture from shaker ~2:00PM

- Filled cuvette with 1 mL of base medium for blank, measured
- Filled cuvettes with 1 mL of culture and measured

Abs600 Measurements 2

	A	B
1	**Sample**	**OD Reading**
2	Blank	ZERO (0.1335)
3	S. one.	1.1197

Comp Cells Cont.

- Added all remaining C. glutamicum liquid culture to 1L of LB + glycine we made
- Added 20 mL LB to a small flask
- Added 200 uL of Shew. culture
- Placed both flasks in shakers: S. in 30C and C. in 37C

Made 15% Glycerol

Goal: We need 15% glycerol for our washes of the cells, so we need to make more.

- Added 150mL of glycerol to a 1L flask
- Added 850mL of di-water
- Covered flask with foil and autoclave tape
- Placed in autoclave (setting 3)
  - Retrieved later

Summary: The process so far is slightly confusing and difficult, but I think as I do this more it will become easier. It's a lot of small things that go into a single large one. We didn't run into any issues.

Blue Light Analysis of Plates

Goal: Check the plates for GFP, if GFP is seen then we can assume the constructs are wrong since the GFP sequences have not been replaced.

Procedure

- Left plates on bench all day
- Placed plates under blue light (same one used for reading gels) and observed
    - 7 had GFP -- the sequence is wrong
    - 2, 3, 4, 6, 9, 10 did not have GFP -- seems like the sequences are right

Summary: Since some of the plates didn't have GFP we can assume the part has been replaced with our plasmid, despite some of them not being red. The plates with red colonies correspond to the plasmids with the highest expression, so those that appear white, may just have low expression to the point where we cannot see the red fluorescence.

Made New Cultures

Goal: For plates with red colonies, make liquid cultures using a red colony. For plates with colonies, but not visibly red, make 3 liquid cultures from white colonies. Replate liquid cultures that did not grow.

Materials

- LB
- Kan 50 plates
- Falcon tubes

Procedure

Worked in hood

- Labeled tubes: ___ iGEM 8/7
    - 2-1, 2-2, 2-3
    - 3-1, 3-2, 3-3
    - 10-1, 10-2, 10-3
    - 4r, 6r, 9r
- Pipetted 4mL of LB into 2, 3, 10 tubes
- Pipetted 5mL of LB into 4, 6, 9 tubes
- Used toothpicks to pick up a red colony from plates 4, 6, and 9. Placed into respective tubes
- Used toothpicks to pick up 3 white colonies from plates 2, 3, 10

- Labeled plates:
    - 8/7 iGEM LB Kan ##
- Took 400uL from the 8 and 11 cultures
- Pipetted onto respective plates
- Used beads to spread
    - Made sure to shake for a long time and in different angles
- Placed in 37C incubator/shaker at ~6:15PM

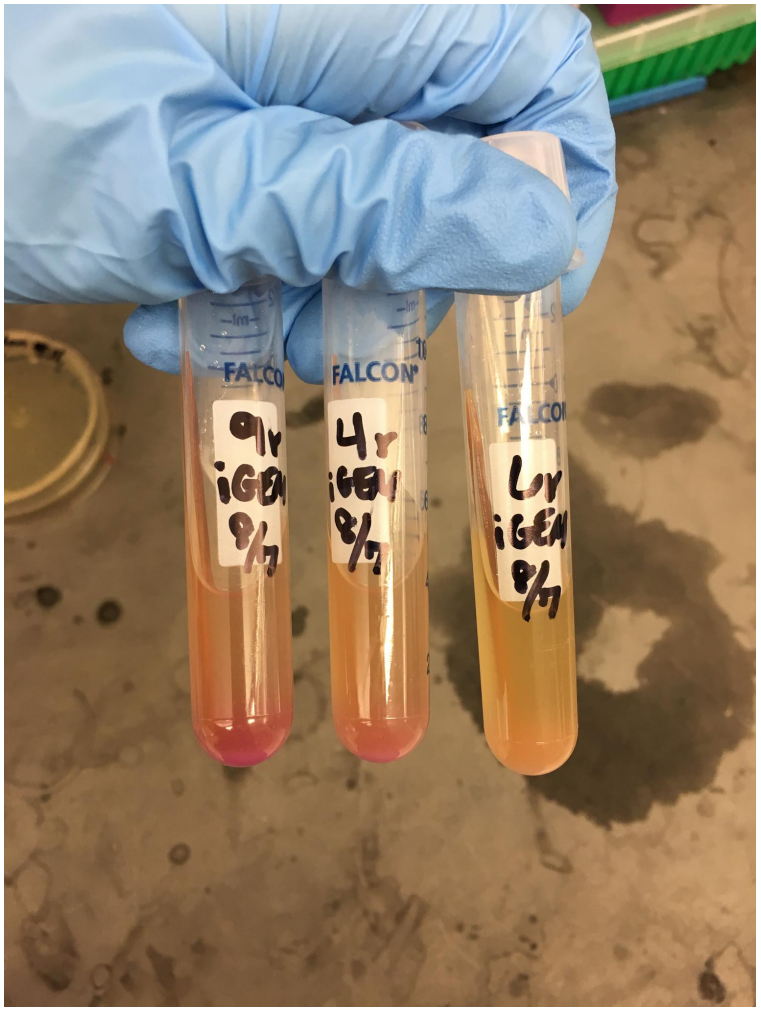
Summary: It was difficult getting colonies from the plates because they were grouped together and no colonies were separate from others. They all were growing into one another. I tried to make sure to get only 1 colony (looking for the faint line between 2 colonies and tried not to touch it), but I'm not sure how it went. Aside from that difficulty we didn't run into any problems.

WEDNESDAY, 8/8/2018

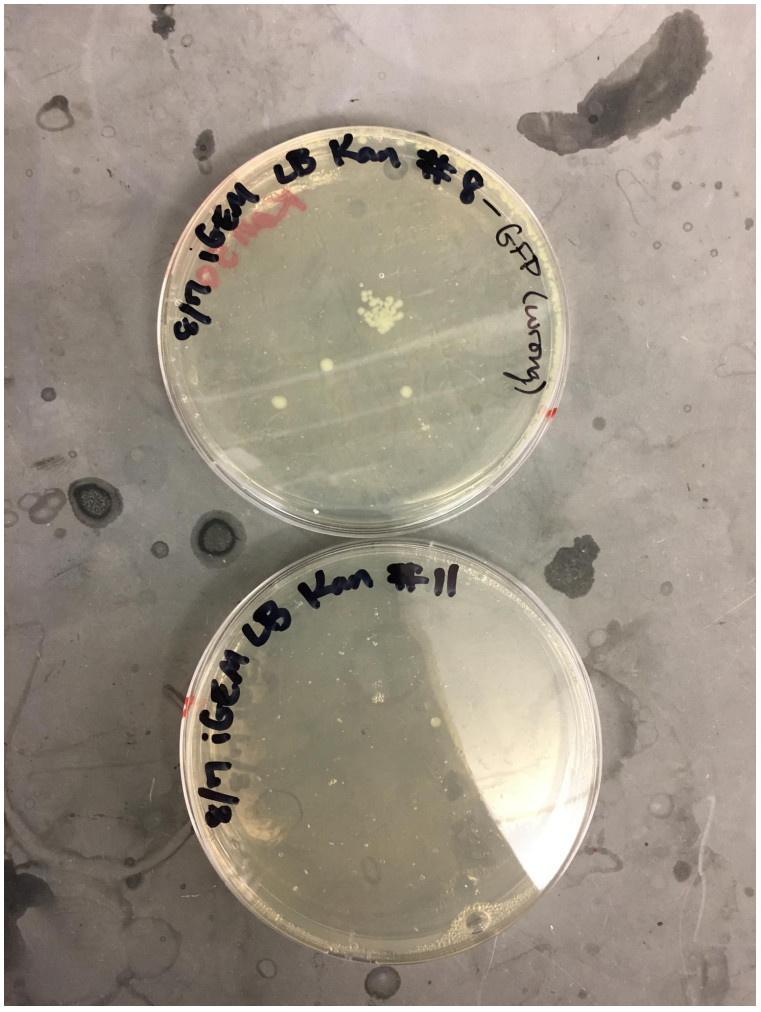
People present: Stefanie, Albert
Time: 9:45AM-3:30PM and 4:15-PM
Location: Keck 201
Goals: Continue making competent cells, continue working with H.W. sequenecs

Removed yesterday's liquid cultures and plates from the incubator/shaker (~10:00AM).
Results: Plates 8 and 11 both have at least 1 colony, but neither plate has red colonies. All liquid cultures look turbid.

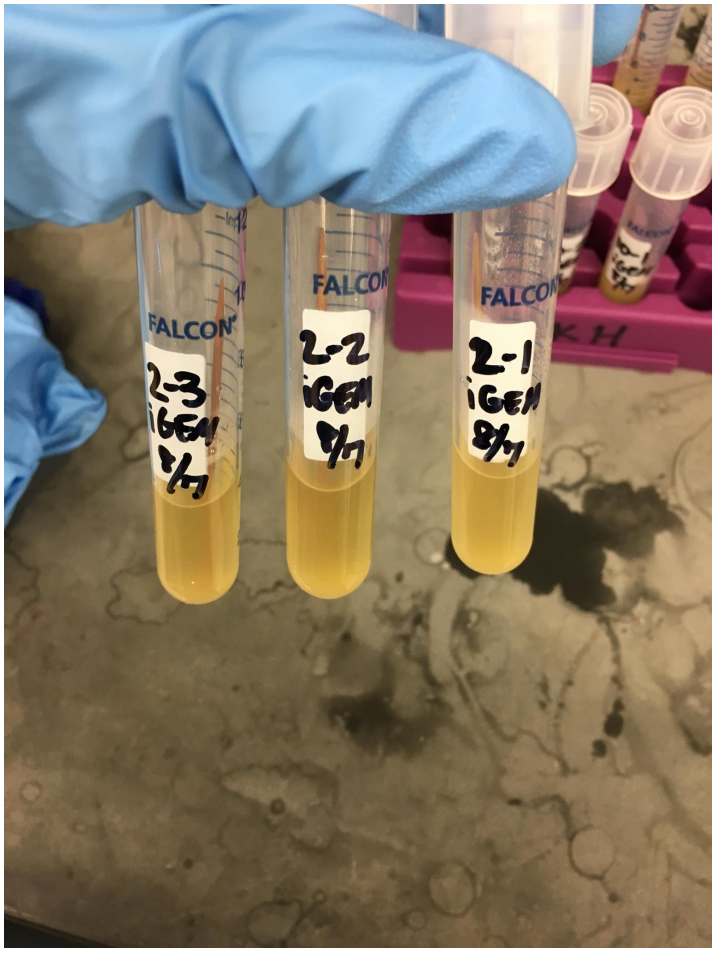
IMG_8471.JPG



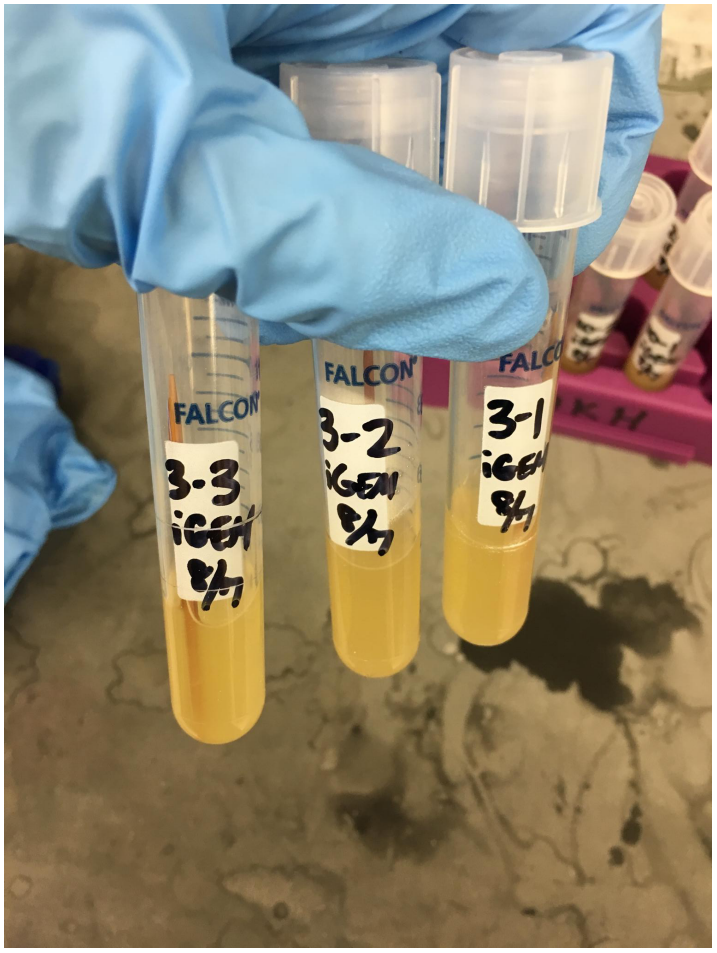
IMG_8472.JPG



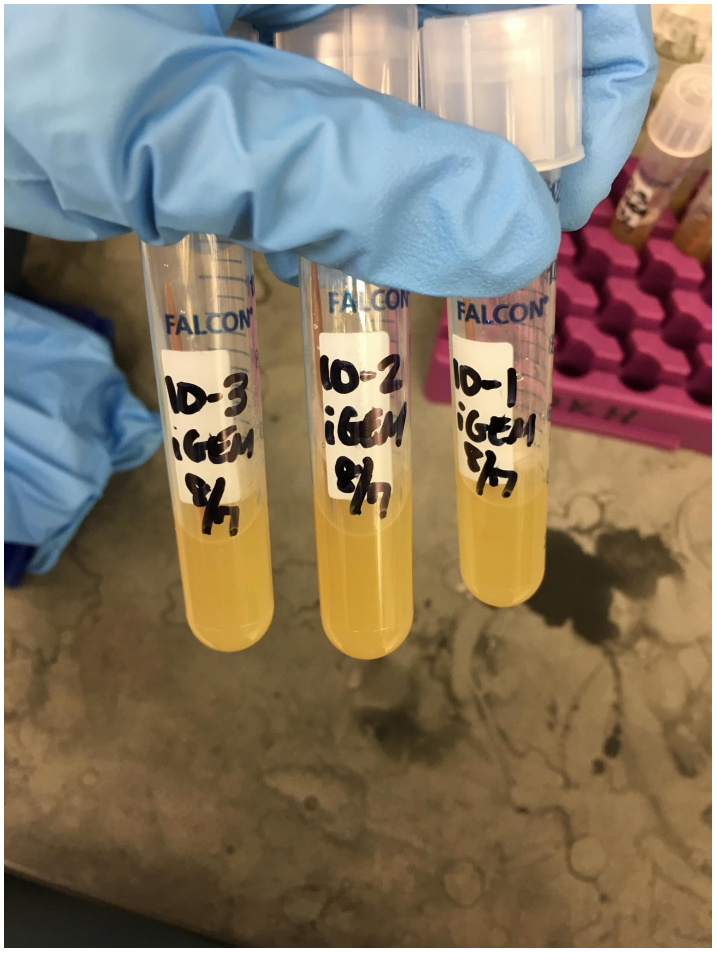
IMG_8474.JPG



IMG_8475.JPG



IMG_8473.JPG



Blue Light Analysis of Plates

Procedure

- Placed plates under blue light and observed
    - 8 had GFP -- the sequence is wrong
    - 11 did not have GFP -- seems like the sequence is right

Competent Cells Cont.

Materials

- 1M D. sorbitol
- Liquid culture

Procedure

- Placed C. glutamicum culture into 37C shaker at 9:52AM
- Removed S. oneidensis culture from 30C shaker
- Measured the amount of culture in the flask using a 25mL pipette
- Distributed the culture equally between 2 15mL falcon tubes using the pipette
- Weighed the tubes on the balance to make sure they were equal (15.62g and 15.63g)
- Placed in the big centrifuge in Keck 201
    - Entered the rotor number (5.3)
    - Time 10 minutes
    - Closed door -- DO NOT grab it by the edges

    - Signed in
- Removed cultures
    - Sat for a few minutes
- Discarded the supernatant
- Resuspended the pellets in 3mL of 1M D. sorbitol
- Transferred 1mL of the resuspended cells into 1.5mL microcentrifuge tubes
- Centrifuged for 1 minute at 14,000rpm to pellet the cells again
- Discarded the supernatant
- Resuspended the cells in 1mL 1M D. sorbitol*
- Centrifuged for 1 minute at 14,000rpm. Discarded supernatant*
- Repeated starred bullets(*) 2 times
- Added 200uL of 1M D. sorbitol to the cells and resuspended
- Took 100uL of the resuspended cells and added them to another microcentrifuge tubes
- Put the aliquots in a box labeled iGEM Shewanella EC
- Put in -80C freezer in Keck 201 (middle section top right section-- above E. coli MG1655 cells)

Summary: YAYYY! We did it! Shewanella competent cells are done. The process was easy after we got them to grow. We didn't run into any issues.

Miniprep Red Liquid Cultures

Goal: I need to miniprep the cultures to get their DNA which some of which will be sent for sequencing later to verify the H.W. sequences

Materials

- Liquid cultures: 4r, 6r, 9r
- Qiagen Miniprep Kit
- Primer: B17
- nfWater

Procedure

- Transferred part of cultures to microcentrifuge tubes and centrifuged for 30 seconds at 14,000rpm. Repeated 2/3 times.
    - I'm not sure what happened the 1st time I centrifuged. I ran the centrifuge, went to get a beaker and when I came back it was at ~5 seconds.
- Labeled spin columns as above^
- Resuspended bacteria in 250uL of buffer P1 by pipetting up and down
- Added 250uL of buffer P2 to tube and inverted tube 6 times
- Added 350uL of buffer N3 to tube and inverted 6 times
    - 9r needed some more inversions
- Centrifuged for 10 minutes at 13,000rpm
- Applied supernatant (liquid result from previous step) to spin column
- Centrifuged for 30 seconds at 13,000rpm; discarded flow-through
- Centrifuged for 30 seconds at 13,000rpm; discarded flow-through
- Added 750uL of DNA Wash Buffer to the spin column
- Centrifuged for 30 seconds at 13,000rpm; discarded flow-through
- Centrifuged for 1 minute at 13,000rpm; discarded flow-through
- Placed the spin column in a new micro centrifuge tube
    - Labeled as above^^ with miniprep iGEM
- Added 50uL of nfH2O to the spin column
- Let stand for 1 minute
- Centrifuged for 1 minute at 13,000rpm

Nanodrop

Procedure

- Took 1uL nfH2O and nanodropped as a blank

- Wiped nano drop with kimwipe
- Took 1uL of _____ and measured:
  - 4r = 128.0 ng/uL
  - 6r = 357.7 ng/uL
  - 9r = 93.9 ng/uL

Sequencing (6r & 9r)

Procedure

- Labeled tubes: 1, 2
- Added respective amounts of water, DNA to tubes, primer

Sample Calculations: 6r_piG206
500ng DNA / 357.7 ng/uL = 1.397 uL DNA

Table3

	A	B	C	D	E	F	G	H	I
1	**Sample #**	**Sample**	**Sample Length**	**Primer**	**[DNA] (ng/uL)**	**DNA Amount (uL)**	**Primer Amount (uL)**	**Water Amount (uL)**	**Total (uL)**
2	1	6r_piG206	6001-8000	B17	357.7	1.40	2.5	11.1	15
3	2	9r_piG209	6001-8000	B17	93.9	5.32	2.5	7.18	15

- Printed out order confirmation
- Placed in bag along with samples
- Left outside Keck 201

Summary: The process was easy and normal. No issues.

Update: Corynebacterium is not growing.

Measure Fluorescence of Liquid Other Cultures

Goal:

Materials

- Liquid cultures
- 10x PBS

Procedure

- Transferred part of cultures to microcentrifuge tubes, centrifuged for 30 seconds at 13,000rpm, discarded supernatant. Repeated 2/3 times.
- Resuspended in 100uL of 10x PBS

Summary:

Making Competent Corynebacterium

1. Grew 1/200 dilution of C. glut in 1 L LB, 0.5% glycerol, 2.5 % glycine
   a. OD = 0.8764 (supposed to be 0.200)
2. Pelleted by centrifuging; 15 min, 4C, 4000xg
   a. Discarded supernatant
3. Resuspended in 1 L 15% glycerol
4. Pelleted again; 10 min, 25C, 4000xg
   a. discarded supernatant
5. Resuspended in 500 mL 15% glycerol
6. Pelleted again; 10 min, 25C, 4000xg
   a. discarded supernatant

7. Resuspended in 200 mL 15% glycerol
8. Pelleted again; 10 min, 25C, 4000xg
    a. discarded supernatant
9. Resuspended in 200 mL 15% glycerol
10. Pelleted again; 10 min, 25C, 4000xg
    a. discarded supernatant
11. Resuspended in 2 mL 15% glycerol
12. aliquotted 100 uL into 1.5 mL microcentrifuge tubes
13. Stored in -80C freezer

Genome Prep of P. putida and S. meliloti

1. Streaked out P. putida and S. meliloti onto universal medium plate
2. In 30C incubator at 8:00 pm

THURSDAY, 8/9/2018

HW cloning

1. Took out 11-1 at 11:00 am
2. read fluorescence
    a. no red fluorescence
3. Will have to redo cloning
4. Sequencing results for piG206 and 209 are back
    a. 206 and 209 have nonspecific priming
        I. maybe 2 colonies were picked
    b. To test if 2 colonies were picked: transformed MG1655 with 0.2 uL ea. of 206 and 209
        I. let rest in SOB, 37C shaking; start 4:45 pm end 5:45 pm
        II. plated 1:10 dilution, 100 uL onto LB Kan50 plate
        III. in 37C incubator at 6:00 pm

Retry cloning pIG203/7/8/10/11

Multigene vectors

	A	B	C	D	E
1					
2	**mKate with HW elements**		**Mastermix**		
3	**Part**	**µL**			
4	cassette vector RSF1010 piG223	0.65	3.25		
5	pSPB501	0.3	1.5		
6	HW elements	0.8			
7	pSPB463	0.3	1.5		
8	pSPB460	0.3	1.5		
9	pSPB512	0.3	1.5		
10					116.8
11	**BsaI**	0.5	2.5		
12	**T4 DNA Ligase**	0.5	2.5		
13	T4 Buffer	1.5	7.5		
14	10X BSA	1.5	7.5		
15	H20	8.14	40.7		
16			69.95	13.99	

Genome Prep of P. putida and S. meliloti

1. Took out plate of P. putida and S. meliloti at 11:00 am
2. P. putida has no pickable colonies; S. meliloti did not grow
3. Restreaked P. putida and S. meliloti onto plain universal medium plates
    a. in 30C at 6:47 pm
4. Nanodropped existing genomes; Shyam said they all looked fine

FRIDAY, 8/10/2018

Genome Prep of P. putida and S. meliloti

1. P. put and S. mel both did not grow on plate
2. "Plain" universal medium plate turned out to be Kan50 plates

HW Cloning

1. Zymo cleaned goldengate products (piG203, 207, 208, 210, 211)
2. Transformed 5 uL ea. into MG1655
    a. electroporated, let rest in 1 mL SOB in 37C shaking incubator (start 7:00 pm end 8:00 pm)
3. Dropped 50 uL of each culture onto a Kan50 plate; streaked out
4. In 37C incubator at 8:30 pm

Origin Testing

1. Electroporated 50 ng ea. of RSF1010 mob- into MG1655, C. glut, S. onei, S. mel, P. putida; transformed B. subtilis using heat shock
2. Did same for pWV01 and pBHR1
3. Let rest in appropriate incubator for amount of time specified in transformation protocols in 1 mL of universal medium
    a. MG1655: 37C, 1 hr
    b. C. glut: 37C, 1 hr
    c. B. sub: 37C, 40 min
    d. P. put: 30C, 1 hr
    e. S. mel: 30C, 3 hr
    f. S. onei: 30C, 90 min
4. Plated 25 uL of ea. transformant, undiluted, onto both Kan and plain universal medium plates
    a. also plated 25 uL of ea. strain of bacteria, undiluted onto Kan plates (neg. control)
        I. plates in appropriate incubator at 8:00 pm
        II. used inoculation loops to spread out ea. culture on plate
        III. Kan to test if origin is working
        IV. plain to see if electroporating/transformation killed all the bacteria (pos. control)

SATURDAY, 8/11/2018

HW Cloning

1. Took out plates from Friday at 12:00 pm
2. 203, 207, and 208 had visibly red colonies
3. 210 and 211 did not have visibly red colonies
4. Picked 2 colonies of each plasmid into 4 mL LB + Kan
    a. in 37C shaking at 5:05 pm

Genome prep of P. putida and S. meliloti

1. Streaked (for the third time) P. put and S. mel onto plain LB agar plates
2. Plates in 30C incubator at 4:50 pm

Making Competent Corynebacterium

1. Streaked C. glut onto plain LB agar plate
2. in 37C at 4:50 pm

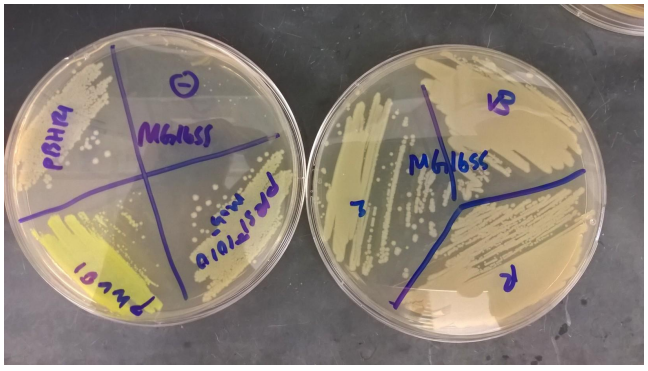
Origin Testing

1. Took out plates at 12:00 pm
2. Results:
    a. Kan plates
        I. MG1655: growth for all 3 origins tested, very little growth for untransformed bacteria
        II. C. glut: growth for all 3 origins tested; untransformed bacteria grew; colonies don't look like C. glut
            1. **Probably contaminated the growth medium when making C. glut competent cells; will have to make new competent cells**
        III. B. sub: growth for all 3 origins tested; untransformed bacteria also grew
            1. **B. sub resistant to Kan50? Maybe try making plates with higher kan concentration**
        IV. P. put: growth for only RSF1010 mob-; no other colonies
        V. S. onei: no growth for any origin; no growth for untransformed bacteria
            1. either origins don't work or plasmids weren't taken up
        VI. S. mel: no growth for any origin; no growth for untransformed bacteria
    b. Plain plates

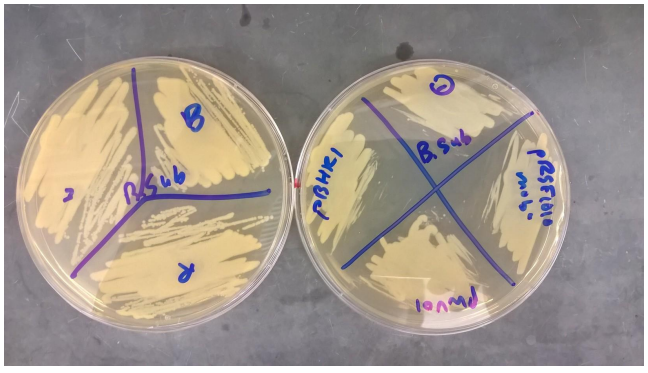
I. MG1655: growth for all 3 origins tested
II. C. glut: growth for all 3 origins tested
III. B. sub: growth for all 3 origins tested
IV. P. put: growth for all 3 origins tested
V. S. onei: growth for all 3 origins tested
VI. S. mel: no growth for any origin

3. Left S. onei and S. mel in 30C incubator
4. Picture proof:
    a. plates divided into quadrants = Kan plates
    b. plates divided into thirds = plain LB plates

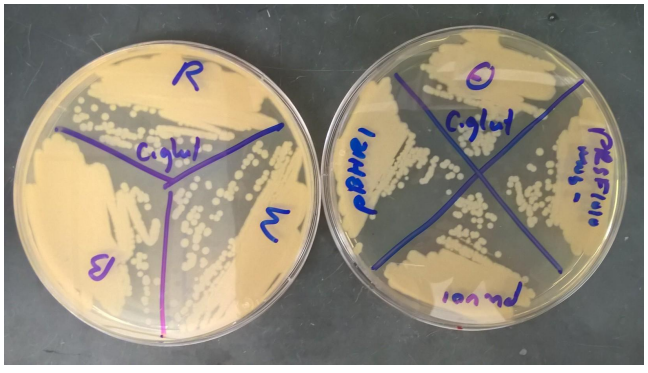
MG1655 origin testing.jpg



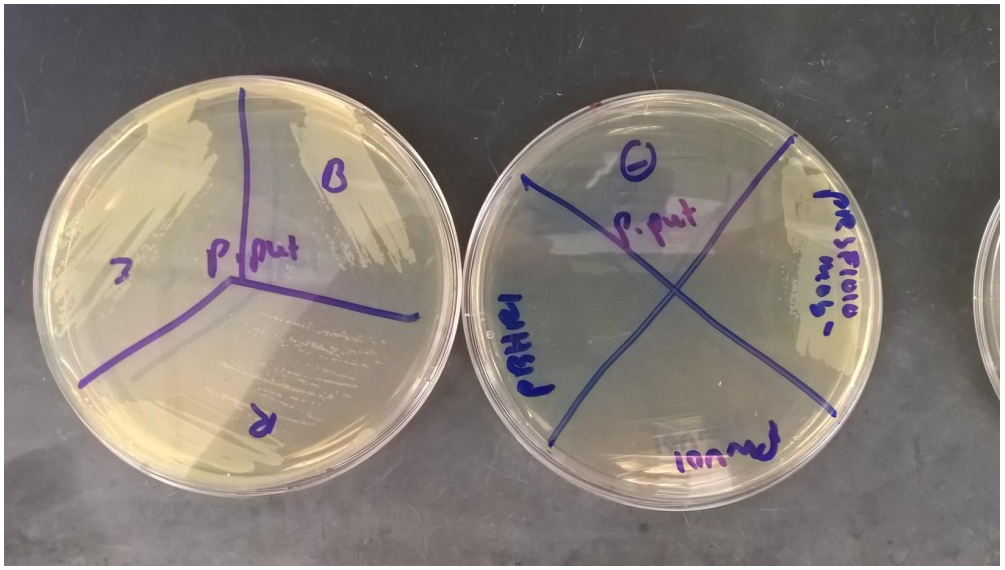
B. sub origin testing.jpg



C. glut origin testing.jpg



P. put origin testing.jpg



Week of August 12

SUNDAY, 8/12/2018

1. Took out all plates and cultures at 11:00 am

HW Cloning

1. All cultures grew.
2. All 207 and 208 cultures were deep red
3. 203, 210 and 211 cultures were not obviously red
4. Pelleted all cultures
5. Resuspended all cultures of 203, 210, and 211 in 100 uL of 1X PBS
6. measured fluorescence of 100 uL of 1/10 dilution
    a. 203 had no fluorescence over background
    b. 210 and 211 cultures had slight fluorescence over background
7. Bleached 203 cultures; repelleted 210 and 211 cultures
8. Miniprepped 207, 208, 210 and 211; eluted with 50 uL dH2O

Genome prep of P. putida and S. meliloti

1. P. putida grew + had pickable colonies
2. S. mel does not have pickable colonies
    a. put plate back in 30C incubator

Origin Testing

1. Still no colonies for S. mel on either Kan50 or plain plates
2. S. oneidensis: about 10 colonies on all quadrants of Kan 50 plate

Preparing C. glut competent cells

1. Picked a colony of C. glut into 5 mL universal medium + ascorbate
2. In 37, shaking at 3:30 pm

Genome prep of P. put and S. mel

1. Picked a colony of P. put into 5 mL of LB
    a. in 30C shaking at 3:30 om
2. No colonies grew on S. mel plate; will leave in and see if pickable colonies grow tomorrow

MONDAY, 8/13/2018

1. Took out all plates and cultures at 11:15 am

HW cloning

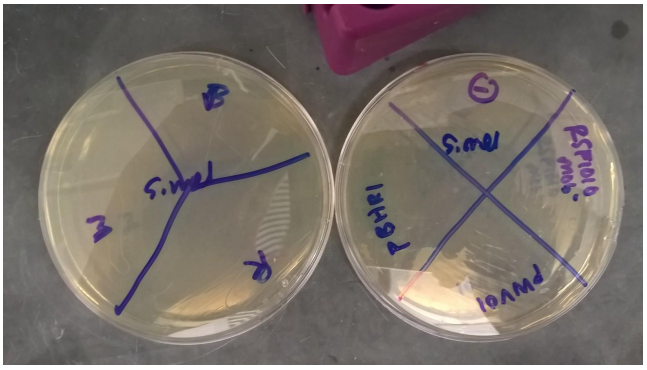
1. Nanodropped all minipreps from yesterday
    a. [piG207 1] = 21.1 ng/uL
    b. [piG207 2] = 21.9
    c. [piG208 1] = 6.5 (absorbance spectrum looked really jagged)
    d. [piG208 2] = 37.0

e. [piG210 1] = 32.5
f. [piG210 2] = 31.0
g. [piG211 1] = 39.6
h. [piG211 2] = 38.5
i. [piG221 1] = 44.7

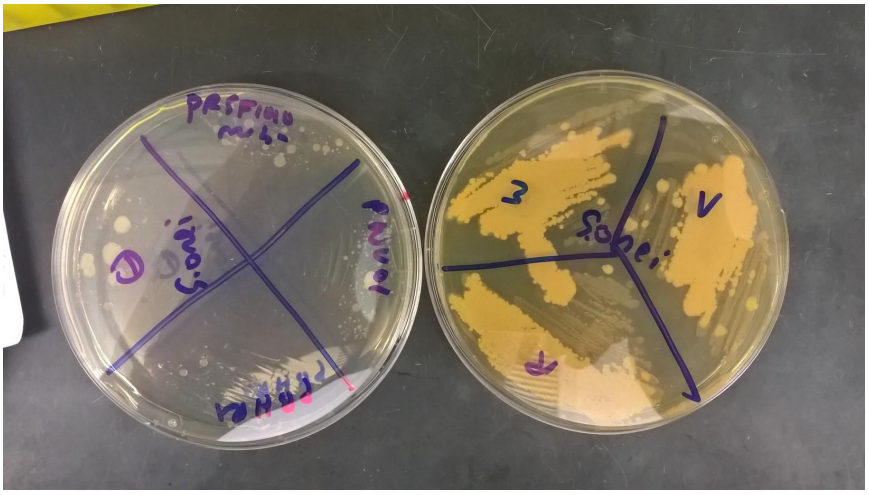
Origin Testing

1. Results for S. mel and S. onei:
    a. no colonies on S. mel plates
    b. S. oneidensis plates have growth in every condition tested, even untransformed S. mel on Kan50 plate
        I. there are some large looking colonies that don't look like S. oneidensis, though; contamination?

S. mel origin testing.jpg

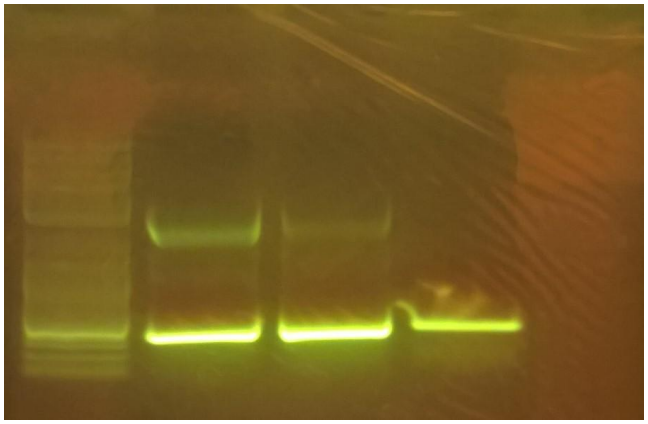


S. onei origin testing.jpg



3. Shyam gave us PCRs for origin acceptor parts for testing
    a. 3 PCRs: 1-8, D, Gc
    b. Ran on 0.8% agarose gel, 100V
    c. left to right: 2-log ladder, 1-8, D, Gc

Shyam origin acceptor PCR 20180813.jpg



d. Cut out 2.5 kB band (topmost band) of 1-8 and D; tried cutting out part of gel where Gc should have been, but dropped the gel;
e. Zymo purified the origin acceptor parts; eluted with each w/ 11 uL H2O; combined; nanodrop showed that it's contaminated with something
f. goldengate; BsaI; origin acceptor and origins

Table1

	A	B	C	D
1	**Reactant**	**Volume (uL)**	**pSPB448**	0.32
2	origin acceptor	~4 uL	**pSPB449**	0.84
3	pSPB4___	see next columns	**pSPB478**	0.16
4	BsaI	0.5	**pSPB479**	1.1
5	T4 ligase	0.5		
6	T4 ligase buffer	1.5		
7	BSA	1.5		
8	H2O	7		
9	**Total**	**~15 uL**		

Genome prep of P. putida and S. meliloti

1. Purified genome of P. putida according to Gram negative bacteria protocol; eluted with 200 uL nfH2O.
2. [P. putida genome] = 81.1; lots of contaminant; cloudy
3. S. mel does not have pickable colonies yet (too small); wait another day

TUESDAY, 8/14/2018

HW Cloning

1. No test digests yet b/c no NotI
2. Do BsaI digest (for tomorrow)

Genome prep of S. meliloti

1. Took out plate of S. mel at 10:45 am
2. Picked colony of S. mel into 2 mL of plain LB (not universal medium)
    a. in 30C incubator at 6:30 pm

Making competent C. glut

1. Added 2.5 mL of C. glut culture to 250 mL of universal medium + 0.5% glucose + ascorbate
2. Let grow in 37C shaker until high OD (overgrown, > 1.0; supposed to be 0.200)
3. Pelleted in centrifuge; 4000g, 15 min, 25C
4. Washed with 250 mL 15% glycerol
5. Pelleted in centrifuge; 4000g, 15 min, 25C
6. repeated steps 4 + 5 two more times
7. Resuspended pelleted C. glut in 1.0 mL 15% glycerol
8. Aliquotted 50 uL into 1.5 mL microcentrifuge tubes
9. Stored in -80C freezer
10. To check for contamination: chose a sacrificial aliquot to streak onto plain LB plate
    a. in 37C incubator at 6:50 pm

Making competent Shewanella

1. Streaked out S. oneidensis from glycerol stock onto plain LB plate
    a. in 30C at 6:30 pm

pT7 mKate cloning (piG226 and piG227)

1. In PCR tube, added all of the following:

Table2

	A	B	C	D	E
1	**for piG226**			**for piG227**	
2	**Reactant**	**Volume (uL)**		**Reactant**	**Volume (uL)**
3	pSPB438	25 fmol		pSPB 438	25 fmol
4	pSPB501	25 fmol		pSPB503	25 fmol
5	pSPB646	25 fmol		pSPB629	25 fmol
6	pSPB624	25 fmol		pSPB520	25 fmol
7	pSPB619	25 fmol			
8	pSPB460	25 fmol			
9	pSPB512	25 fmol			
10					
11	BsaI	0.5		BsaI	0.5
12	T4 ligase	0.5		T4 ligase	0.5
13	T4 ligase buffer	1.5		T4 ligase buffer	1.5
14	BSA	1.5		BSA	1.5
15	H2O	6		H2O	9
16	**Total**	**~15 uL**		**Total**	**~15 uL**

2. Put in thermocycler:

Table3

	A	B	C
1		**Temp**	**Time**
2		37C	10:00
3	25X	37C	1:30
4		16C	3:00
5		50C	5:00
6		80C	5:00
7		4C	hold

Origin testing

1. no electroporation cuvettes = no testing possible

WEDNESDAY, 8/15/2018

1. Took out all plates and cultures at 12:15 pm

HW Cloning

1. Cloning diagnostics: BsaI digest of pSPB223, 501, 463, 460, 512, 003, 007, 008, 010, 011

a. Ea. = 1.5 uL DNA, 1 uL cutsmart, 0.2 uL BsaI
b. 1 hr in 37C incubator

Genome prep of S. meliloti

1. prepared genome of S. mel according to kit instructions; eluted with 200 uL of nfH2O
2. [S. mel genome] = 10.2 ng/uL (why is this so low?!)

Origin Testing

1. Transformed 0.5 uL goldengate product of ori acceptor + either pSPB448, 449, 478, and 479 into MG1655
   a. electroporation; let rest in 1 mL SOB, 37C shaking
2. Placed 25 uL of ea. transformant onto both Kan50 and plain universal medium plate
3. Spread out 25 uL spot with inoculation loop
4. Plates in 37C incubator at 7:10 pm

pT7 mKate cloning

1. Transformed 0.5 uL of piG226 and piG227 goldengate product into MG1655
   a. electroporation; let rest in 1 mL SOB, 37C shaking
2. Placed 50 uL of ea. transformant onto Amp100 plate
3. Spread out with inoculation loop
4. Plates in 37C incubator at 7:10 pm

THURSDAY, 8/16/2018

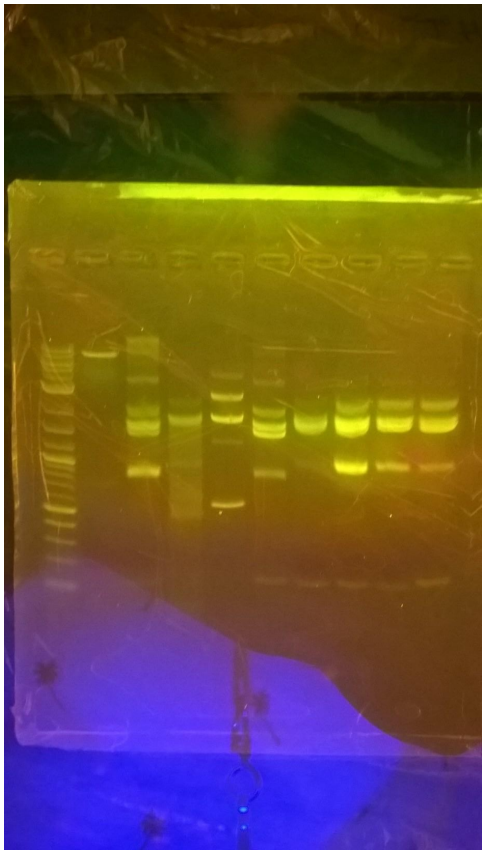
1. Took out all plates at 10:40 am

HW Cloning

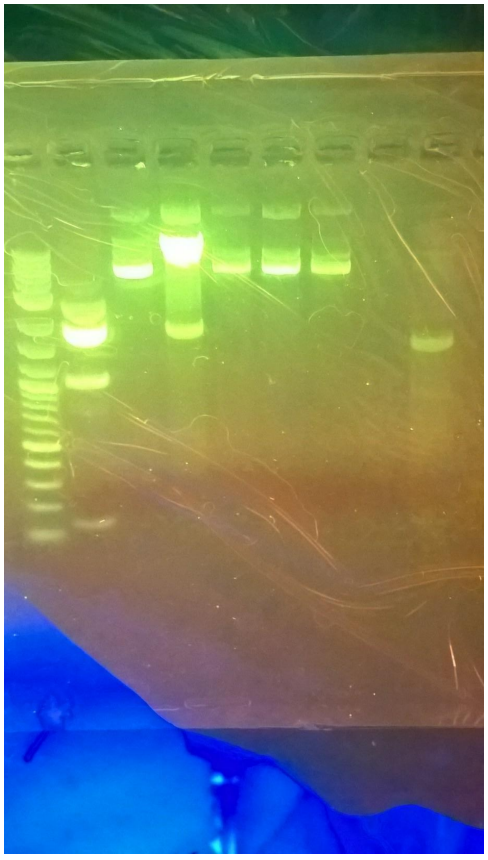
1. Ran digest from yesterday on 1% agarose gel, 100 V
2. Wells from left to right: 2-log ladder, 223, 501, 463, 460, 512, 003, 007, 008, 010, 011
   a. gel isn't very clear; redo digest
3. Test digest (may have left BsaI to work too long)
   1st gel
   2-log ladder, 223, 501, 463, 460, 512, 003, 007, 008, 010

   2-log ladder, 011, 204, 206, 207, 208, 209, 210, 211 (20X plasmids don't have BsaI sites; should have checked before digesting them)

20180816 gel 1.jpg



20180816 gel 2.jpg



Origin testing

1. Picked one colony of ea. construct (ori acceptor + pSPB448, 449, 478, 479) from yesterday into 4 mL LB + Kan
2. In 37C shaking incubator at 7:15 pm

pT7 mKate cloning

1. Plate is suspiciously dense with colonies. Is MG1655 resistant to amp?
2. Picked a colony of piG226 and piG227 into 4 mL LB + Amp
3. In 37C shaking incubator at 7:15 pm

FRIDAY, 8/17/2018

Took cultures out at 3:00 pm

Origin testing

1. Cultures of ori acceptor + pSPB448, 449, and 479 grew; 448 and 449 were red; 479 was not
2. 478 did not grow
3. Miniprepped all cultures that grew; eluted with 50 uL dH2O
    a. all concentrations around 50 ng/uL

pT7 mKate cloning

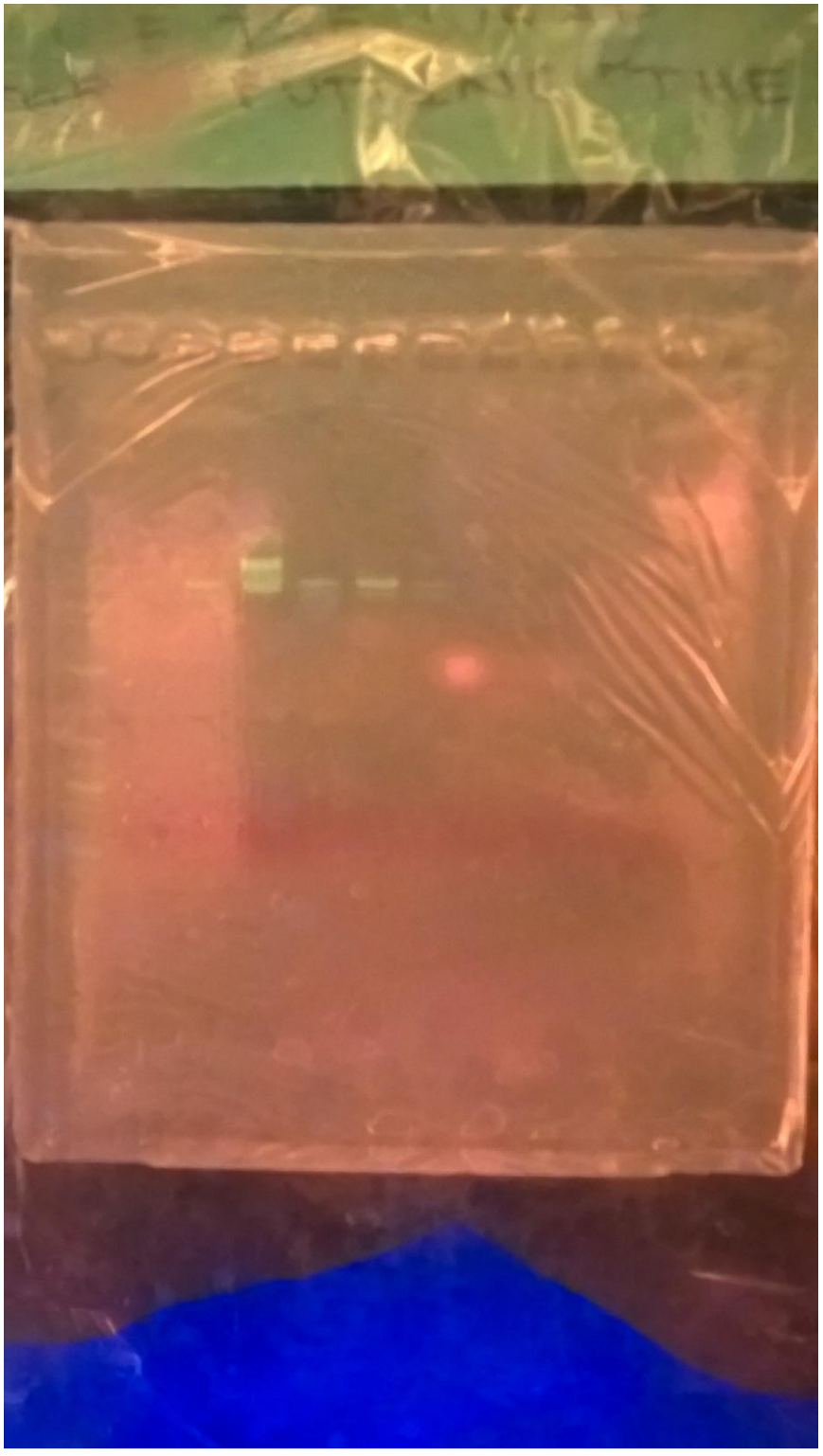
1. piG226 culture grew but piG227 did not
2. miniprepped piG226 culture; eluted w/ 50 uL dH2O

3. streaked untransformed MG1655 onto Amp100 plate to make sure it isn't ampicillin resistant and that colonies on plates contain plasmid
    a. in 37C at 5:30 pm

HW Cloning and pT7 mKate cloning

1. Test digested piG204, 206, 207, 209, 210 (1 and 2), and 211 (1 and 2) and 226 w/ NotI
2. Ran on 0.8% agarose gel; 100V
3. Results: left to right: 2-log ladder, 226, 204, 206, 207, 208, 209, 210 1, 210 2, 211 1, 211 2

20180817 gel.jpg



Week of August 20

TUESDAY, 8/21/2018

Origin testing: C. glutamicum

1. Transformed 50 ng ea. of piG221, pBHR1, and pWV01 into C. glutamicum
    a. Transformed by electroporation
    b. Let rest in 1 mL SOB, in 37C shaking incubator for 1 hr; start 4:05 pm, end 5:05 pm
2. Plate 25 uL of ea. transformant onto Kan50 and plain universal medium plate
    a. also, on kan50 plate, plated 25 uL of untransformed C. glut
3. In 37C incubator at 5:15 pm

pT7-mKate Cloning

1. Plated 100 uL of transformed piG226 and 227 (from Aug 15) onto freshly made Amp100 plates
2. in 37C incubator at 5:15 pm

Digesting HW-mKate cassettes with NotI

Table1

	A	B	C	D	E	F
1	**sample**	**concentration**	**DNA**	**NotI**	**NEB buffer 3.1**	**water**
2	piG204	112.7	1.5	1	1.5	11
3	piG206	357.7	1.5	1	1.5	11
4	piG207	90.1	1.5	1	1.5	11
5	piG208	166.6	1.5	1	1.5	11
6	piG209	115.5	1.5	1	1.5	11
7	piG202	54.7	4	1	1.5	8.5
8	piG210 (1)	32.5	4	1	1.5	8.5
9	piG210 (2)	31.0	4	1	1.5	8.5
10	piG211 (1)	39.6	4	1	1.5	8.5
11	piG211 (2)	38.5	4	1	1.5	8.5

Digesting UBER multigenes (224 and 225) with Esp3I

Table2

	A	B	C	D	E	F
1	**sample**	**concentration**	**DNA**	**Esp3I**	**CutSmart**	**water**
2	piG224	98.6	6	1	3	20
3	piG225	96.6	6	1	3	20

HW-mKate cassettes transformations

Transformed piG204, 202, 207, 208, 209, 210, 211 into MG1655. Plated the transformations at 8:30

WEDNESDAY, 8/22/2018

Origin Testing in S. mel and B. sub

pT7 mKate cloning

1. Redo Goldengate, using different terminator and vectors (438, 439, 224, 225)

Table3

	A	B	C	D	E
1	**for piG226**			**for piG227**	
2	**Reactant**	**Volume (uL)**		**Reactant**	**Volume (uL)**
3	pSPB438	25 fmol		pSPB 438	25 fmol
4	pSPB501	25 fmol		pSPB503	25 fmol
5	pSPB646	25 fmol		pSPB629	25 fmol
6	pSPB624	25 fmol		pSPB520	25 fmol
7	pSPB619	25 fmol			
8	pSPB460	25 fmol			
9	pSPB512	25 fmol			
10					
11	BsaI	0.5		BsaI	0.5
12	T4 ligase	0.5		T4 ligase	0.5
13	T4 ligase buffer	1.5		T4 ligase buffer	1.5
14	BSA	1.5		BSA	1.5
15	H2O	6		H2O	9
16	**Total**	**~15 uL**		**Total**	**~15 uL**

Table4

	A	B	C	D	E
1	**for piG226**			**for piG227**	
2	**Reactant**	**Volume (uL)**		**Reactant**	**Volume (uL)**
3	pSPB439	25 fmol		pSPB439	25 fmol
4	pSPB501	25 fmol		pSPB503	25 fmol
5	pSPB646	25 fmol		pSPB629	25 fmol
6	pSPB624	25 fmol		pSPB520	25 fmol
7	pSPB619	25 fmol			
8	pSPB460	25 fmol			
9	pSPB512	25 fmol			
10					
11	BsaI	0.5		BsaI	0.5
12	T4 ligase	0.5		T4 ligase	0.5
13	T4 ligase buffer	1.5		T4 ligase buffer	1.5
14	BSA	1.5		BSA	1.5
15	H2O	6		H2O	9
16	**Total**	**~15 uL**		**Total**	**~15 uL**

Table5

	A	B	C	D	E
1	**for piG226**			**for piG227**	
2	**Reactant**	**Volume (uL)**		**Reactant**	**Volume (uL)**
3	pSPB224	25 fmol		pSPB224	25 fmol
4	pSPB501	25 fmol		pSPB503	25 fmol
5	pSPB646	25 fmol		pSPB629	25 fmol
6	pSPB624	25 fmol		pSPB520	25 fmol
7	pSPB619	25 fmol			
8	pSPB460	25 fmol			
9	pSPB512	25 fmol			
10					
11	BsaI	0.5		BsaI	0.5
12	T4 ligase	0.5		T4 ligase	0.5
13	T4 ligase buffer	1.5		T4 ligase buffer	1.5
14	BSA	1.5		BSA	1.5
15	H2O	6		H2O	9
16	**Total**	**~15 uL**		**Total**	**~15 uL**

Table6

	A	B	C	D	E
1	**for piG226**			**for piG227**	
2	**Reactant**	**Volume (uL)**		**Reactant**	**Volume (uL)**
3	pSPB225	25 fmol		pSPB225	25 fmol
4	pSPB501	25 fmol		pSPB503	25 fmol
5	pSPB646	25 fmol		pSPB629	25 fmol
6	pSPB624	25 fmol		pSPB520	25 fmol
7	pSPB619	25 fmol			
8	pSPB460	25 fmol			
9	pSPB512	25 fmol			
10					
11	BsaI	0.5		BsaI	0.5
12	T4 ligase	0.5		T4 ligase	0.5
13	T4 ligase buffer	1.5		T4 ligase buffer	1.5
14	BSA	1.5		BSA	1.5
15	H2O	6		H2O	9
16	**Total**	**~15 uL**		**Total**	**~15 uL**

Sequencing

piG023 is the same as pSPB 639. Used Shyam's normalized sample

Table7

	A	B	C	D	E	F	G	H	I
1	**Sample #**	**Sample**	**Sample Length**	**Primer**	**[DNA] (ng/uL)**	**DNA Amount (uL)**	**Primer Amount (uL)**	**Water Amount (uL)**	**Total (uL)**
2	1	piG012	5795	B17	322.3	1.5513496742	2.5	10.9486503258	15
3	2	piG012	5795	B18	322.3	1.5513496742	2.5	10.9486503258	15
4	3	piG012	5795	E12	322.3	1.5513496742	2.5	10.9486503258	15
5	4	piG012	5795	E13	322.3	1.5513496742	2.5	10.9486503258	15
6	5	piG012	5795	E14	322.3	1.5513496742	2.5	10.9486503258	15
7	6	piG023	6198	B17	634.8	0.7876496534	2.5	11.7123503466	15
8	7	piG023	6198	B18	634.8	0.7876496534	2.5	11.7123503466	15

Table8

	A	B	C	D	E	F	G	H	I
1	Sample #	Sample	Sample Length	Primer	[DNA] (ng/uL)	DNA Amount (uL)	Primer Amount (uL)	Water Amount (uL)	Total (uL)
2	1	210 (1)	7714	B26	32.5	12.5	2.5	0	15
3	2	210 (2)	7714	B26	31.0	12.5	2.5	0	15
4	3	211 (1)	7714	B26	39.6	12.5	2.5	0	15
5	4	211 (2)	7714	B26	38.5	12.5	2.5	0	15

Origin Testing: S. Mel

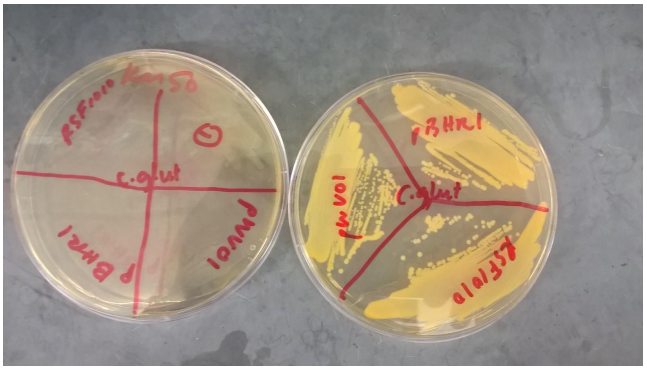
1. Electroporated 50 ng of DNA (RSF1010, pWV01, pBHR1 origins) into S. meliloti (2.1 kV/cm)
2. let rest in 1 mL LB for 3 hours, 30C shaking
3. Plated 25 uL on both Kan50 and plain LB plates
    a. In 30C incubator at 11:00 pm

THURSDAY, 8/23/2018

Origin Testing: C. glut results

1. After 2 days growth in 37C incubator: no growth on kan plate for any origin and neg. control; growth on plain plate
2. Cloudiness in Kan plate quadrants containing neg. control and pWV01

C. glut origin testing 2.jpg



pT7 mKate cloning

1. Transformed 0.5 uL of uncleaned goldengate reaction of each variant of pT7 mKate and spacer cassettes into Rosetta (piG226 using 438, 439, 224, and 225 as backbone; piG227 using 438, 439, 224, and 225 as backbone)
    a. Electroporated; let rest for 1 hr in SOB in 37C shaking incubator
2. Plated 25 uL of ea. transformant on Amp100 plates; in 37C incubator at 4:15 pm

FRIDAY, 8/24/2018

pT7 mKate Cloning

1. Took out all plates at 8:40 am
2. Only piG226 and 227 w/ mKate 438 vector grew
3. Plated more culture for the rest (piG226 and piG227 w/ 439, 224, 225 vectors)
    a.

HW-mKate origin acceptor + origins (478,479)

- Albert has cloned RSF1010-mob+ with origin acceptor
- pBBR1-mob+ and pBBR1-mob- did not work on the first try

Table9

	A	B	C	D	E
1	**for piG226**			**for piG227**	
2	**Reactant**	**Volume (uL)**		**Reactant**	**Volume (uL)**
3	HW-mKate origin acceptor	2.4		HW-mKate origin acceptor	2.4
4	478	0.5		479	0.5
5					
6					
7	BsaI	0.5		BsaI	0.5
8	T4 ligase	0.5		T4 ligase	0.5
9	T4 ligase buffer	1.5		T4 ligase buffer	1.5
10	BSA	1.5		BSA	1.5
11	H2O	8.1		H2O	8.1
12	**Total**	**15**		**Total**	**15**

amounts of DNA are for 25 fmol

PT7 mKate cloning with pre-digested 224, 225

Table10

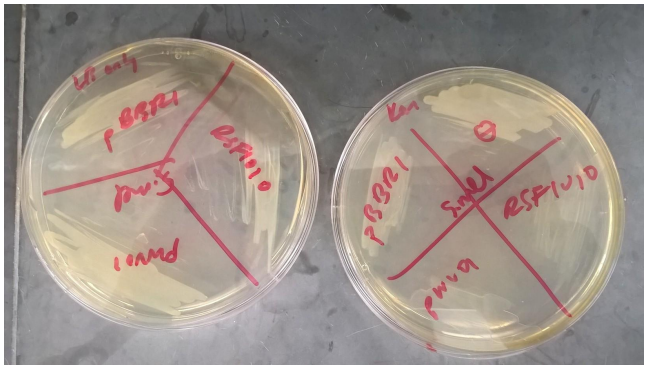
	A	B	C	D	E
1	**for piG237**			**for piG238**	
2	**Reactant**	**Volume (uL)**		**Reactant**	**Volume (uL)**
3	pSPB224	0.5		pSPB225	0.5
4	pSPB501	0.5		pSPB501	0.5
5	pSPB646	0.5		pSPB646	0.5
6	pSPB624	0.5		pSPB624	0.5
7	pSPB619	0.5		pSPB619	0.5
8	pSPB460	0.5		pSPB460	0.5
9	pSPB512	0.5		pSPB512	0.5
10					
11	BsaI	0.5		BsaI	0.5
12	T4 ligase	0.5		T4 ligase	0.5
13	T4 ligase buffer	1.5		T4 ligase buffer	1.5
14	BSA	1.5		BSA	1.5
15	H2O	11.5		H2O	11.5
16	**Total**	**15**		**Total**	**15**

Volumes of DNA are for 25 fmol (except 224 and 225 due to very low concentration)

Origin Testing: S. meliloti Results

1. S. mel is resistant to Kan (whoops)

S. mel origin testing 2.jpg



1. On Kan50 plate, neg. control grew; cells transformed with pBBR1 grew more than pWVO1 and RSF1010
    a. Not sure if this is because that culture contained more cells or if resistance marker/origin is working (but plain plate looks like all relatively similar concentrations)

HW elements characterization

made liquid cultures for the HW-mKate cassettes in 96-well plate in 1 mL of LB and Kan50.

SATURDAY, 8/25/2018

PT7 mKate cloning with pre-digested 224, 225

Both 237 and 238 had colonies. Picked 2 colonies per plate.
(Later I noticed that some of the colonies on the plates were green (???))

HW-mKate origin acceptor + origins (478,479)

Very few colonies grew for 479 and one of them was red. No colonies observed for 478, so plated the rest of recovery culture.

HW elements characterization

Measured fluorescence of the cultures (100 uL per samples in 96-well plate) using TECAN

Week of August 27

SUNDAY, 8/26/2018

People present: Soohyun, Anna, Stefanie
Time: 9:15AM-
Location: Keck 201
Goals: Make these plasmids today: 230, 231, 235, 236, 239,

Miniprep

Miniprepped:

Used 30 uL EB warmed to 60 deg

NotI digest

In ABL, combined:

- 1 uL NotI
- 2.5 uL 3.1 NEB buffer
- 250 ng DNA (amounts in column C below)

Table1

	A	B	C	D
1	**DNA**	**Concentration**	**Volume**	**Total volume**
2	226 a	467.3	0.5349882303	20.9650117697
3	226 b	490.1	0.5100999796	20.98990002...
4	238 a	159.4	1.5683814304	19.9316185696
5	237 a	66.6	3.7537537538	17.7462462462
6	213	93.4	2.6766595289	18.8233404711

Carried back to ABL on ice to digest at 37 deg in Thermocycler for 1.5 hours

Benchling

- Took piG230 and 235, selected 16S rRNA sequence and replaced with piG017 spacer. (Also, removed promoter and terminator from 16S)
- Saved in the cassette folder as 240 and 239 respectively

Cloning orthogonal translation constructs

1. Assembled components below in PCR tubes and placed in thermocycler in Keck 201

GGA Component Volumes-1

	A	B
1		**piG230**
2	**Component**	**Volume (uL)**
3	diWater	8
4	BsaI	0.5
5	T4 DNA Ligase	0.5
6	10x T4 Ligase Buffer	1.5
7	10x BSA	1.5
8	pSPB438	0.5
9	pSPB503 ConL2	0.5
10	pSPB613	0.5
11	piG015	0.5
12	pSPB463	0.5
13	pSPB513	0.5

GGA Component Volumes-2

	A	B
1		**piG231**
2	**Component**	**Volume (uL)**
3	diWater	8
4	BsaI	0.5
5	T4 DNA Ligase	0.5
6	10x T4 Ligase Buffer	1.5
7	10x BSA	1.5
8	pSPB438	0.5
9	pSPB501	0.5
10	pSPB476	0.5
11	piG025	0.5
12	piG019	0.5
13	pSPB460	0.5
14	pSPB462	0.5
15	pSPB512	0.5

GGA Component Volumes-3

	A	B
1		**piG235**
2	**Component**	**Volume (uL)**
3	diWater	8
4	BsaI	0.5
5	T4 DNA Ligase	0.5
6	10x T4 Ligase Buffer	1.5
7	10x BSA	1.5
8	pSPB438	0.5
9	pSPB503	0.5
10	piG022	0.5
11	piG015	0.5
12	piG013	0.5
13	pSPB513	0.5

GGA Component Volumes-4

	A	B
1		**piG236**
2	**Component**	**Volume (uL)**
3	diWater	8
4	BsaI	0.5
5	T4 DNA Ligase	0.5
6	10x T4 Ligase Buffer	1.5
7	10x BSA	1.5
8	pSPB438	0.5
9	pSPB501	0.5
10	piG022	0.5
11	piG025	0.5
12	piG019	0.5
13	pSPB460	0.5
14	piG021	0.5
15	pSPB512	0.5

GGA Component Volumes-5

	A	B
1		**piG239**
2	**Component**	**Volume (uL)**
3	diWater	9
4	BsaI	0.5
5	T4 DNA Ligase	0.5
6	10x T4 Ligase Buffer	1.5
7	10x BSA	1.5
8	pSPB438	0.5
9	pSPB503	0.5
10	piG017	0.5
11	pSPB513	0.5

MONDAY, 8/27/2018

Cloning pT7-mKate Cassette

1. Sequenced piG226 a and b

Table2

	A	B	C
1	Reagent	Volume (uL) BB01	BB02
2	DNA	1	1
3	Primer (B17)	2.5	2.5
4	dH2O	11.5	11.5
5	Total	15	15

Making Competent S. oneidensis

1. Picked 1 colony of S. oneidensis from plate from 8/14/18 into 4 mL LB
    a. in 30C shaking incubator at 4:30 pm
    b. out at 9:00 pm
    c. centrifuged to pellet (4000xg, 5 min)
    d. discarded supernatant
    e. resuspend w/ 6 mL 1 M D-sorbitol
    f. centrifuged to pellet (14,000 rpm, 1 min in microcentrifuge)
    g. discarded supernatant

h. repeat e.-g. 3 more times (4 total washes)
i. resuspend in 100 uL D-sorbitol

224, 225 Digest with Esp3I, 213 (1,2,3) digest with NotI

Repeat the digest of 224 and 225 and try to get higher yields
Repeat digest of 213 to check whether any of the colonies are red

Cloning orthogonal translation constructs

Huge lawns of green colonies were observed, which indicates that digestion did not work or something of the sort. Will repeat the assemblies tomorrow.

Origin Testing S. onei and B. sub

1. Transformed S. mel and B. sub w/ 200 ng of origin plasmids (RSF1010, pBHR1, pWV01)
    a. S. mel = electroporation; 1.8 kV; let rest in 1 mL LB for 1 hr in 30C shaking incubator
    b. B. sub = heatshock; thawed aliquots w/ 37C heatblock, added 50 uL SpII + DNA to aliquot; let rest in 37C shaking incubator for 40 min.
    c. Plated 25 uL of each transformant onto Kan plates + plain LB plates; spread by streaking
        I. used Kan50 plate for S. mel
        II. used Kan100 plate for B. sub
    d. In appropriate incubator at 11:00 pm

WEDNESDAY, 8/29/2018

Cloning orthogonal translation constructs

Repeated the assemblies, following the same protocol as on Sunday

Cloning UBER + RSF1010 + mKate

a) Pre-digested UBER + RSF1010 mutigene vectors (224, 225) + mKate gBlock

Table3

	A	B	C	D	E
1		**piG239**			**piG239**
2	**Component**	**Volume (uL)**		**Component**	**Volume (uL)**
3	diWater	5.48		diWater	5.48
4	BsaI	0.5		BsaI	0.5
5	T4 DNA Ligase	0.5		T4 DNA Ligase	0.5
6	10x T4 Ligase Buffer	1.5		10x T4 Ligase Buffer	1.5
7	10x BSA	1.5		10x BSA	1.5
8	mKate gBlock	0.52		mKate gBlock	0.52
9	224	5		225	5

b) Pre-digested 224, 225 + all components (as previously)

Table10

	A	B	C	D	E	F	G
1	**for piG237**			**for piG238**			
2	**Reactant**	**Volume (uL)**		**Reactant**	**Volume (uL)**		**MM**
3	pSPB224	0.5		pSPB225	0.5		1.65
4	pSPB501	0.5		pSPB501	0.5		1.65
5	pSPB646 (piG014)	0.5		pSPB646	0.5		1.65
6	pSPB624 (025)	0.5		pSPB624	0.5		1.65
7	pSPB619 (020)	0.5		pSPB619	0.5		1.65
8	pSPB460	0.5		pSPB460	0.5		1.65
9	pSPB512	0.5		pSPB512	0.5		1.65
10							
11	BsaI	0.5		BsaI	0.5		2.15
12	T4 ligase	0.5		T4 ligase	0.5		2.15
13	T4 ligase buffer	1.5		T4 ligase buffer	1.5		6.45
14	BSA	1.5		BSA	1.5		6.45
15	H2O	7.5		H2O	7.5		32.25
16	**Total**	**15**		**Total**	**15**		

c) PT7-mKate cassette and spacer cassette

Table4

	A	B	C	D	E
1	**for piG226**			**for piG227**	
2	**Reactant**	**Volume (uL)**		**Reactant**	**Volume (uL)**
3	pSPB438	0.5		pSPB 438	0.5
4	pSPB501	0.5		pSPB503	0.5
5	pSPB646	0.5		pSPB629	0.5
6	pSPB624	0.5		pSPB520	0.5
7	pSPB619	0.5			
8	pSPB460	0.5			
9	pSPB512	0.5			
10					
11	BsaI	0.5		BsaI	0.5
12	T4 ligase	0.5		T4 ligase	0.5
13	T4 ligase buffer	1.5		T4 ligase buffer	1.5
14	BSA	1.5		BSA	1.5
15	H2O	7.5		H2O	9
16	**Total**	**~15 uL**		**Total**	**~15 uL**
17					

Origin Testing S. onei and B. sub

1. looked at plates at 8:30 am
2. No colonies on Kan100 B. subtilis plate
3. colonies on Kan50 S. onei for RSF1010 transformant, none for the rest
4. All transformants on plain LB plate had lots of growth
5. Left in incubator to see if anything else grows

Making unmethylated DNA for origin tests w/ C. glut and S. mel

1. Got EC aliquots of methylase-lacking E. coli from Prashant
    a. ATCC BAA 525
    b. NEB dam- bcl-
2. Transformed 50 ng of plasmids w/ RSF1010, pBHR1 and pWV01 origins into ea. strain (one plasmid origin per aliquot for a total of 3 aliquots used for each strain)
    a. Electroporated; let rest in 1 mL SOB, in 37C shaking incubator for 1 hr
    b. Plated 100 uL of ea. transformant onto Kan50 plate; spread out by streaking w/ inoculation loop
    c. plates in 37C incubator at 6:30 pm

Making electrocompetent ATCC BAA 525 and NEB dam- bcl-

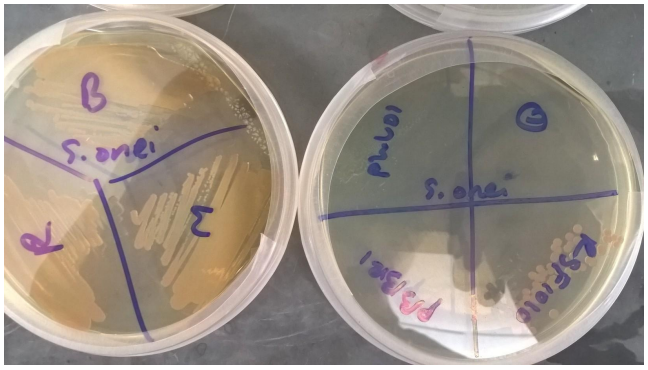
1. Streaked aliquot of ATCC BAA 525 and NEB dam- bcl- onto plain LB plate
2. In 37C at 6:30 pm

THURSDAY, 8/30/2018

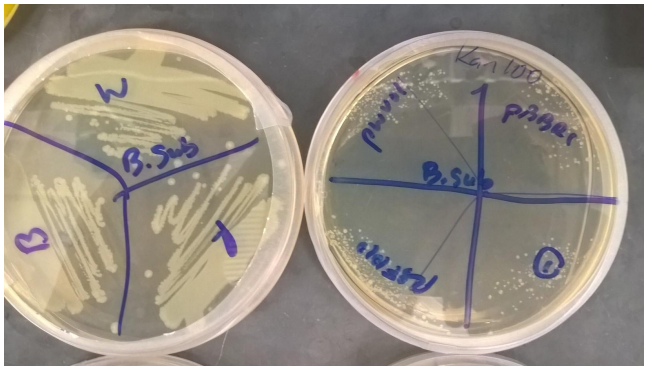
Origin Testing S. onei and B. sub Results

1. Took out plates at 1:00 pm
2. For S. onei Kan plate:
    a. Big colonies on RSF1010 part of plate
    b. small colonies on pWV01 part of plate; green fluorescence under blue light
    c. no colonies on pBHR1 part of plate
    d. no colonies for neg. control
3. For B. sub Kan plate
    a. equal number of colonies on all quadrants of plate (RSF1010, pBHR1, pWV01)
4. Large amount of growth for all transformants on plain plates

Origin testing S. onei 2.jpg



Origin testing B. sub 2.jpg



Making unmethylated DNA for origin tests w/ C. glut and S. mel

1. Took out plates at 9:30 am
2. Picked colony from ea. plate into 4 mL LB + 50 ug/mL Kan
3. In 37C shaking incubator at

Making electrocompetent ATCC BAA 525 and NEB dam- bcl-

1. Took out plates at 9:30 am
2. PIcked colony from ea. plate into 5 mL plain LB
3. In 37C shaking incubator at 6:00 pm

Oligo Anneal for oRBSs (universal, OR1, OR4)

anneal the two by combining the oligos at 1 µM in water, 1 µL of each in 100 µL
Run the rxn on the annealing program: 98° and cool to 25° at the minimum ramp rate, 0.1°/s.

make a 10–15 µL rxn with 1× T4 ligase buffer, 0.5 µL T4 PNK (polynucleotide kinase), and a tenth volume of the oligo anneal (final 100 nM). Incubate 30 min 37°. Use 0.5 µL in the Golden Gate (50 fmol).

16S rRNA PCR

Table5

	A	B	C	D	E	F	G	H
1		Phusion HF buffer	dNTPs	Fwd Primer	Rev Primer	Phusion DNA polymerase	template	H2O
2	PCR components	10	1	2.5	2.5	0.5	0.5	33

Primers:
Short

- universal: 76-52; 57 C
- El1: 77-52; 57C
- OR1: 73-52; 57C

Long

- universal: 50-75, 68C
- El1: 50-78, 68C
- OR1: 50-74, 68C

FRIDAY, 8/31/2018

Making EC ATCC BAA 525 and NEB dam- bcl-

1. Inoculated 2 flasks of 500 mL plain LB with 5 mL culture of BAA 525 or dam- bcl-
2. Grew until turbid, about 4 hrs (overgrown)
3. Pelleted by centrifugation; 4000xg, 10 min, 25C
4. Discarded supernatant
5. Resuspended pellets of ea. strain in 500 mL 10% glycerol
6. Pelleted by centrifugation; 4000xg, 10 min, 25C, discarded supernatant
7. Resuspended pellets of ea. strain in 250 mL 10% glycerol
8. see step 6.
9. resuspended in 100 mL 10% glycerol
10. see step 6
11. resuspended in 10 mL 10% glycerol
12. see step 6
13. final resuspension in 1 mL 10% glycerol
14. flash froze in liquid nitrogen

15. store in -80C

SUNDAY, 9/2/2018

People present: Katherine, Anna, Soohyun
Goals: Biobrick assembly

Biobrick Assembly Procedure

Backbone:
5 uL CutSmart
0.5 ul EcoRI-HF
0.5 ul PstI
linearized plasmid backbone for 500ng- 25 ng/uL so use 20 uL
H20 to 50 uL (24uL of NF water)
Digest 37C/1 hour, heat kill 80C/20 min

Parts: (Doing three parts to make sure I'm doing it right, digesting only 002, 004, 006)
Digest part plasmid with EcoRI and PstI:
5 uL CutSmart
0.5 uL EcoRI-HF
0.5 ul PstI
DNA for 1000 ng
- 002- 114 ng/uL so add 8.77 uL and add 35.23 uL H2O
- 004- 112.6 ng/uL so add 8.88 uL and add 35.12 uL H2O
- 006- 153.7 ng/uL so add 6.51 uL and add 37.49 uL H2O

Digest 37C/1 hour, heat kill 80C/20 min

Running in thermocycler in Keck at 37* for 1 hour
Made more gel (300 l TAE 3 g pure agarose powder)
Running on a gel, added