MIT Team Cloning Lab Notebook

Wednesday 6/12/19

Protocol

- Transformation:
    1. Obtain bacteria cells on ice - thaw from -80 C (thaw 2-4 min)
    2. Obtain DNA plasmids
        a. L0s = SPEC
        b. L1s = CARB
    3. Label tubes of cells
        a. One tube per plasmid + PUC19 control
    4. Add 5 uL of bacteria into tube
    5. Add 1 uL of DNA into tube
    6. Incubate on ice for 30 minutes
    7. Heat shock at 42 C for 30s
    8. Ice for 2 minutes
    9. 750 microliters of SOC media to tube
    10. Grow w/ shaking at 280 rpm, 37 C for 30 min
    11. Take appropriate antibiotic plate and pre-warm to room temp - label plates
    12. Add 10 uL of cells to center of plate
    13. Add beads and shake
    14. Dump beads + put plate in 37 C

Lab

Transformed Plasmids

- pIG_001-pIG_003 and puc19 as control (Margaret)
- pIG_004-pIG_006 and puc19 as control (Ye Cheng)
- pIG_007-pIG_009 and puc19 as control (Miles)
- pIG_010-pIG_012 and puc19 as control (Malik)
- pIG_013-pIG_015 and puc19 as control (Vanessa)
- pIG_016-pIG_017 and puc19 as control (Maisha)
- pIG_018-pIG_019 and puc19 as control (Lab)
- pIG_020-pIG_022 and puc19 as control (Gabi)

Thursday 6/13/19

Protocol

- Inoculation
    1. Obtain your plates.

2. Count colonies on each of plates
3. Obtain 14mL falcon tubes and label each tube with date, your initials, the plasmid name, and the instance.
4. Add 4mL of LB media into each 14mL tube from 50mL aliquots (blue conicals). Once you open a blue conical, write your group number, initials, and date on the top.
5. Each antibiotic solution is in 1000x concentration such that you'll need to add 4uL of the correct antibiotic to the appropriate 4mL liquid culture tubes. Make sure you pipette the antibiotic solution into the liquid and NOT the side of the tube. Also make sure you do not lower the tip too far below the surface of liquid to avoid contaminating the pipet.
6. For each tube + plate:
7. Using a p-200 pipette, obtain a clean new tip, open the petri dish and slowly scrape the bacterial colony onto the edge of your tip.
8. Take the tip and lower it into the 4mL culture being careful not to submerge the entire tip.
9. Gather tubes and ask TA for directions to the 37C shaking incubator to leave overnight to grow.

Lab

Inoculated plates

- Maya and Krissy helped with protocol
- Ye Cheng
    - pUC_19: 8
    - pIG_004: 712
    - pIG_005: 456
    - pIG_006: 244
- Malik
    - pUC-19: 27
    - pIG_010: 10
    - pIG_011: 616
    - pIG_012: 184
- Miles
    - pUC_19: 57
    - pIG_007: 810
    - pIG_008: 664
    - pIG_009: 792
- Vanessa
    - pUC_19: 0
    - pIG_013: 9
    - pIG_014: 14
    - pIG_015: 45
- Maisha
    - pIG_016 : 104

    - pIG_017: 4
    - pUC_19: 30
- Margaret
    - pIG_001: 1680
    - pIG_002: 1400
    - pIG_003: 440
    - pUC_19: 70
- Gabi
    - pUC_19: 4
    - pIG_020: 600
    - pIG_021: 800
    - pIG_022: 200

Friday 6/14/19

MiniPrep Protocol

- **QIAprep Spin Miniprep Protocol (Krissy wrote it)**
    - Notes before starting
        - Optional: Add LyseBlue reagent to Buffer P1 at a ratio of 1 to 1000.
        - Add the provided RNase A solution to Buffer P1, mix and store at 2–8°C.
        - Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
    - All centrifugation steps are carried out at 13,000 rpm (~17,900 x g) in a conventional
    - table-top microcentrifuge.

    1. Pellet 1–5 ml bacterial overnight culture by centrifugation at >8000 rpm (6800 x g) for 3 min at room temperature (15–25°C).
    2. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.

    3. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than 5 min. If using LyseBlue reagent, the solution will turn blue.
    4. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times. If using LyseBlue reagent, the solution will turn colorless.
    5. Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.
    6. Apply 800 µl supernatant from step 5 to the QIAprep 2.0 spin column by pipetting. For centrifuge processing, follow the instructions marked with a triangle (). For vacuum manifold processing, follow the instructions marked with a circle (). Centrifuge for 30–60 s and discard the flow-through, or apply vacuum to the

manifold to draw the solution through the QIAprep 2.0 spin column and switch off the vacuum source.

7. Recommended: Wash the QIAprep 2.0 spin column by adding 0.5 ml Buffer PB. Centrifuge for 30–60 s and discard the flow-through, or apply vacuum to the manifold to draw the solution through the QIAprep 2.0 spin column and switch off the vacuum source. Note: This step is only required when using endA+ strains or other bacteria strains with high nuclease activity or carbohydrate content.
8. Wash the QIAprep 2.0 spin column by adding 0.75 ml Buffer PE. Centrifuge for 30–60 s and discard the flow-through, or apply vacuum to the manifold to draw the solution through the QIAprep 2.0 spin column and switch off the vacuum source. Transfer the QIAprep 2.0 spin column to the collection tube.
9. Centrifuge for 1 min to remove residual wash buffer.
10. Place the QIAprep 2.0 column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM TrisCl, pH 8.5) or water to the center of the QIAprep 2.0 spin column, let stand for 1 min, and centrifuge for 1 min.
11. If the extracted DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5
    volumes of purified DNA. Mix the solution by pipetting up and down before loading the
    Gel.

Glycerol Stock

Introduction

While miniprepping, remaining miniprep culture should be have been kept in the 4 degree C fridge. After sequencing and confirmation, the miniprep culture of the cells you've chosen to midiprep should be made into a glycerol stock and put into the -80 iGEM glycerol box.

Materials

- Cryo Tubes (orange caps, in bottom drawer)
- 50% Glycerol solution (on shelves above bench) 500uL (for each miniprep culture)
- Miniprep cultures 500uL
- Label Printer and other components
- P1000 and tips

Procedure

Initial Set Up

1. First make sure that the tape printer located on top of the computer tower has enough tape. If out of tape, contact Brian for more.
2. Take out your miniprep cultures and aspirate the ones that you will not be using for midiprep
3. Check that you have enough glycerol. If out, contact Brian.
4. Take out the number of cryo tubes equal to the amount of minipreps cultures you'll be using for midiprep. If out, check the Weiss Lab cabinets located in hall.

Labels

1. Labels should be made first before making the glycerol stock so you don't confuse tubes.

2. The label making program, P-touch Editor 5.0, can be found on the desktop of the computer
3. If the iGEM label template is not open, click File--->Open---->iGEM 2016 Template Label
4. Follow template of label. Fill in date, your initials, plasmid name, cell type, and add 'iGEM 2016'
5. Once finished, print the labels using the printer named Brother PT-2430PC
6. The labels are peel stickers. Stick labels onto cryo tubes

Making Glycerol Stock

1. Pipette 500uL glycerol into each cryo tube.
2. Pipette 5

Lab

Miniprep plasmids

- (Maya and Krissy helped with protocol)

Completed miniprep

- pIG_001-pIG_003 with replicates of 3 (Margaret)
    - Note: pIG_003 replicate 3 was dropped in step 6
- pIG_020-pIG_022 with replicates of 3 (Gabi)
    - Note: Half of pIG_020 replicate 1 was poured out in step 2
- pIG_013-pIG_015 with replicate of 3 (Vanessa)

Began miniprep

- pIG_004-pIG_006 (Ye Cheng)
- pIG_007-pIG_009 (Miles)
- pIG_010-pIG_012 (Malik)

Made Glycerol Stocks

- pIG_001-pIG_022 (Krissy and Maya)

Monday 6/17/19

Protocol

- **Blanking Nanodrop and Measuring Samples**
    1. If it's not running, start the Nanodrop 2000 software. Select "Nucleic Acids."
    2. Ensure that the Type drop-down box on the right-hand side reads DNA.
    3. Ensure that the Use cuvette box on the left-hand side is off.
    4. Raise the Nanodrop arm.
    5. Squirt a Kimwipe with a little water and gently wipe off both the measurement surfaces (the pedestal and the light aperture.)
    6. Use a dry Kimwipe to gently wipe off both measurement surfaces.
    7. Pipette 1.5 ul of Buffer EB onto the pedestal.
    8. Gently lower the Nanodrop arm.
    9. Click the Blank button. Wait a few seconds for the instrument to blank.
    10. Gently wipe off both measurement surfaces.
    11. Pipette 1.5 ul of your sample onto the pedestal.

12. Lower the Nanodrop arm.
13. Click the Measure button.
14. Record the concentration on the side of the tube and in the plasmid's notebook page.
15. Gently wipe off both measurement surfaces.
16. You do not need to use water to clean the surfaces between measurements; the measurement surfaces are hydrophobic and there is very little sample carryover.
17. Repeat steps 11-15 for each sample.

Lab

Completed miniprep from Friday

- pIG_003-pIG_0012 with replicates of 3 (Kristina, Malik, Miles, Ye Cheng, Ethan)
    - Note: pIG_005 replicate 3 was dropped in step 4

Completed nano dropping: Everyone

- pIG-001-pIG_022 with three replicates

Tuesday 6/18/19

Protocol:

pL1 MoClo Golden Gate protocol

	For each rxn	Master Mix amount
Backbone pL1 (ex. ST1-2)	0.5 ul	6ul
T4 ligase	0.5 ul	6ul
10x T4 buffer	2 ul	24ul
BsaI-HFv2 enzyme	1 ul	12ul
10x BSA buffer (NEB B9001)	2 ul	24ul
DEPC H20	6 ul	72ul
pIG_001, pIG_005, pIG_012, pIG_013 (Inerts)	4 ul	24 ul
TOTAL	16 ul	120ul

Note: We can also make a master mix for multiple constructs (i.e. everything that's used*(however many constructs) with a little extra!!)

6/18 note: we added the Master Mix we made first (16ul) and then added the DNA amounts

4. Thermal cycler (use one on my bench): ~2.5 hours for 10x cycles

iGEM folder --> "golden gate (BsaI, BsmbI)"

Heat lid	110 C	
Temp	37 C	20 min
Start cycle	10x	
	37 C	2 min
	16 C	5 min
Close cycle		
Temp	37 C	15 min
Temp	50 C	5 min
Temp	80 C	20 min
Store	8 C	

5. Transform bacteria as before and grow colonies on agar plates (Carb resistance), grow at 37C overnight

6. Miniprep

7. Test digest and run gel, send good clones to sequencing
NOW WE CAN USE THE PLASMID!!! :)

Lab
Aliquoted DNA and sent to sequencing
- pIG_001 - pIG_022 (Margaret, Maisha, Gabi, Vanessa)

Made DNA working stocks for Golden Gate Cloning
- pIG_001 - pIG_022 (Krissy, Ethan, Margaret, Vanessa, Melody, Maya)

Put golden gates in thermocycler for mMoClo
- pIG_030 - pIG_034 (Krissy, Ethan, Margaret, Vanessa, Melody, Maya)

Wednesday 6/19/19

Protocol:
pL1 MoClo Golden Gate protocol

Note: all buffers, enzymes, backbones and DNA parts stored at -20C

1. Identify your necessary parts and plan the construction

Example: Inert_hEF1a_Inert_EYFP_Inert_SynthPA

Plasmid L1 ID	I	P	5	G	3	T
pIG_030	pIG_0001 Inert 1 ul	pIG_002 hEF1a 1 ul	pIG_005 Inert 1 ul	**pIG_009** **iRFP720** **1 ul**	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_031	pIG_0001 Inert 1 ul	pIG_002 hEF1a 1 ul	pIG_005 Inert 1 ul	**pIG_022** **mKO2mod** **1ul**	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_032	pIG_0001 Inert 1 ul	pIG_002 hEF1a 1 ul	pIG_005 Inert 1 ul	**pIG_011** **Tag-TetR** **1ul**	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_033	pIG_0001 Inert 1 ul	pIG_002 hEF1a 1 ul	pIG_005 Inert 1 ul	**pIG_008** **TagBFP** **1ul**	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_034	pIG_0001 Inert 1 ul	pIG_002 hEF1a 1 ul	pIG_005 Inert 1 ul	**pIG_010** **TetR-VP64** **1ul**	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul

2. Mix the DNA parts (1ul each of 40 fmol/ul stocks) -- should be a total of 6 ul

3. Add the following reagents (should all be kept on ice/ice block)

	For each rxn	Master Mix amount
Backbone pL1 (ex. ST1-2)	0.5 ul	3ul
T4 ligase	0.5 ul	3ul
10x T4 buffer	2 ul	12ul
BsaI-HFv2 enzyme	1 ul	6ul
10x BSA buffer (NEB B9001)	2 ul	12ul
DEPC H20	6 ul	36ul
TOTAL (including DNA above)	20 ul	120ul

Note: Can also make a master mix for multiple constructs

6/18 note: we added the Master Mix we made first (16ul) and then added the DNA amounts

4. Thermal cycler (use one on my bench): ~2.5 hours for 10x cycles

iGEM folder --> "golden gate (BsaI, BsmbI)"

Heat lid	110 C	
Temp	37 C	20 min
Start cycle	10x	
	37 C	2 min
	16 C	5 min
Close cycle		
Temp	37 C	15 min
Temp	50 C	5 min
Temp	80 C	20 min
Store	8 C	

5. Transform bacteria as before and grow colonies on agar plates (Carb resistance), grow at 37C overnight

6. Miniprep

7. Test digest and run gel, send good clones to sequencing

Lab
Put golden gates in thermocycler for mMoClo

pIG_023-pIG_029 (Krissy, Ye Cheng, Malik, Miles, Gabi, Maisha)
pIG_035-pIG_038 (Krissy, Ye Cheng, Malik, Miles, Gabi, Maisha)

Protocol:
Bacterial Transformation

1. Obtain bacteria cells on ice - thaw from -80 C (thaw 2-4 min)
2. Obtain DNA plasmids
3. Label tubes
4. One tube per plasmid + PUC19 control + negative control (no DNA)
5. Add 5 uL of bacteria into tube
6. Add 1 uL of DNA (GG rxn/plasmid) into tube

7. Incubate on ice for 30 minutes
8. Heat shock at 42C for 30s
9. Ice for 2 minutes
10. ~~750 uL~~ 100 uL of SOC media to tube
11. Grow w/ shaking at 280 rpm, 37 C for 45 min
12. Take appropriate antibiotic plate and pre-warm to room temp - label plates
    a. L0s = SPEC
    b. L1s = CARB
13. Add ~~10 uL~~ 50 uL of cells to center of plate
14. Add beads (about 4-10, enough so when moving will cover plate) and shake
15. Dump beads + put plate in 37 C

Lab:

- Transformed pL1s (pIG_023 - pIG_038)
    - Note that pIG_037 went missing
    - Plate was put on 2nd shelf in 37C incubator
    - Remaining SOC media left over is in -20 iGEM fridge.
- Will inoculate tomorrow.

Thursday 6/20/19

Protocol

Bacterial Transformation

1. Obtain bacteria cells on ice - thaw from -80 C (thaw 2-4 min)
2. Obtain DNA plasmids
3. Label tubes
4. One tube per plasmid + PUC19 control (from -80C fridge) + negative control (no DNA)
5. Add 5 uL of bacteria into tube
6. Add 1 uL of DNA (GG rxn/plasmid) into tube
7. Incubate on ice for 30 minutes
8. Heat shock at 42C for 30s
9. Ice for 2 minutes
10. 100 uL of SOC media to tube
11. Grow w/ shaking at 280 rpm, 37 C for 45 min
12. Take appropriate antibiotic plate and pre-warm to room temp - label plates
    a. L0s = SPEC
    b. L1s = CARB
13. Add 50 uL of cells to center of plate
14. Add beads (about 4-10, enough so when moving will cover plate) and shake
15. Dump beads + put plate in 37 C

Lab

- Pulled the plates → Transformation of pL1s (pIG_023 - pIG_038) failed (Vanessa, Gabi, Krissy, Margaret, and Melody)
- Redid the transformations for pIG_023 to pIG_038
    - This time we put in 100 uL SOC media and 50 uL onto the plate
    - Note: plates were incubated for 10 minutes to warm up to room temperature
    - Note: all plates were put in bottom left shelf of the incubator!

Friday 6/21/19

Protocol

- Inoculation protocol
    - No change

Lab

- Created 100 mM Carb stock (Maya and Krissy)
    - Is in the -4 fridge in the antibiotic stock box
- Inoculated our transformations
    - pIG_023 to pIG_038 (Miles, Malik, Vanessa, Ye Cheng, Ethan)

Monday 6/24/19

Protocol

Miniprep

https://drive.google.com/open?id=19Gytvr-2QzGY8_IGe94JNQl_eMYFGwoz

Making and pouring an agarose gel

1. Measure 200ml of TAE into a glass bottle or flask
2. Add UltraPure agarose to a final concentration of 1% (mass / volume), 2 grams for 200ml gel
3. Swirl the bottle or flask to distribute the agarose.
4. Heat the solution in the microwave with frequent stirring to dissolve the agarose homogeneously. ~45, make sure to not let it bubble
5. Let sit until cool enough to handle -- if it's too hot, it can warp the casting trays.
6. Add 15 µl SYBRSafe (0.5X ; stock solution is 1000X) per 100 ml of the solution and mix well.
7. Set up the gel tray in the casting stand. Make sure the rubber gaskets are flat up against the edges of the casting tray.
8. Set up the gel combs to form the wells. Tape the edge of the comb to make sure it does not touch the bottom of the tray.
9. Rinse the combs with water and wipe dry.
10. Note for combs: 15-well combs hold about 6 ul liquid per well, 12-well combs hold about 15 ul per well, 8-well combs hold about 20 ul per well, Taping two 8-well comb wells

together results in a well that holds up to 100 ul, Taping three 8-well comb wells together result in a well that holds up to 200 ul

11. Pour the molten agarose into the casting tray.
12. If bubbles form around the combs, remove and re-insert.
13. Wait 45 minutes for the gel to solidify.
14. Use immediately, or place in a plastic zip-lock bag with a little 1X TAE and store at 4°C

Gel digest:
https://docs.google.com/document/d/1R3S90FE2ZTrWog5A84cqElLWR5uzAVEPwShIoPibVmA/edit
From Nika:

1. take 2ul of each pL1 you miniprepped, add 0.5 ul NcoI-HF (or ApaL1) and 1 ul Cutsmart buffer and 6.5 ul DEPC H20, leave @ 37C (in incubator) for 30 min-1 hour.
2. after incubating, you need to add DNA dye (it is a 6x solution, so you add 2 ul dye + 10 ul DNA), then load that 12ul volume into the gel. don't forget to run the appropriate gel ladder. I recommend you have a look at the ladders you have in the box from previous years and google them to see if they're appropriate for the lengths you want to see. Then run in 1% agarose gel, 40 min at 150V

Lab

Miniprep notes: (Krissy, Ethan, Melody, Margaret)

- 3ml of culture were spun down (1.5ml twice), 1ml saved for glycerol stocks
- pIG_023 - pIG_038 (16 plasmids, 2 clones = 32 cultures total)
    - Note: these were accidentally transferred into a spin column after adding P1, but we took them out and back into a microcentrifuge tube pIG_023 - pIG_025 (#1 and #2s)
    - Note: 33_1 - 35_2 may have accidentally had 300ul P1 put in instead of the recommended 250ul
    - Note: between 35_1 and 35_2 forgot tip change
- Future notes: remember that the blue tube thing is the spin column and the plastic is the microcentrifuge tube!

Digest our L1s: (Krissy, Ethan, Melody, Margaret)

- For pIG_023 - pIG_038

Nanodrop: (Krissy, Ethan, Melody, Margaret)

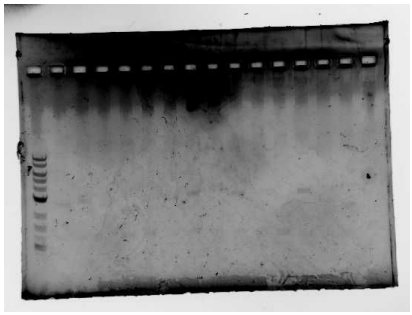
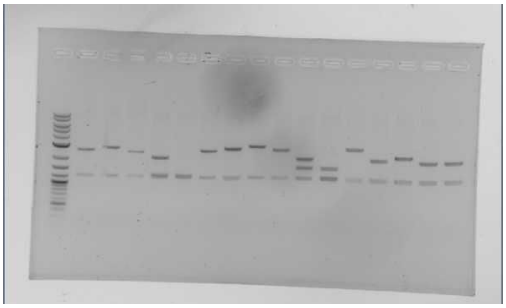
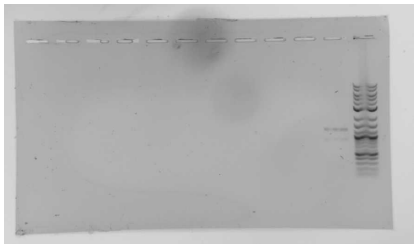
- pIG_023 - pIG_038 were nano dropped during restriction digest
- See concentrations here: https://docs.google.com/spreadsheets/d/1NZj-h7bA1Q3kJuHLNF4nCaY6EmRjNdR0mOiDujds-Qs/edit#gid=0

Make a gel to check the digest (Gabi, Vanessa)

- Initially made 300 ml of gels with 0.5% agarose
- Remade 200 ml of gels with 1% agarose
    - 1.5 ul of SYBRsafe was added before microwaving (SYBRsafe is heat sensitive)
    - Added an additional 1.5 ul of SYBRsafe after the mixture cooled down
    - Gel was in the open for 90 minutes instead of 45 minutes
    - Stored in 4°C with 1X TAE
    - Suggestion: make gels while the plasmid digest
-

Gel Placement																	
Well	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	Ladder	23-1	24-1	25-1	26-1	27-1	28-1	29-1	30-1	31-1	32-1	33-1	34-1	35-1	36-1	37-2	38-1
Well	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
	Ladder	23-2	24-2	25-2	26-2	27-2	28-2	29-2	30-2	31-2	32-2	33-2	34-2	35-2	36-2	37-1	
Well	1	2	3	4	5	6	7	8	9	10	11	12					
	Ladder	38-2															

- Gel with 17 wells were ran in the white electrophoresis apparatus at 100 volts for 30 minutes
- Gel with 16 wells were ran in the blue electrophoresis apparatus at 150 volts for 40 minutes
- Gel with 12 wells were ran in the white electrophoresis apparatus at 100 volts for less than 30 minutes
- The results of gel with 17 wells was clear and well distributed, gel with 16 wells was blurry and changed the settings to see the bands, gel with 12 wells was visible but clumped
- Future Reference: running in the white electrophoresis apparatus at 100 volts for 30 minutes
- Imaged the bio-rad gel under UV illumination
- Images saved in Jan’s server

- 



Tuesday 6/25/19

Protocol:
Inoculation of Liquid Cultures from Solid Media
Sent in pL1s for Sequencing:
A Sending Samples for Sequencing How-To:

1) Open GeneWiz.
2) Go to My GeneWiz
3) Find order/determine what you are ordering (for sequencing, go to Sanger Sequencing → Plasmid:
    a) DNA Type: Plasmid
    b) Service Type: Custom
    c) # of Samples (dependent on how many plasmids sequencing * each primer = total number of sequencing rxns/samples)
    d) Order Name (pIG/Plasmid ID in spreadsheet)
    e) Order Comments: Use Banked Oligos (name if possible?)
    f) Then fill out excel spreadsheet and upload
    g) Make sure you click tube/plate format (>48 samples = plate) and follow **Sample Submission Guidelines**
4) Pass by a mentor to make sure you did correctly
5) Put in order form with samples into a bag and put bag in GeneWiz box

Lab

- Sent in plasmids for sequencing (three were sent in with a little more because concentrations were slightly lower than the recommended total of 400ng)

Sent L1s pIG_023 - pIG_038 to sequencing at Genewiz (Melody, Krissy)
Inoculated L1s pIG_023 - pIG_038 (Margaret, Ethan, Krissy, Melody)

- Trying to get better nanodrop scores
- Did a third clone, but actually should have taken old cultures from first transformation
- Tried inoculation cultures with 2.5 mL and took out 1.5 mL for miniprepping

Wednesday 6/26/19

Protocol:
Making glycerol stock protocol:
https://docs.google.com/document/d/19qRfn-OeixmC5Z8pPIamiryFkk3u-nB7994RQAXZ5eA/edit

Lab:
Pulled the inoculation tubes→ Transformation of pL1s (pIG_023 - pIG_038) #2 (Krissy)

Transformed iGEM plasmids pIG_079 - pIG_087 that Deepak requested (Krissy, Gabi, Vanessa)
- Made concentrated stocks with 6O, 8A, 8C, 8G, 8E, 8I, 8M, 8G, 8O on plate 5
- Less stock in 80C (pIG_)
- Transformation protocol as used previously
- Incubated on ice for 1 hour
- Four of the samples were heat shocked twice

Inoculated cultures from old cultures (pIG_023-pIG_038) (6/21) -- only took clones that were verified by gel digest and sequencing (Margaret, Ethan, Melody)

Made glycerol stocks of pIG_023_1 to pIG_036_1 and pIG_037_2 and pIG_038_2 (sequenced clones; Margaret, Melody, Ethan)
- pIG_33 had 250 ul of culture and 250 ul of glycerol stock

Thursday 6/27/19

Protocol
Miniprep

Nanodrop

Transformation
https://docs.google.com/document/d/1RP03OeeD7FuVT5N4exJN-G_SlSu39oREHRksMt67qPI/edit

Lab

Pulled the inoculation tubes (from yesterday pIG_023-pIG_038) (Krissy and Ethan)

- pIG_031 looks very slightly tinted pink? -- for future reference -- hope there's not contamination! -- sequencing results turned up fine though?
- Took 1.5ml from 2.5ml for miniprepping

Pulled plates after transformation of pL1s (pIG_079 - pIG_087) (Krissy and Ethan)

Miniprepped (pIG_023-pIG_038) (Maya, Krissy, Melody, Margaret, Ethan)

- Some tubes were pink after pelleting (25, 31, 37) = mKO2 → supposed to happen/there may have been leaking of the promoter according to Shiva
- "The reddish color is due to leaky expression, with some promoters this leakiness happens but it is not a concern in this context of DNA amplification"
- Nanodropped results after! ~3x the original amount!! (compared to Monday's)

Redid transformation of pL1s (pIG_079 - pIG_087) (Krissy, Ethan, Margaret, Melody)

- Note: changes made to protocol this time around include: incubate during rescue period in shaker for 1 hr instead (step 11) and change to 10ul bacteria and 2ul DNA plasmids (steps 5 and 6)
- During incubate on ice step (60 minutes as recomm. By Deepak), went to lunch
- May have forgotten 87 on ice for more than 2 minutes… :(→ incubated in shaker at 37 for another 20 minutes by itself
- All were put in the 37C overnight (as of 3:30pm)

Friday 6/28/19

Protocol

Lab

- Transformations of pIG_79-87 have been pulled.
    - 5 plates with no growth (including no dna) - 82, 85, 86, 87
    - 5 plates with low amounts of colonies (4-20)
    - 1 plate with over a hundred colonies, no lawns (pUC19)
- Organized lab and took photos for safety forms (Krissy and Ethan)
- We sent the safety form for iGEM (Everyone)

Monday 7/1/19

Protocol

Making gels -
https://docs.google.com/document/d/14qF-wdTUSmiqHemgCrwHfq55DbjasAWXi317TRuka4c/edit

pL0-G bb digest & running gel/gel purification-

https://docs.google.com/document/d/1LVV5uXHj6HOyx4qopynUfUVtPxtwshHrtTxqmXxrBTQ/edit

Lab
Inoculated pIG_017 (pL0-G bb) from glycerol stock (Krissy, Melody, Margaret, Vanessa) in shaking culture overnight

Made Spec 1000x stock- (Krissy, Melody, Margaret, Vanessa)(Some chem basics: https://www.researchgate.net/post/What_volume_of_solvent_to_add_to_compound_powder_to_obtain_a_mM_stock)
https://www.goldbio.com/documents/1089/Spectinomycin+Stock+Solution.pdf -- says to use 0.5g-1g/10mL

Making gels notes:

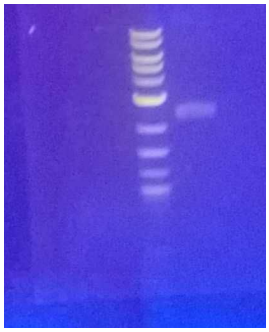
- Made 200 ml of gel
- Gels turned out softer than usual -- could be because of TAE?
- TAE + agarose mixture was a little foggier than usual after microwaving
- Ran gel and no bands showed up
- Remade 200ml of gels, better wells but it is still soft
- Added 5 ul of SYBRSafe for 100 ml of solution
    - This was later clarified to be 1000x SYBRSafe and so we should put in 10ul/100ml = 20ul for the 200ml solution
- Remade 500 ml of 1x TAE Buffer
- Remade 200ml of gel with 20 ul of SYBRSafe total
- Remade 500 ml of 1x TAE Buffer
- Gel with 20 ul of SYBRSafe are sturdy and the wells are clear
- The gel may have been better because we used newly made TAE Buffer instead of ones made in the previous week (although they should be stable for up to two years)
- Currently have 3 large gel and 3 small gels for future experiments stored in TAE Buffer under the iGEM bench
- Used agarose from the large Ultrapure bag for successful run

Gel digest & purification notes:

- Should do this again tomorrow because a little sketchy (i.e. no insert seen/may have run off gel, full plasmid also not there, but backbone seems to be correct size although band is a bit thick)
- Gels worked well 2nd digest around
- 111ng/ul was the concentration of the stock for pIG_017 (pL0-G bb)
- Took out gel with band of interest using tool Nika gave us (with some difficulty)
- ~0.08g was the gel mass, and we added 240ul ADB
- Band was cut out and made into working stock

Culturing HEK293: See Culture Notebook

Digested, Ran gel, and Gel purified the pIG_017 (Krissy, Melody, Margaret, Ethan, Vanessa)

- 
- **Image above**: pIG_017 digested with BsaI, the thick band is a little below the 3 kb (where the digested version of the backbone should be), this is what we extracted
- Purified pIG_017 from gel and put in pL0s concentrated stock

Tuesday 7/2/19

Protocol

Inoculation from Glycerol Stock

1) Add 2.5ml LB + 2.5ul antibiotic to culture tube
2) Use pipette with tip to pick off a small chunk of ice from glycerol stock (from -80).
3) Pipette up and down to mix glycerol stock with culture in tube.
4) Place culture tube in shaking incubator at 37C overnight.
5) Return glycerol stock to -80.

Lab

Pulled the inoculated pIG_017 (Krissy and Ethan)

- The Inoculated pIG_017 (pL0-G bb) left overnight was found clear (little to no growth).
- We will try to grab more of the glycerol stock next time
- We check the antibiotic, it was correct

Inoculation of pIG_017 from glycerol stock (Krissy, Melody, Maya)

- Grew on both plate and culture
- Colonies on plate (if grown) should be inoculated tomorrow
- Glycerol stock was out of ice for more than a minute - We need to retransform

Wednesday 7/3/19

Protocol

Lab

Learned how to transfect HEK293

- pIG_029 (YFP), pIG030 (RFP), pIG033 (BFP)

Monday 7/8/19

Protocol

Construction of pL0s with g-Blocks:

1. Resuspend gBlock in 20 uL DEPC H20
2. Digest with BsaI
    a. 8.5 uL gBlock
    b. .5 uL BsaI - Hfv2
    c. 1 uL cutsmart
3. Incubate @ 37 C, 1 hr
4. PCR purification -> to remove excess
    a. See PCR Purification protocol
5. SHOULD TRY TO NANODROP AFTER TO MAKE SURE RATIO IS RIGHT WITH BACKBONE!
6. Ligate to predigested pL0-G backbone (see pL0-G digestion protocol -- completed last week)
    a. 4 uL purified gBlock x
    b. 4 uL DEPC H20 x
    c. 0.5 uL T4 ligase x
    d. 1 uL 10x T4 buffer x
    e. 0.5 uL pL0-G digested backbone x
7. Leave at room temperature for 20-30 minutes
8. Ready to transform

Transformation (https://www.addgene.org/protocols/bacterial-transformation/)

1. Obtain bacteria cells on ice - thaw from -80 C (thaw 2-4 min)
2. Obtain DNA plasmids
3. Label tubes
4. One tube per plasmid + PUC19 control + negative control (no DNA)
5. Add 5 uL of bacteria into tube
6. Add 1 uL of DNA (GG rxn/plasmid) into tube
7. Incubate on ice for **30 minutes (for pIG_017 (for glycerol stock) and pIG_047-049)** and 60 minutes (for iGEM plasmids)
8. Heat shock at 42C for 30s
9. Ice for 2 minutes
10. 100 uL of SOC media to tube
    a. Put 2ml tube in shaking (15ml) tubes before putting in incubating shaker
11. Grow w/ shaking at 280 rpm, 37 C for ~~45~~ 60 min (this is the outgrowth step)
12. Take appropriate antibiotic plate and pre-warm to room temp - label plates
    a. L0s = SPEC

b. L1s = CARB
c. iGEM Biobricks = CHLOR
d. Puc19 = CARB

13. Add ~~50 uL~~ 100ul of cells to center of plate
    a. If necessary, can also plate all 100ul if transformed on all of full plate → usually don't want too much liquid so bacteria float around, but 100ul is okay
14. Add beads (about 4-10, enough so when moving will cover plate) and shake
15. Dump beads + put plate in 37 C

MoClo Golden Gate Assembly Protocol
pL1 MoClo Golden Gate protocol

Note: all buffers, enzymes, backbones and DNA parts stored at -20C

1. Identify your necessary parts and plan the construction

Example: Inert_hEF1a_Inert_EYFP_Inert_SynthPA

Plasmid ID	**I**	**P**	**5**	**G**	**3**	**T**
pIG_053	pIG_0001 Inert 1 ul	pIG_002 hEF1a 1 ul	pIG_005 Inert 1 ul	MIT_gB012 IL8 1 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_055	pIG_0001 Inert 1 ul	pIG_002 hEF1a 1 ul	pIG_005 Inert 1 ul	MIT_gB013 IL8 Flag 1 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_057	pIG_0001 Inert 1 ul	pIG_002 hEF1a 1 ul	pIG_005 Inert 1 ul	MIT_gB014 IL8-NeonGreen 1 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_054	pIG_0001 Inert 1 ul	pIG_004 CMV 1 ul	pIG_005 Inert 1 ul	MIT_gB012 IL8 1 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_056	pIG_0001 Inert 1 ul	pIG_004 CMV 1 ul	pIG_005 Inert 1 ul	MIT_gB013 IL8 Flag 1 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul

pIG_058	pIG_0001 Inert 1 ul	pIG_004 CMV 1 ul	pIG_005 Inert 1 ul	MIT_gB014 IL8-NeonGreen 1 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_097	pIG_0001 Inert 1 ul	pIG_003 TRE 1 ul	pIG_005 Inert 1 ul	MIT_gB012 IL8 1 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_098	pIG_0001 Inert 1 ul	pIG_003 TRE 1 ul	pIG_005 Inert 1 ul	MIT_gB013 IL8 Flag 1 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_099	pIG_0001 Inert 1 ul	pIG_003 TRE 1 ul	pIG_005 Inert 1 ul	MIT_gB014 IL8-NeonGreen 1 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul

2. Mix the DNA parts (1ul each of 40 fmol/ul stocks) -- should be a total of 6 ul

3. Add the following reagents (should all be kept on ice/ice block)

		For MM
Backbone pL1 (ex. ST1-2)	0.5 ul	5ul
T4 ligase	0.5 ul	5ul
10x T4 buffer	2 ul	20ul
BsaI-HFv2 enzyme	1 ul	10ul
10x BSA buffer (NEB B9001)	2 ul	20ul
DEPC H20	8 ul	80ul
TOTAL (including DNA above)	14ul	140 ul

Note: We can also make a master mix for multiple constructs (i.e. everything that's used*(however many constructs) with a little extra!!)

4. Thermal cycler (use one on my bench): ~2.5 hours for 10x cycles

iGEM folder --> "golden gate (BsaI, BsmbI)"

Heat lid	110 C	
Temp	37 C	20 min
Start cycle	10x	
	37 C	2 min
	16 C	5 min
Close cycle		
Temp	37 C	15 min
Temp	50 C	5 min
Temp	80 C	20 min
Store	8 C	

5. Transform bacteria as before and grow colonies on agar plates (Carb resistance), grow at 37C overnight

6. Miniprep

7. Test digest and run gel, send good clones to sequencing
NOW WE CAN USE THE PLASMID!!! :)

Lab

- ✓ gblocks (pIG_047-49; IL8) from IDT arrived
- ✓ Resuspended gBlocks pIG_012 - pIG_014
- ✓ Digested gBlocks pIG_012-pIG_014
- ✓ PCR Purified pIG_012-pIG_014
    - ❏ A bit of dH2O/NFW was added to 47-49 after PCR purification step
- ✓ Ligated gBlocks pIG_012-pIG_014 each with L0 bb (pIG_017)
    - ❏ Buffer was also accidentally added to 47, but after we put everything in a new tube for ligation
    - ❏ Nanodropped and found that it was 2-3 ng for all three after purification
- ✓ Transformed
    - ❏ pIG_079-pIG_087
        - ❏ iGEM plasmids for Deepak
    - ❏ pIG_017
        - ❏ b/c we need to make a new glycerol stock for the L0 bb (pIG_017)
    - ❏ pIG_012-pIG_014
        - ❏ Making the chemokine L0s ready to be put into the L1s
    - ❏ Notes → Plates: 9 chlor (for 79-87), 6 spec (for 47-49, pIG_017 L0-G bb, pUC19, and no DNA)

Golden gate MoClo notes:

- ❏ For IL8 plasmids:
  - ✓ pIG_053
  - ✓ pIG_054
  - ✓ pIG_055
  - ✓ pIG_056
  - ✓ pIG_057
  - ✓ pIG_058
  - ✓ pIG_097
  - ✓ pIG_098
  - ✓ pIG_099

- ✓ Culture-related notes:
  - ❏ pIG_029 and pIG_033 were diluted to 100ng/ul and put in pL0 concentrated stocks (for transfection use) (Margaret)

Tuesday 7/9/19

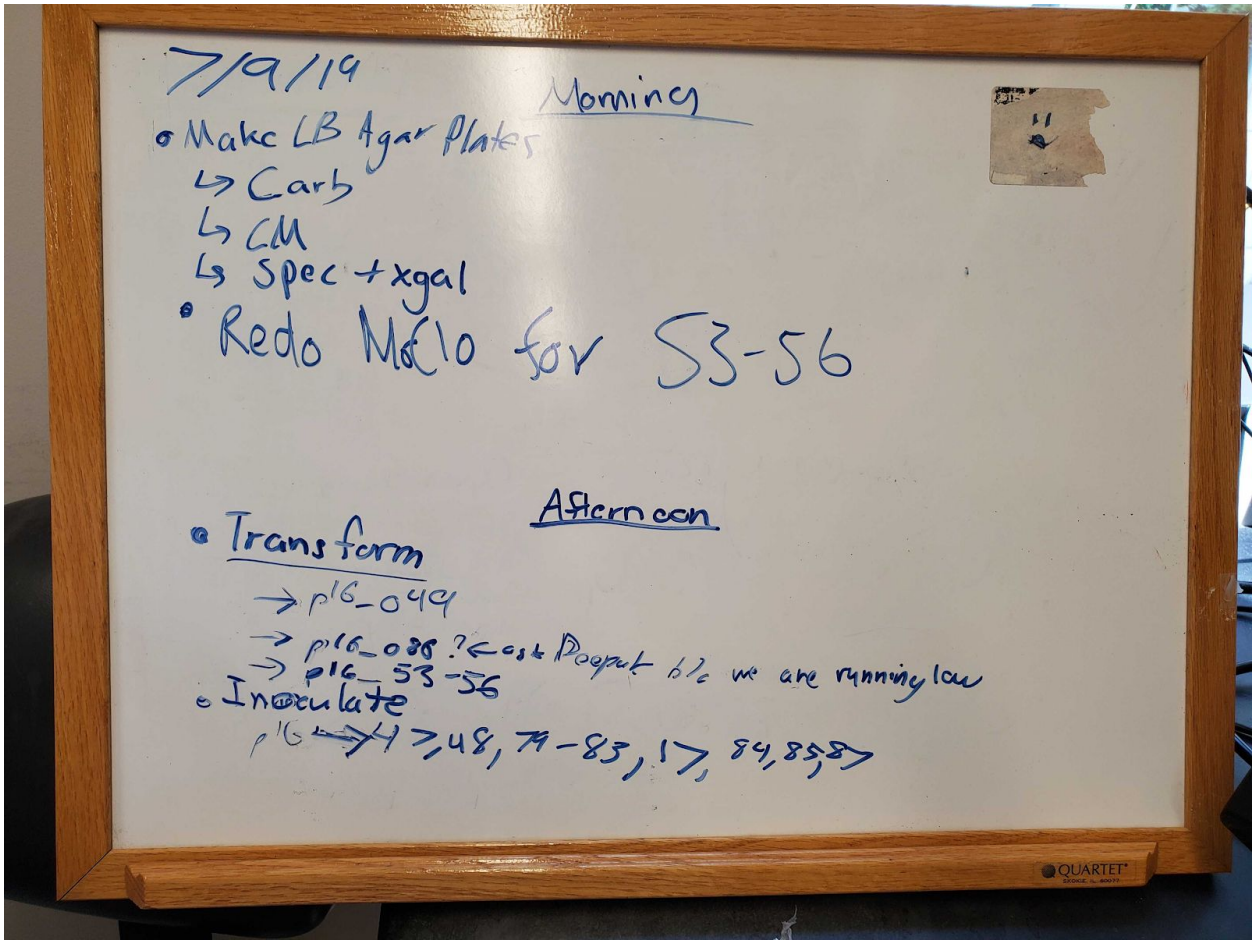
Protocol

Transformation

1. Obtain DH5a bacteria cells on ice - thaw from -80 C (thaw **2-4 min - temp sensitive so be quick!**)
2. Obtain DNA plasmids (pL0s, pL1s, etc.)
3. Label tubes.
4. One tube per plasmid + PUC19 control + negative control (no DNA)
5. Add 5uL of bacteria into tube
   a. If earlier transformation resulted in few colonies, use 10ul bacteria, 2uL DNA
6. Add 1uL of DNA (GG rxn/plasmid) into tube
   a. Again, if earlier transformation resulted in few colonies, use 10ul **bacteria**, 2uL **DNA**
7. Incubate on ice for:
   a. 30 minutes (typically for pL0s/pL1s)

    b. 60 minutes (for iGEM plasmids)
8. Heat shock at 42C for 30s-45s
9. Ice for 2 minutes
10. Add 100 uL of SOC media to tube
    a. In the case where you are performing the optional outgrowth steps, put in 700uL and then see note at Step 11 (ii)
11. Grow w/ shaking at 280 rpm, 37 C for 45-60 min
    a. This is the outgrowth step; if fewer colonies/no growth perform the following steps:
        i. **Outgrow longer (>60 minutes -- preferrably 2 hours)**
        ii. Spin down the outgrowth (in SOC media) for 5 minutes in centrifuge at 13000rpm, then take out 600ul, and resuspend the pellet in the remaining 100ul.
12. Take appropriate antibiotic plate and pre-warm to room temp - label plates
    a. L0s = SPEC
    b. L1s = CARB
    c. iGEM plasmids = CHLOR
13. Add 50 uL of cells to center of plate
    a. If necessary, can also plate all 100ul if transformed on all of full plate → usually don't want too much liquid so bacteria float around, but 100ul is okay
14. Add beads (about 4-10, enough so when moving will cover plate) and shake
15. Dump beads + put plate in 37 C

Lab

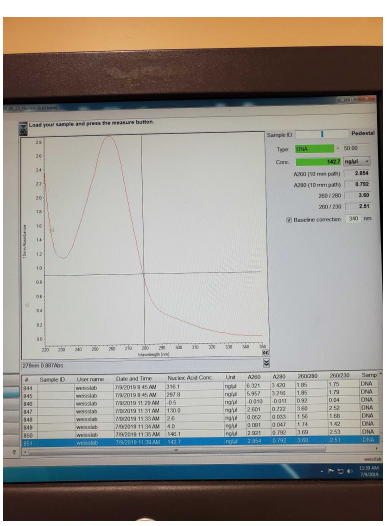
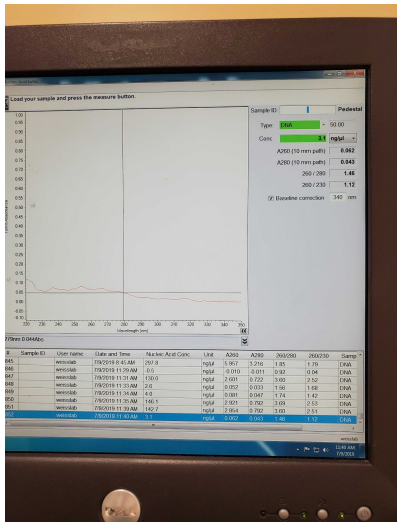
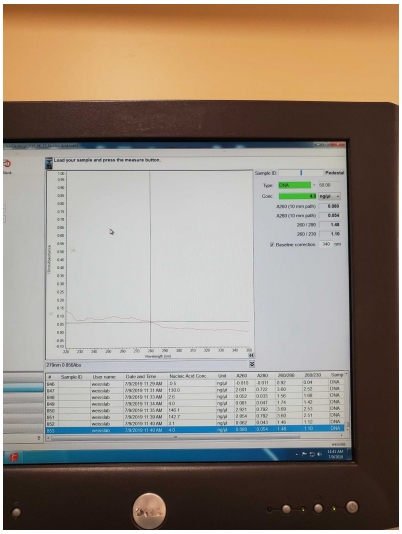


- ✓ Checked transformed plates: (Krissy, Melody, Margaret, Ethan)
  - Plenty of colonies: PUC19, 47 (1st gblock), 48 (2nd gblock), pIG_79,80,81,82,83 and 17 (Backbone, very blue)
  - Few colonies:pIG_84,85,87
  - No Colonies: no DNA, **pIG_86**, **49** (3rd gblock)
- ✓ Made LB Agar Plates (Krissy, Melody, Margaret)
  - Carb (1 sleeve)
  - Spec + Xgal (1 sleeve)
  - CM (1 sleeve)
- ✓ GG MoClo (Melody, Margaret, Krissy, Ethan): Redo for pIG_053 - pIG-056; finished 57-58 and 97-99
  - See protocol on Monday
- ✓ Transformations (Margaret, Ethan, Krissy, Melody,):
  - Redo for pIG_049 (3rd gblock) since no colonies from yesterday -- using reaction from yesterday
  - 86 and the rest of the pL1s (91-99)

- Transform pIG_049 digestion-ligation reaction with modified longer outgrowth step (step 11 of Transformation protocol); same goes for 86 and the rest of the pL1s (91-99) - Ethan, Margaret, Melody, Krissy

✓ After talking with mentors over Slack-- remade To-Do list (Krissy, Melody, Margaret, Ethan):

✓ Nanodrop purified gBlock(Krissy, Melody, Margaret, Ethan)

- (see step 5 of construction of pL0 plasmids with predigested backbone from Monday's protocol)
-  
- 

✓ pIG_047: ng/ul

✓ pIG_048: ng/ul

✓ pIG_049: ng/ul

✓ Design new primers for the gBlocks (12-14) (Ye Cheng)

- so we can PCR and make more in case we run out of gBlocks

        - How to design primers: https://docs.google.com/document/d/12Oui3hy0y0yUIhaiRZi8wpxmRPccESlVmxndPcQaq4Y/edit
- ✓ Set up new ligation reaction (proxy GG) and put more purified gBlock (~6uL) than protocol says (depending on Nanodrop results) - (Krissy)
- ✓ Inoculated plate with colonies (Margaret, Krissy, Ethan)
    - 2 colonies for growing cultures (make sure you put correct antibiotic into 2.5mL LB for each culture!)
    - PUC19, 47 (1st gblock), 48 (2nd gblock), pIG_79, 80, 81, 82, 83 and 17 (Backbone, very blue), pIG_84, 85, 87

(Logistic/Kind of Stupid) Questions?

- What really is 1000x for antibiotics/reagents?
    - 1 uL antibiotics to 1 mL media
- Does the LB Agar need to cover the whole plate?
    - Yes
- What is the purpose of incubating on ice before heat shock? What really happens to the bacteria? -- check transformation protocols?
    - Incubating on ice same times within a 10 minute range difference is okay
    - Outgrowth:
        - 60 minutes for carb/spec/kan
        - >60 minutes for chlor
- Does solute affect final volume solution? Partial molar volume (a little lesson on Gen Chem) and solubility impacts
    - Nope, negligible here

Wednesday 7/10/19

Protocol
Transformation
Inoculation
Miniprep
Nanodrop
How to design primers

Lab

- Transformation Results:
    - Many Colonies: pIG_53,54,55,56,97,98,99
    - Few Colonies: pIG_57,58 - 90-100% red colonies, effectively did not transform correctly
    - No Colonies: pUC19 (accidentally used spec instead of carb plate), no dna, pIG_49 (third g-block), 86

056	19r
057	14r
058	10r, 3 w (on edge of plate)
097	15 r, maybe 1 w

- Inoculation Results:
    - Tubes were very cloudy, inoculation appeared successful
- ✓ Miniprep (2 copies of each) :
    - ✓ pIG_017 - Krissy
    - ✓ pIG_47-48 - Krissy
    - ✓ pIG_79, 80, 81, 82 - Margaret
    - ✓ pIG_83, 84, 85, 87 - Melody
- ✓ Nanodrop:
    - ✓ Results were low (avg. 90-100ng/ul for highest clones) → can we improve miniprepping protocol?
- ✓ Inoculate: (do two copies!!!!)
    - ✓ pIG_053 - pIG_055, pIG_098, pIG_099 (many colonies) - Ethan
    - ✓ Could not inoculate pIG_056, pIG_057, and pIG_058 because basically all colonies were red -- need to transform again
- ✓ Transform:
    - ✓ Ligation reaction for pIG_48 and pIG_49 - Krissy, Ethan, Melody, Margaret
    - ✓ pIG_086 needs to be redone, need to redo - Krissy, Ethan, Melody, Margaret
- ✓ Order primers after verifying by mentors - Yecheng
    - Nika has ordered, should come July 12th

Questions?

- Should we always do the recommended wash step (says note: this step is only req. When using endA+ strains or other bacteria strains with high nuclease activity)?
- For miniprepping, trying to maybe put in less EB buffer at the end? (~30ul?)
- Should we do 47-49 from the very beginning but with the predigested backbone (since that has been verified)? If so, we will need gBlock, so we need to wait for the primers (which come in tomorrow)?
- Should we still send in 48 for sequencing?

Thursday 7/11/19

Protocol

Transformation

1. Obtain DH5a bacteria cells on ice - thaw from -80 C (thaw **2-4 min - temp sensitive so be quick!**)
2. Obtain DNA plasmids (pL0s, pL1s, etc.)
3. Label tubes.
4. One tube per plasmid + PUC19 control + negative control (no DNA)
5. ~~Add 5uL of bacteria into tube~~
6. ~~Add 1uL of DNA (GG rxn/plasmid) into tube~~
    a. **If earlier transformation resulted in few colonies, use 10ul bacteria, 2uL DNA**
7. Incubate on ice for:
    a. 30 minutes (typically for pL0s/pL1s)
8. Heat shock at 42C for 30s-45s
9. Ice for 2 minutes
10. Add 100 uL of SOC media to tube
    a. In the case where you are performing the optional outgrowth steps, put in 700uL and then see note at Step 11 (ii)
11. Grow w/ shaking at 280 rpm, 37 C for 45-60 min
    a. This is the outgrowth step; if fewer colonies/no growth perform the following steps:
        i. Outgrow longer (>60 minutes -- preferrably 2 hours)
        ii. Spin down the outgrowth (in SOC media) for 5 minutes in centrifuge at 13000rpm, then take out 600ul, and resuspend the pellet in the remaining 100ul.
12. Take appropriate antibiotic plate and pre-warm to room temp - label plates
    a. L0s = SPEC
    b. L1s = CARB
    c. iGEM plasmids = CHLOR
13. Add 50 uL of cells to center of plate
    a. **If necessary, can also plate all 100ul if transformed on all of full plate → usually don't want too much liquid so bacteria float around, but 100ul is okay**
14. Add beads (about 4-10, enough so when moving will cover plate) and shake
15. Dump beads + put plate in 37 C

Lab

- Transformation Results:
    - Many Colonies: pUC19
    - No Colonies:no dna, pIG_48 (second4 g-block), 49 (third g-block), 86
- Inoculation Results:
    - Tubes were very cloudy, inoculation appeared successful

✓ **Enter minipreps that were made yesterday into inventory**

✓ Miniprep cultures (2 copies of each) for pL1s - Krissy, Ethan, Melody

- pIG_053 → hEF1a IL-8

- pIG_054 → CMV IL-8
- pIG_055 → hEF1a IL-8-Flag
- pIG_086 → iGEM plasmid -- only 1 copy
- pIG_098 → TRE IL-8-Flag
- pIG_099 → TRE IL-8-NeonGreen

✓ Transform GG reactions of IL8 pL1s - Melody
- pL1 for IL-8 (Made using gBlocks not pL0s):
  - pIG_056 (pL1) → CMV IL-8Flag
  - pIG_057 (pL1) → hEF1a IL-8-NeonGreen
  - pIG_058 (pL1) → CMV IL-8-NeonGreen
  - pIG_097 (pL1) → TRE IL-8

✓ Gel digest and verification - Krissy, Ethan
- pL1s for IL8 (Made using gBlocks not L0-G) :
  - pIG_053 → hEF1a IL-8
  - pIG_054 → CMV IL-8
  - pIG_055 → hEF1a IL-8-Flag
  - pIG_098 → TRE IL-8-Flag
  - pIG_099 → TRE IL-8-NeonGreen

Gel

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Ladder	53#1	53#2	54#1	54#2	55#1	55#2	86	99#1	99#2	98#1	98#2	Ladder			

- ✓ Send to sequencing - Krissy, Ethan, Melody
  - Backbone:
    - pIG_17 L0 bb
  - pL0s for IL8:
    - pIG_047→ L0 IL-8
    - pIG_048 → L0 IL-8-Flag
  - pL1s for IL8 (Made using gBlocks not L0-G):
    - pIG_053 → L1s hEF1a IL-8
    - pIG_054 → L1s CMV IL-8
    - pIG_055 → L1s hEF1a IL-8-Flag
    - pIG_098 → TRE IL-8-Flag
    - pIG_099 → TRE IL-8-NeonGreen
- ❑ Tell Deepak about iGEM plasmids: give him minipreps of pIG_79-85, pIG_87; 86 actually had one colony which we cultured and miniprepped, but no more DNA in kit :(- Miles

Tomorrow:

- ❑ Analyze sequencing results, determine which to make glycerol stocks of (if not all); think about how to proceed!

        - ❏ What happens if good results but nanodrop shows a bit of contamination? Remembering to do centrifuge 2 min. step to remove any residual wash buffer before final elution.
    - ❏ PCR gBlocks
        - ❏ Will these need to be sequenced?
    - ❏ Ligation reaction of gBlocks 13 and 14 into pL0-G bb to make pIG_48 & 49

Friday 7/12/19

Protocol

PCR gBlocks

- Annealing temp of primers: 65C

Lab

- ✓ Check the sequencing - Krissy, Ethan, Margaret, Melody
    - Backbone:
        - X pIG_17 L0 bb (failed)
    - pL0s for IL8:
        - pIG_047→ L0 IL-8 (reverse primer failed)
        - pIG_048 → L0 IL-8-Flag (reverse primer failed)
    - pL1s for IL8 (Made using gBlocks not L0-G):
        - pIG_053 → L1s hEF1a IL-8 (weird error)
        - pIG_054 → L1s CMV IL-8 (weird error)
        - ✓ pIG_055 → L1s hEF1a IL-8-Flag (fine i think)
        - ✓ pIG_098 → TRE IL-8-Flag (Good)
        - pIG_099 → TRE IL-8-NeonGreen (Good)
- ❏ Inoculate IL8 pL1s -
    - pL1 for IL-8 (Made using gBlocks not pL0s):
        - pIG_056 (pL1) → CMV IL-8Flag
        - pIG_057 (pL1) → hEF1a IL-8-NeonGreen
        - pIG_058 (pL1) → CMV IL-8-NeonGreen
        - pIG_097 (pL1) → TRE IL-8

Inventory check? Anything we need to order/make more of?

- Transformation Results:
    - Many Colonies: pIG_56,57,58,97
    - pIG_97 had only red colonies, must be redone

Monday 7/15/19

Protocol

PCR gBlocks

- Annealing temp of primers: 65C

- Elongation time: 45 sec

PCR Purification Protocol

Lab

- ❏ PCR gBlocks (Cloning Team):
  - ■ MIT gBlock_012 [IL-8 (CXCL-8)] - don't have reverse primer
  - ✓ MIT gBlock_013 [IL-8-Flag (CXCL-8)] - Ethan, Margaret, Krissy, Melody
  - ■ MIT gBlock_014 [IL-8-NeonGreen (CXCL-8)] - don't have reverse primer
    - ✓ Annealing: 65C
    - ✓ Elongation time:45 sec
- ✓ PCR purify gblock 13 PCR
  - ■ Notes:
    - PCR purification seemed weird, redoing PCR again with 2 reactions (30 cycles): one with annealing at 58C and one with annealing at 65C
- ■ Made gels
- X Gel purification after running PCR in gel
  - ■ Notes:
    - Gel bands likely ran off gel, we did it again running for 20 minutes
    - 2nd time PCR band showed up (really thick one) but not ladder?
- X Redo GG with digested backbone + PCR-ed gBlocks
- X Transformation of Golden Gates (tomorrow?)
- ✓ Check the sequencing - Krissy, Ethan, Margaret, Melody
  - ■ Backbone:
    - X pIG_17 L0 bb (failed)
  - ■ pL0s for IL8:
    - pIG_047→ L0 IL-8 (reverse primer failed)
    - pIG_048 → L0 IL-8-Flag (reverse primer failed)
  - ■ pL1s for IL8 (Made using gBlocks not L0-G):
    - pIG_053 → L1s hEF1a IL-8 (Missing Reverse Primer)
    - pIG_054 → L1s CMV IL-8 (weird error)
    - pIG_055 → L1s hEF1a IL-8-Flag (fine i think)
    - pIG_098 → TRE IL-8-Flag (Good)
    - pIG_099 → TRE IL-8-NeonGreen (NeonGreen not showing up, redoing golden gate with pcr product)
      - Is actually 97 not 99 ??→ will redo golden gates? May have been a mistake in labeling/reading writing
- ✓ Inoculate IL8 pL1s -
  - ■ pL1 for IL-8 (Made using gBlocks not pL0s) (# of colonies):
    - pIG_056 (pL1) → CMV IL-8Flag (1)
    - pIG_058 (pL1) → CMV IL-8-NeonGreen (2)
  - ■ pIG_017 (pL0-G bb) (1)
- X Inventory check? Anything we need to order/make more of?
- ✓ Made LB Agar Plates (Krissy, Ethan)

✓ Carb (34 plates)

Tuesday 7/16/19

Protocol

PCR gBlocks

- Annealing temp of primers: 65C
- Elongation time: 45 sec

PCR purification protocol

a. Add 3 volumes of DNA binding buffer (Zymo) to each volume double stranded DNA
b. Load into Zymo spin column and place in 2 ml collection tube
c. Centrifuge @ max speed, 30 sec
d. Discard flow through
e. Add 200 ul Buffer PE (QIAGEN) and centrifuge @ max speed, 30 sec
f. Repeat step e
g. Discard flow through and spin for 1 min
h. Place column in new 1.5 ml eppendorf tube
i. Add 10 ul DEPC H20, wait 1 min (elute in same volume as started)
j. Spin @ max speed, 1 min

MoClo Golden Gate Assembly Protocol
pL1 MoClo Golden Gate protocol

Note: all buffers, enzymes, backbones and DNA parts stored at -20C

1. Identify your necessary parts and plan the construction

Plasmid ID	I	P	5	G	3	T
pIG_055	pIG_0001 Inert 1 ul	pIG_002 hEF1a 1 ul	pIG_005 Inert 1 ul	MIT_gB013 IL8 Flag 1 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_056	pIG_0001 Inert 1 ul	pIG_004 CMV 1 ul	pIG_005 Inert 1 ul	MIT_gB013 IL8 Flag 1 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul

pIG_098	pIG_0001 Inert 1 ul	pIG_003 TRE 1 ul	pIG_005 Inert 1 ul	MIT_gB013 IL8 Flag 1 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_100	pIG_0001 Inert 1 ul	pIG_002 hEF1a 1 ul	pIG_005 Inert 1 ul	pIG_006 mKate2 1 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_101	pIG_0001 Inert 1 ul	pIG_004 CMV 1 ul	pIG_005 Inert 1 ul	pIG_006 mKate2 1 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_102	pIG_0001 Inert 1 ul	pIG_003 TRE 1 ul	pIG_005 Inert 1 ul	pIG_006 mKate2 1 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul

Also making pL0s:

pIG_048	MIT_gB013 IL8-Flag 1 ul (PCR-purified) 2ul (not PCR-purified)	pIG_017 L0-G bb 1 ul

2. Mix the DNA parts (1ul each of 40 fmol/ul stocks) -- should be a total of 6 ul

3. Add the following reagents (should all be kept on ice/ice block)

		MM (for pL1s)	for each 48
Backbone pL1 (ex. ST1-2)	0.5 ul	6ul ~6.6ul	
T4 ligase	0.5 ul	6ul ~6.6ul	0.5 ul
10x T4 buffer	2 ul	24ul ~26.4ul	2 ul

BsaI-HFv2 enzyme	1 ul	6ul ~6.6ul	1 ul
10x BSA buffer (NEB B9001)	2 ul	12ul ~13.2ul	2 ul
TOTAL	**6ul**	**6ul/tube = 72ul**	**5.5ul/tube**
DEPC H20		pL1s purified, 100-102 - 8ul pL1s not purified - 7ul	pL0 48 purified - 12.5ul x pL0 48 not purified - 11.5ul x

Note: We can also make a master mix for multiple constructs (i.e. everything that's used*(however many constructs) with a little extra!!)

4. Thermal cycler (use one on my bench): ~2.5 hours for 10x cycles

iGEM folder --> "golden gate (BsaI, BsmbI)"

Heat lid	110 C	
Temp	37 C	20 min
Start cycle	10x	
	37 C	2 min
	16 C	5 min
Close cycle		
Temp	37 C	15 min
Temp	50 C	5 min
Temp	80 C	20 min
Store	8 C	

5. Transform bacteria as before and grow colonies on agar plates (Carb resistance), grow at 37C overnight

6. Miniprep

7. Test digest and run gel, send good clones to sequencing
NOW WE CAN USE THE PLASMID!!! :)

8. For pL0s (Jan's suggestion): Add an extra T4 ligation step:
- Add 1 ul T4 ligase
- Let sit at room temp for ~30 minutes (or overnight if still not good)

Lab

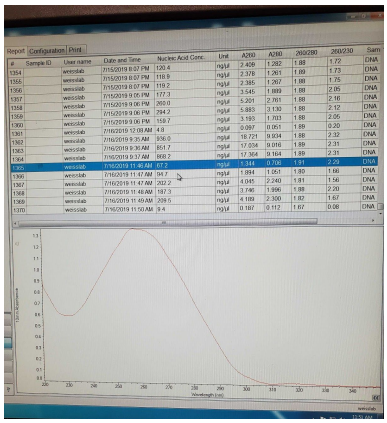
- Inoculation Results:
    - Tubes (pIG_017 1 and 2, 56, 58 1 and 2) were very cloudy, inoculation appeared successful

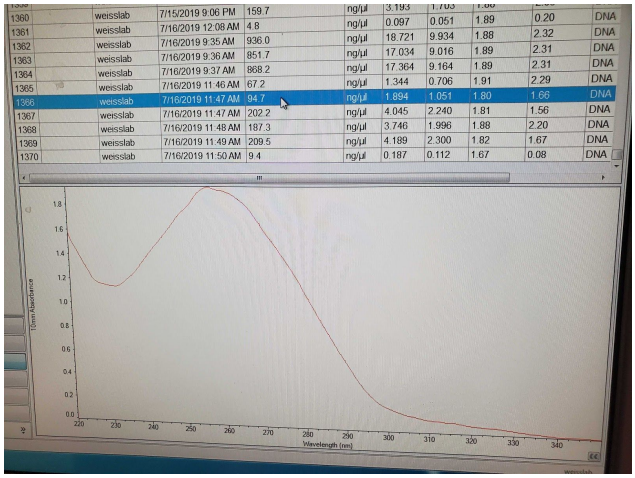
Miniprep	**Nanodrop**	**Gel Verify**	**Sent to sequencing**
pIG_017 #1	pIG_017 #1 (67.2)	pIG_017 #1	pIG_017 #2
pIG_017 #2	pIG_017 #2 (94.5)	pIG_017 #2	
pIG_056	pIG_056 (202.2)	pIG_056	pIG_056
pIG_058 #1	pIG_058 #1 (187.3)	pIG_058 #1	pIG_058 #2
pIG_058 #2	pIG_058 #2 (209.5)	pIG_058 #2	
	pIG_017 digested (9.4) → need to re-digest from pIG_017 #2		

Run Gel	**PCR purify**	**Nanodrop**	**GG**
GB 13	GB 013 (PCR 65^0C)	GB 013 (PCR 65^0C) (47.3)	pIG_055 p pIG_056 p pIG_098 p p=purified
	GB 013 (PCR 58^0C)	GB 013 (PCR 58^0C) (45.9)	
		GB 013 (PCR 65^0C) non purified	pIG_055 pIG_056 pIG_098
		GB 013 (PCR 58^0C) non purified	

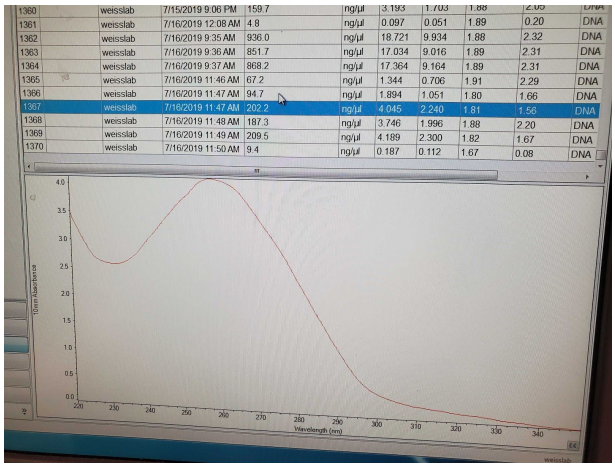
GG	**Transform**
100	100
101	101
102	102

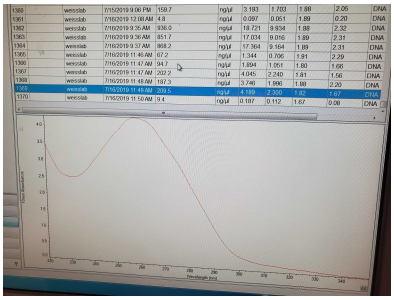
- Miniprep
    - pIG_17 (1,2), 56, 58 (1,2)
- Nanodrop:
    - Results were low for pIG_017 (avg. 90-100ng/ul) → can we improve miniprepping protocol?
    - High for pIG 56-58
    - pIG_17 digested had awful curve, must've caused bad pL0s

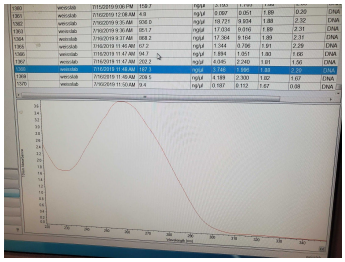


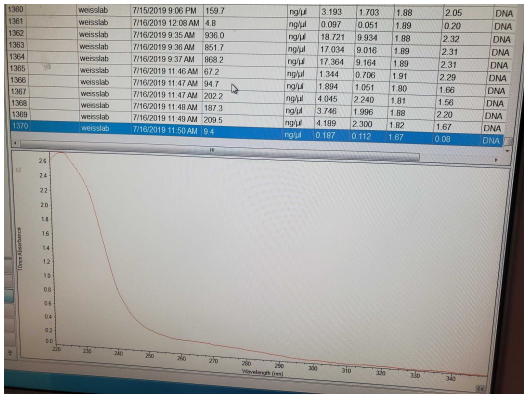


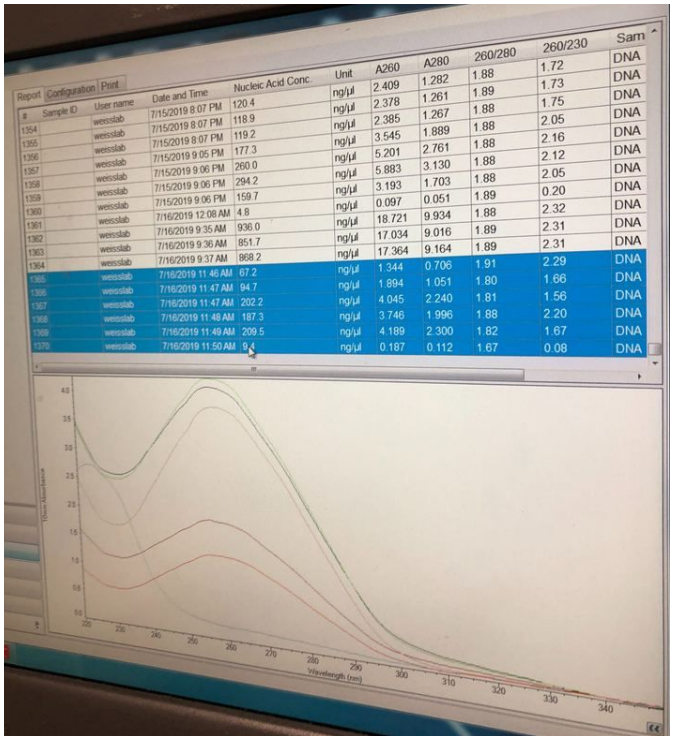
1360	weisslab	7/15/2019 9:06 PM	159.7	ng/µl	3.193	1.703	1.88	2.05	DNA
1361	weisslab	7/16/2019 12:08 AM	4.8	ng/µl	0.097	0.051	1.89	0.20	DNA
1362	weisslab	7/16/2019 9:35 AM	936.0	ng/µl	18.721	9.934	1.88	2.32	DNA
1363	weisslab	7/16/2019 9:36 AM	851.7	ng/µl	17.034	9.016	1.89	2.31	DNA
1364	weisslab	7/16/2019 9:37 AM	868.2	ng/µl	17.364	9.164	1.89	2.31	DNA
1365	weisslab	7/16/2019 11:46 AM	67.2	ng/µl	1.344	0.706	1.91	2.29	DNA
1366	weisslab	7/16/2019 11:47 AM	94.7	ng/µl	1.894	1.051	1.80	1.66	DNA
1367	weisslab	7/16/2019 11:47 AM	202.2	ng/µl	4.045	2.240	1.81	1.56	DNA
1368	weisslab	7/16/2019 11:48 AM	187.3	ng/µl	3.746	1.996	1.88	2.20	DNA
1369	weisslab	7/16/2019 11:49 AM	209.5	ng/µl	4.189	2.300	1.82	1.67	DNA
1370	weisslab	7/16/2019 11:50 AM	9.4	ng/µl	0.187	0.112	1.67	0.08	DNA



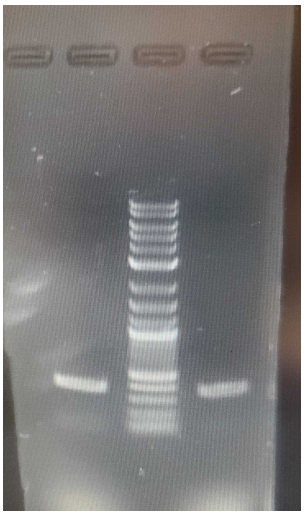








INSERT pIG_086 results

- Ran gel with both PCR reactions, both showed up!: Margaret, Melody
  -  From Left to Right: GB 013 (PCR 58^{0}C), DNA ladder, GB 013 (PCR 65^{0}C)
- PCR purification completed (eluted in 10ul); nanodrop results: Margaret, Melody

GB 013 (PCR 65^{0}C) (47.3)
GB 013 (PCR 58^{0}C) (45.9)

✓ Miniprep inoculated cultures (17, 56, 58), nanodrop, gel digest verification for 56 and 58 (make sure these weren't flipped this time!) - Ethan, Krissy

✓ Nanodrop digested pIG_017 - Ethan, Krissy

- ✓ Rerun gel for gBlock 13 PCR + PCR purify gblock 13? - Margaret, Melody
- ✓ Nanodrop pIG_086 and PCR Purified gBlock 13s - Margaret, Melody
- ✓ Running 56 and 58 in gel
    - Notes:
        - Ladder, 58 1 ,58 2, 56
- ✓ Send to sequencing after gel digest - Ethan, Krissy
    - 17 #2
    - 56
    - 58 #2
- ✓ Redo GG with PCR-ed gBlocks -- should we go ahead and do just anything with gBlock 13 - pIG_048 and pIG_055, pIG_056, and pIG_098? -- yes! - Margaret, Melody
    - Notes: make sure to ligate 48 for another 15 minutes with 2ul T4 ligase @room temp
- ✓ GG with mKate2 (pIG_100-102)

Wednesday 7/17/19

Protocol

PCR Protocol

Glycerol Stocks

Protocol for Sequencing gBlock 14

gBlock14 [50ng/ul]	0.8ul
gB014_R [10uM]	2.5ul
ddH2O	11.7ul
Total	**15ul**

Send this to sequencing!

Lab

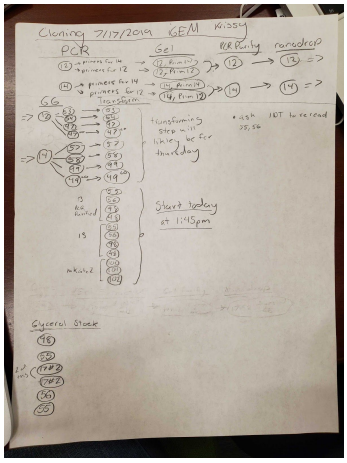
- ✓ Analyze sequencing results
    - Backbone:
        - pIG_17 L0 bb (looked great)
    - pL1s for IL8 (Made using gBlocks not L0-G):
        - X pIG_058 → CMV IL-8 with Neongreen (no NeonGreen part, still good as pure IL8)
        - ✓ pIG_056 → CMV IL-8-Flag (Great)
- PCR notes:
    - Resuspended gB012_R in 380ul NFW
    - Resuspended gB014_R in 317ul NFW
- Notes for culture team:

    - Made 100ng/ul samples of the transfection plasmids (29 and 33)... need to make more DNA (@Cloning team) of all of these -- take glycerol stocks, transform, inoculate and culture, and then miniprep for more!!
- Notes for sequencing gB014:
    - Always remember to vortex primer stock!
❑ Figure out what to throw out/Inventory CHECK!!!
✓ Made Glycerol Stocks - Margaret, Krissy
    ✓ pIG_017 #2 (2 tubes) [Note: one tube was 300 uL glycerol and 300 uL pIG_017 #2]
    ✓ pIG_055, pIG_056, pIG_098
✓ PCR remaining gBlocks (Ethan, Melody)-- Annealing: 65C, Elongation time: 45 sec
    ✓ MIT gBlock_012 [IL-8 (CXCL-8)] - don't have reverse primer
        ✓ Run with 12's reverse primer
        ✓ Run with 14's reverse primer
    ~~✓ MIT gBlock_013 [IL-8-Flag (CXCL-8)] - Ethan, Margaret, Krissy, Melody~~
    ✓ MIT gBlock_014 [IL-8-NeonGreen (CXCL-8)] - don't have reverse primer
        ✓ Run with 12's reverse primer
        ✓ Run with 14's reverse primer
✓ Run PCRs in gel to verify
    - 12 w/ 12pr
    - 14 w/ 14pr
    - 12 w/ 14pr
    - 14 w/ 12pr

DNA ladder	12 w/ 12 pr	12 w/ 14 pr	14 w/ 12 pr	14 w/ 14 pr	14 w/14 pr	DNA ladder

✓ PCR purify gBlocks after PCR (12, 14) - Margaret
✓ Transform GG reactions
    ✓ (100-102, 48-pcr purified and 48-not pcr purified)
✓ Gel looks weird, decided that gBlock 14 needs to be sequenced for us to be absolutely sure; sent in for reverse reaction sequencing
    ✓ Protocol above

- ❏ Golden Gate with PCR gBlock 12 and 14 (verified after running in gel + sequencing)



Thursday/Friday:

- ❏ If other Redo digestion ligation reaction with digested backbone 17 + gBlock 13 (for pIG_048, pL0-IL8-Flag)
- ❏ Digest backbone 17 next week if we want?

Thursday 7/18/19

Protocol

1) PCR mix gBlock 12 + 14 (total is 25ul/reaction in tube)
    a) H_2O (NFW) 9 ul
    b) Primer forward 1.25 ul
    c) Primer reverse 1.25 ul (gB012R for 12, gB014R for 14)
    d) Template (gBlock) 1 ul (only want 1ng/50ul reaction -- make sure this is the case/you know the DNA template concentration)
    e) Q5 Mix 12.5 ul

Lab

- ✓ Check GeneWiz sequencing results; both redos and gBlock 13
- ✓ PCR gBlock 12 and gBlock 14 (Melody, Margaret, Krissy)
- ✓ Run gel with PCR products: gBlock 12 and gBlock 14

DNA Ladder	gBlock 12	gBlock 14

    - First gel was weird/was an old gel so this was rerun!

- ✓ Inoculate Golden Gate reactions for 100-102
  - Select multiple of the red colonies + sequence tomorrow to distinguish between Mkate2 vs backbone
  - 3 copies of each
- ✓ Make glycerol stocks:

Glycerol Stocks
L1 54 (labeled 58) - x
L0 47 (7.9.19) - x
L0 48 (7.9.19) - x
L1 57 (labeled 53) (7.10.19) - x
L1 58 (labeled 54) (7.10.19)- x
L1 97 (labeled 99) (7.10.19) - x

- ✓ Inventory CHECK and **throw out** incorrect sequences!!!
- ✓ Transformation Results:
  - No Colonies: No DNA
  - All red colonies: pIG_100-102 (Mkate2)
  - All Blue colonies: pIG_48, 48p (IL-8 Flag)
  - Many colonies: puc19

Change this	To this
~~Working Stock gBlock 12~~	~~14~~
~~Working Stock gBlock 14~~	~~12~~
~~DNA pIG_058_2 (7.16.19)~~	~~pIG_054~~
~~DNA pIG_053 (7.11.19)~~	~~pIG_057_1~~
~~DNA pIG_054 (7.11.19)~~	~~pIG_058~~
~~DNA pIG_099 (7.11.19)~~	~~pIG_097~~

Friday 7/19/19

Protocol

PCR purification protocol

a. Add 3 volumes of DNA binding buffer (Zymo) to each volume double stranded DNA
b. Load into Zymo spin column and place in 2 ml collection tube
c. Centrifuge @ max speed, 30 sec
d. Discard flow through
e. Add 200 ul Buffer PE (QIAGEN) and centrifuge @ max speed, 30 sec
f. Repeat step e
g. Discard flow through and spin for 1 min
h. Place column in new 1.5 ml eppendorf tube
i. Add 10 ul DEPC H20, wait 1 min (elute in same volume as started)
j. Spin @ max speed, 1 min

MoClo Golden Gate Assembly Protocol

pL1 MoClo Golden Gate protocol

Note: all buffers, enzymes, backbones and DNA parts stored at -20C

1. Identify your necessary parts and plan the construction

Plasmid ID	I	P	5	G	3	T
pIG_053	~~pIG_0001 Inert~~ ~~1 ul~~	~~pIG_002 hEF1a~~ ~~1 ul~~	~~pIG_005 Inert~~ ~~1 ul~~	~~MIT_gB012 IL8~~ ~~1 ul~~	~~pIG_012 Inert~~ ~~1ul~~	~~pIG_013 Synthetic PA~~ ~~1 ul~~

pIG_105	~~pIG_0001 Inert~~ ~~1 ul~~	~~pIG_002 hEF1a~~ ~~1 ul~~	~~pIG_005 Inert~~ ~~1 ul~~	~~pIG_103/pL0 NeonGreen~~ ~~1 ul~~	~~pIG_012 Inert~~ ~~1ul~~	~~pIG_013 Synthetic PA~~ ~~1 ul~~
pIG_106	~~pIG_0001 Inert~~ ~~1 ul~~	~~pIG_004 CMV~~ ~~1 ul~~	~~pIG_005 Inert~~ ~~1 ul~~	~~pIG_103/pL0 NeonGreen~~ ~~1 ul~~	~~pIG_012 Inert~~ ~~1ul~~	~~pIG_013 Synthetic PA~~ ~~1 ul~~
pIG_107	~~pIG_0001 Inert~~ ~~1 ul~~	~~pIG_003 TRE~~ ~~1 ul~~	~~pIG_005 Inert~~ ~~1 ul~~	~~pIG_103/pL0 NeonGreen~~ ~~1 ul~~	~~pIG_012 Inert~~ ~~1ul~~	~~pIG_013 Synthetic PA~~ ~~1 ul~~

2. Mix the DNA parts (1ul each of 40 fmol/ul stocks) -- should be a total of 6 ul

3. Add the following reagents (should all be kept on ice/ice block)

		MM
Backbone pL1 (ex. ST1-2)	0.5 ul	2ul ~2.2ul x
T4 ligase	0.5 ul	2ul ~2.2ul x
10x T4 buffer	2 ul	8ul ~8.8ul x
BsaI-HFv2 enzyme	1 ul	4ul ~4.4ul x
10x BSA buffer (NEB B9001)	2 ul	8ul ~8.8ul x
DEPC H20	8 ul	32ul ~35.2 ul x
pIG_001 Inert	1 ul	4ul ~4.4ul x
pIG_005 Inert (5')	1 ul	4ul ~4.4ul x
pIG_012 Inert (3')	1 ul	4ul ~4.4ul x
pIG_013 Synthetic PA	1 ul	4ul ~4.4ul x

TOTAL (including DNA)	18ul total + 2ul unique DNA	

Note: We can also make a master mix for multiple constructs (i.e. everything that's used*(however many constructs) with a little extra!!)

4. Thermal cycler (use one on my bench): ~2.5 hours for 10x cycles

iGEM folder --> "golden gate (BsaI, BsmbI)"

Heat lid	110 C	
Temp	37 C	20 min
Start cycle	10x	
	37 C	2 min
	16 C	5 min
Close cycle		
Temp	37 C	15 min
Temp	50 C	5 min
Temp	80 C	20 min
Store	8 C	

5. Transform bacteria as before and grow colonies on agar plates (Carb resistance), grow at 37C overnight

6. Miniprep

7. Test digest and run gel, send good clones to sequencing
NOW WE CAN USE THE PLASMID!!! :)

Lab

- ✓ Inoculation Results:
  - Tubes (pIG_100 1, 2, and 3, 101 1, 2 and 3, and 102 1, 2, and 3) were very cloudy and pink, inoculation appeared successful
- ✓ PCR Purify gBlock 12 PCR and run gel and purify gBlock 14 PCR from gel
  - 41.7 ng/ul for gBlock 12
- ✓ Golden Gate with PCR gBlock 12 ~~and 14~~ (verified after running in gel + sequencing)
  - Dilute backbone 1:10 with water (*noted from Nika)
  - Golden Gated: pIG_53, 105, 106, 107
  - pL0s that still need to be made: pIG_049
  - pL1s that still need to be made: pIG_099

- ✓ Miniprep Inoculated cultures (100_1-3, 101_1-3, 102_1-3)
- X Send mKate2 pL1s to sequencing

Monday 7/22/19

Protocol
MoClo Golden Gate Assembly Protocol
pL0 and pL1 MoClo Golden Gate protocol

Note: all buffers, enzymes, backbones and DNA parts stored at -20C

1. Identify your necessary parts and plan the construction

Plasmid ID	I	P	5	G	3	T
pIG_099 (pL1)	pIG_0001 Inert 1 ul	pIG_003 TRE 1 ul	pIG_005 Inert 1 ul	MIT_gB014 IL8-NeonGreen 1 ul / 2 ul	pIG_012 Inert 1ul	pIG_013 Synthetic PA 1 ul
pIG_049 (pL0)	Insert: MIT_gB014 IL8-NeonGreen 1 ul / 2 ul					

2. Mix the DNA parts (1ul each of 40 fmol/ul stocks) -- should be a total of 6 ul

3. Add the following reagents (should all be kept on ice/ice block)

Backbone pL1 (ex. ST1-2) / pL0 (pIG_017)	0.5 ul	Already put in
T4 ligase	0.5 ul	2.25
10x T4 buffer	2 ul	9
BsaI-HFv2 enzyme	1 ul	4.5
10x BSA buffer (NEB B9001)	2 ul	9
DEPC H20	8 ul / 7 ul	DETERMINE
TOTAL (including DNA)	20 ul	5.5ul/tube

Note: We can also make a master mix for multiple constructs (i.e. everything that's used*(however many constructs) with a little extra!!)

4. Thermal cycler (use one on my bench): ~2.5 hours for 10x cycles

iGEM folder --> "golden gate (BsaI, BsmbI)"

Heat lid	110 C	
Temp	37 C	20 min
Start cycle	10x	
	37 C	2 min
	16 C	5 min
Close cycle		
Temp	37 C	15 min
Temp	50 C	5 min
Temp	80 C	20 min
Store	8 C	

5. Transform bacteria as before and grow colonies on agar plates (Carb resistance), grow at 37C overnight

6. Miniprep

7. Test digest and run gel, send good clones to sequencing
NOW WE CAN USE THE PLASMID!!! :)

8. For pL0s: Add an extra T4 ligation step:
- Add 1 ul T4 ligase
- Let sit at room temp for ~30 minutes (or overnight if still not good)

Transformation

Fmlp stimulation

1. Coated four plates with 150 microliters of collagen IV.
    a. Let incubate for two hours at room temperature
2. Aspirate liquid from well without touching the collagen
3. Pipette 250 ul of RPMI to each well, pipette onto the wall of the well
4. Shake well with lid on
5. Aspirate RPMI without touching the collagen layer
6. 250 ul of differentiated cell in each well

a. Since collagen fibers did not seem to coat well, 1 ml of RPMI was added twice to wash the surface

Fmlp Stocks

1. Make 10 mM fmlp in DMSO -> 4.3755g per L or 4.3755 mg per ml
2. 5mg per 1.143 ml of water
3. Divide into 50 ul tubes, make ~20 tubes total

Fmlp Working Stocks

- Make 100 uM fmlp in RMPI

1. Add 50 ul of 10mM fmlp to 4.950 ml of RMPI
2. Divide into 200 ul tubes, make ~25 tubes

Lab

✓ Run mKate2 pL1s digest on gel - Krissy and Ethan
- Digest showed all as the L1 vector
  - During inoculation the red colonies were picked should have picked the white ones. Will redo inoculations from the same plates

*

Well #1	Well #2	Well #3	Well #4	Well #5	Well #6
Ladder from Gel Box	100 #2	100 #3	101 #1	101 #2	102 #2

*

Well #1	Well #2
Ladder from Gel box	102 #2

✓ Made new gels - Krissy, Ethan
- ✓ 4 small gels
- ✓ 3 large gels

✓ Inoculated white colonies from pIG_100 - pIG_102 (MKate2 pL1s) - Krissy

✓ Transformation of GGs (pIG_53, 105, 106, 107) - Margaret, Melody, Krissy
- ✓ CARB
  - ✓ pIG_053 (10ul DH5alpha)
  - ✓ pIG_105, pIG_106, pIG_107 (10ul DH5alpha)
  - ✓ pIG_099 v1 *note: 5 uL Stellar cells used
  - ✓ pIG_099 v2 = pcr product *note: 5 uL Stellar cells used
  - ✓ PUC19 (5ul DH5alpha)
  - ✓ No DNA *note: 5 uL Stellar cells used
- ✓ SPEC

        - ✓ pIG_049 v1 (5ul DH5alpha)
        - ✓ pIG_049 v2 = pcr product (5ul DH5alpha)
- ✓ Golden Gate (total: 4 rxns): Margaret, Melody
    - ✓ pIG_099 (L1: TRE_IL-8-NeonGreen) - w/ PCR gel purified gB014 (v2) and OG gB014 40nM stock (v1)
    - ✓ pIG_049 (L0: IL-8-NeonGreen) - w/ PCR gel purified gB014 (v2) and OG gB014 40nM stock (v1)
- ✓ Made fMLP working stocks - Miles, Ethan

Fmlp stimulation

-Left collagen for over two hours
-Collagen did not cover the entire plate, fibers were observed in the plate
-Washed the collage covering with 1ml of RPMI twice
-Use 500 ul of differentiated cells total
-Scratch test with pipette tip, collagen did not plate well
-Abandoned experiment due to bad collagen covering and lack of differentiated cells
-Suggestion: dilute collagen, dissolve in acetic acid, or change temperature

Tuesday 7/23/19

Protocol
MoClo Golden Gate Assembly Protocol (began; incomplete, stopped at adding DNA parts part)
Miniprep
Gel digest
Pouring and making gels
Inoculation

Lab

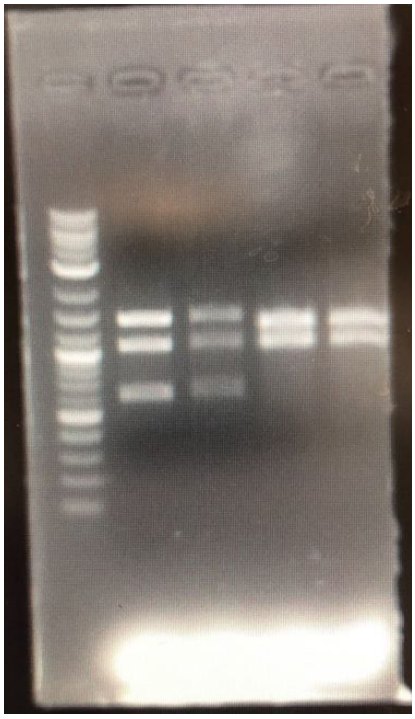
- ✓ Inoculation Results:
    - Tubes (pIG_101 1 and 2, 102 1 and 2) were very cloudy, inoculation appeared successful
- ✓ Transformation Results:
    - ✓ Many colonies: pUC19, pIG_49 v1, 99 v2
    - ✓ Few colonies: pIG_49 v2, 53, 99 v1, 105, 106, 107
    - ✓ No colonies: no dna
    - ✓ Fewer than 3 white colonies: pIG_53, 105, 106, 107
- ✓ Miniprep mKate2 pL1s: pIG_101 #1, pIG_101 #2, pIG_102 #1, pIG_102 #2 - Margaret
    - Note: Used Buffer P2 that may have had tip contamination but should be okay
    - ✓ Nanodrop - Margaret
        - ✓ 101 #1: 130.5 ng/uL
        - ✓ 101 #2: 75.0 ng/uL

- ✓ 102 #1: 126.6 ng/uL
- ✓ 102 #2: 116.7 ng/uL

✓ Gel digest for pIG_101 #1, pIG_101 #2, pIG_102 #1, pIG_102 #2 - Krissy, Margaret

- ✓ Note: used 3 uL miniprep, 1 uL ApaLI, 1 uL CutSmart buffer
- ✓ Note: ran for 25 minutes

DNA ladder	101 #1	101 #2	102 #1	102 #2



✓ Send to sequencing:

- ✓ mKate2 pL1s (101, 102)
- ✓ Copies of pIG_001-pIG_020 (whatever was not sent originally!)

✓ Inoculate transformations (only white colonies, ignore blue/red!) -- 2 copies each if possible: - Ethan

- ✓ L1 53: hEF1a_IL-8
- ✓ L1 99: TRE_IL-8-NeonGreen v1
- ✓ L1 99: TRE_IL-8-NeonGreen v2
- ✓ L0 49: IL-8-NeonGreen v1
- ✓ L0 49: IL-8-NeonGreen v2
- ✓ L1 105: hEF1a-NeonGreen
- ✓ L1 106: CMV-NeonGreen
- ✓ L1 107: TRE-NeonGreen

❏ Retransform mKate2 pIG_101 (L1: hef1a_mKate2) and GG rxns - **saved for tomorrow**

- Note: try with 2ul DNA, 10ul bacteria again; 100ul outgrowth

✓ Resuspend gBlock - Melody, Krissy

- ✓ MIT_gB015 - 50ng/ul

- ✓ MIT_gB016 - 50ng/ul
- ✓ MIT_gB017 - 100ng/ul
- ✓ MIT_gB010a - 100ng/ul
- ✓ MIT_gB001b/10b - 100ng/ul

✓ Also made working stock (40nM) of gBlocks - Melody, Krissy
- ✓ gB015
- ✓ gB016
- ✓ gB017

❏ Begin GGs for other chemokines - Krissy, Melody - **saved for tomorrow**

	CCL5 (gB015)	CCL5-Flag (gB016)	CCL5-NeonGreen (gB017)	C5a-NeonGreen (gB010a and 10b)
pL0	pIG_088	pIG_089	pIG_090	pIG_051
pL1 - hEF1a	pIG_091	pIG_093	pIG_095	pIG_061
pL1 - CMV	pIG_092	pIG_094	pIG_096	pIG_062
pL1 - TRE	pIG_108	pIG_109	pIG_110	pIG_111

- pIG_001 more working stock was made; **need to have more concentrated stock for later use; send other two copies to sequencing**

✓ Make TRE pL1 chemokine plasmid designs (in silico)
- ❏ **C5a-NeonGreen would not work?? - try digest and ligate**

✓ Transformed from glycerol stock:
- ✓ pIG_057 (hEF1a IL8-NeonGreen)

-Made 10 uM working stock of fmlp
- added 200 uL of 100 uM fmlp to 1800 uL rpmi

Wednesday 7/24/19

Protocol

pL1s	**G** ~~MIT_gB015~~ ~~CCL5~~ ~~1 ul~~	**G** ~~MIT_gB016~~ ~~CCL5-Flag~~ ~~1 ul~~	**G** ~~MIT_gB017~~ ~~CCL5-NeonGreen~~ ~~1 ul~~	**G** ~~MIT_gB010a + 10b~~ ~~C5a-NeonGreen~~ ~~1 ul + 1 ul~~
~~P~~ ~~pIG_002~~ ~~hEF1a~~	**pIG_091**	**pIG_093**	**pIG_095**	**pIG_061**

~~1 ul~~				
~~P~~ ~~pIG_003~~ ~~CMV~~ ~~1 ul~~	**pIG_092**	**pIG_094**	**pIG_096**	**pIG_062**
~~P~~ ~~pIG_004~~ ~~TRE~~ ~~1 ul~~	**pIG_108**	**pIG_109**	**pIG_110**	**pIG_111**

pL0s	~~MIT_gB015~~ ~~CCL5~~ ~~1 ul~~	~~MIT_gB016~~ ~~CCL5-Flag~~ ~~1 ul~~	~~MIT_gB017~~ ~~CCL5-NeonGreen~~ ~~1 ul~~	~~MIT_gB010a + 10b~~ ~~C5a~~ ~~1 ul~~
Plasmid ID	**pIG_088 (pL0)**	**pIG_089 (pL0)**	**pIG_090 (pL0)**	**pIG_051 (pL0)**

2. Mix the DNA parts (1ul each of 40 fmol/ul stocks) -- should be a total of 6 ul

3. Add the following reagents (should all be kept on ice/ice block)

		MM for pL1s	MM for pL0s
Backbone pL1 (ex. ST1-2) / pL0 (pIG_017)	0.5 ul	~~pIG_020 - 6ul ~ 6.6ul~~	~~pIG_017 - 2ul ~2.2ul~~
T4 ligase	0.5 ul	~~6ul ~ 6.6ul~~	~~2ul ~ 2.2ul~~
10x T4 buffer	2 ul	~~24ul ~ 26.4ul~~	~~8ul ~8.8ul~~
BsaI-HFv2 enzyme	1 ul	~~12ul ~ 13.2ul~~	~~4ul ~4.4ul~~
10x BSA buffer (NEB B9001)	2 ul	~~24ul ~ 26.4ul~~	~~8ul ~8.8ul~~
~~pIG_0001 - Inert (pL1s only)~~	~~1 ul~~	~~12ul ~13.2ul~~	
~~pIG_0005 - Inert (pL1s only)~~	~~1 ul~~	~~12ul ~13.2ul~~	
~~pIG_012 - Inert (pL1s only)~~	~~1 ul~~	~~12ul ~13.2ul~~	
~~pIG_013 - Synthetic PA (pL1s only)~~	~~1 ul~~	~~12ul ~13.2ul~~	
~~DEPC H20~~	~~7-8 ul / 11-12 ul~~	determine!	determine!
TOTAL (including DNA)	20 ul		

Note: We can also make a master mix for multiple constructs (i.e. everything that's used*(however many constructs) with a little extra!!)

4. Thermal cycler (use one on my bench): ~2.5 hours for 10x cycles

iGEM folder --> "golden gate (BsaI, BsmbI)"

Heat lid	110 C	
Temp	37 C	20 min
Start cycle	10x	
	37 C	2 min
	16 C	5 min
Close cycle		
Temp	37 C	15 min
Temp	50 C	5 min
Temp	80 C	20 min
Store	8 C	

5. Transform bacteria as before and grow colonies on agar plates (Carb resistance), grow at 37C overnight

6. Miniprep

7. Test digest and run gel, send good clones to sequencing
NOW WE CAN USE THE PLASMID!!! :) **(began; incomplete, stopped at adding DNA parts part - 7/23)**

FMLP Stimulation

- 300 ul of gelatin in each well
- sit at room temperature for 5 minutes
- aspirate remaining liquid from the wells
- 250 ul of differentiated cells in one well and 250 ul of undifferentiated cell to another well (control)
    - Have third well of just RPMI media
- Image each well (experimental and control) under zeiss microscope (10x magnification)
    - Timelapse for 2 minutes (10s interval)
    - Add 250 ul of 200 nM FMLP to well
        - Final concentration of 100 nM
    - Timelapse for 8 more minutes (10s interval)

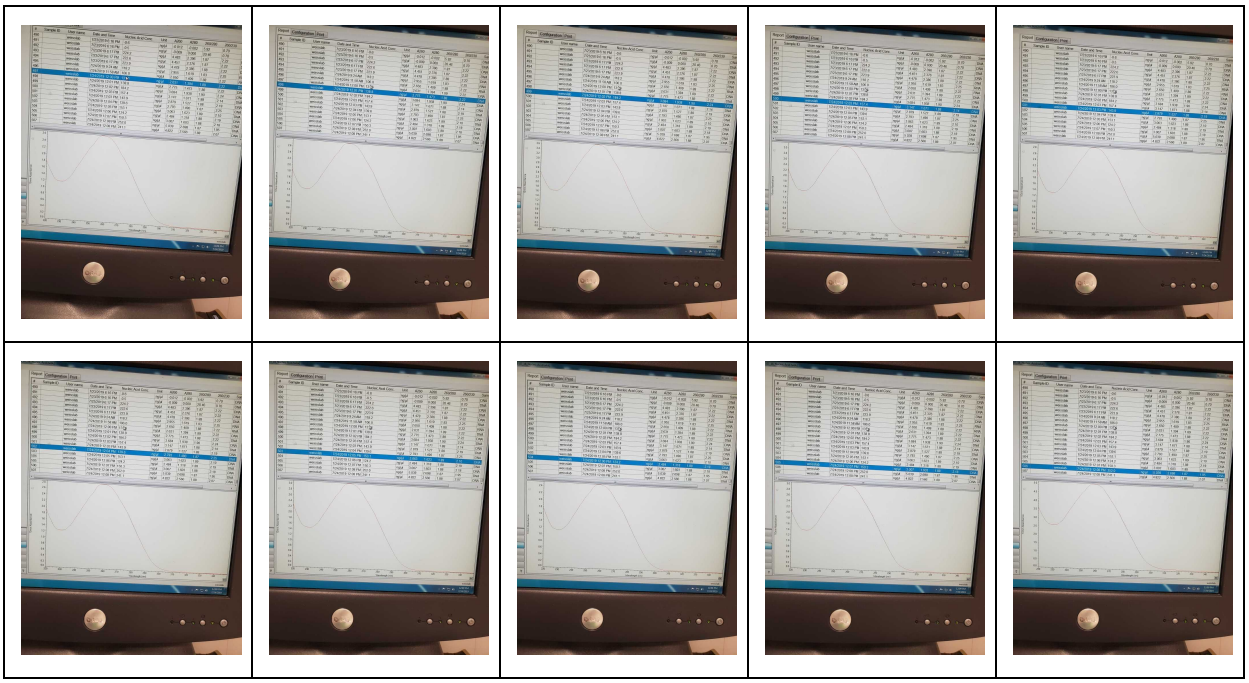
Lab

- ✓ Inoculation Results:
  - Tubes (pIG_53,99 v1, 99 v2, 49 v1, 49 v2, 105, 107) were very cloudy, inoculation appeared successful
  - pIG_53 1 had an orange tint, but 2 had a more cloudy tint like the rest
  - 53_1 and 53_2 were both pink-- thrown out; need to pick more colonies/transform again; 106 also was pink-- thrown out
- ✓ Transformation Results:
  - ✓ Many colonies: pIG_057 (hEF1a IL8-NeonGreen)
- ✓ Sequencing Results:
  - All inerts worked; proceeded to make working stocks of all of them which were then used for GG
  - For mKate2: 101 and 102 → Questionable - we should ask the mentors
- ✓ Golden-Gate other chemokines - Melody

	CCL5 (gB015)	CCL5-Flag (gB016)	CCL5-NeonGreen (gB017)	C5a-NeonGreen (gB010a and 10b)
pL0	pIG_088 (possibly extra master mix)	pIG_089 (possibly extra master mix)	pIG_090	pIG_051
pL1 - hEF1a	pIG_091	pIG_093	pIG_095	pIG_061
pL1 - CMV	pIG_092	pIG_094	pIG_096	pIG_062
pL1 - TRE	pIG_108	pIG_109	pIG_110	pIG_111

- ✓ Miniprep inoculations: pIG_53, 99 v1, 99 v2, 49 v1, 49 v2, 105, 106, 107
  - ✓ Nanodrop

    pIG_49 v1 1:131.6, 2:138.8
    pIG_49 v2 1:184.2, 2:157.4
    pIG_99 v1 1:143.9, 2:139.6
    pIG_99 v2 1:153.1, 2:124.1
    pIG_105: 150.3
    pIG_107: 143.9



- ✓ Gel digest
  - ✓ In order of: Ladder, 49 (v1 #1, v1 #2, v2 #1, v2 #2), 99 (v1 #1, v1 #2, v2 #1, v2 #2,), 105, 107
  - ✓ Image: 
  - ✓ **Gel analysis conclusions: 49 looks right, 99 v2 looks right; 99 v1 #1 definitely looks like pL1 backbone; 105 looks right; 107 looks right**
- ✓ Send for sequencing pIG_49 v1 #2, v2 #1, 99 v2 #1 and #2, 105, 107

✓ Inoculate pIG_57 (select single colonies)

- ✓ 3 cultures picked and shaking in incubator at 37C

-Performed fmlp stimulation with gelatin coating
-3 wells: differentiated cells, rpmi media, and undifferentiated cells
-Time lapse images taken every 10 sec for 10 minutes
    -250 ul of 200uM fmlp added 2 minutes after
    -Final concentration of fmlp is 100 ul
-Images were saved in Malik's dropbox

Tomorrow:

- ❏ Retransform mKate2 pIG_101 (L1: hef1a_mKate2) and transform GG rxns (in -20 in thermocycler for other chemokines and also for 53 and 106)
    - Note: try with 2ul DNA, 10ul bacteria again; 100ul outgrowth
- ❏ Make TRE pL1 chemokine plasmid designs (in silico)
    - **C5a-NeonGreen would not work?? - try digest and ligate -- need to ask Deepak (again)**
- X Inventory check for existing glycerol stocks (make new subsection) and confirm pL1s

Thursday 7/25/19

Protocol

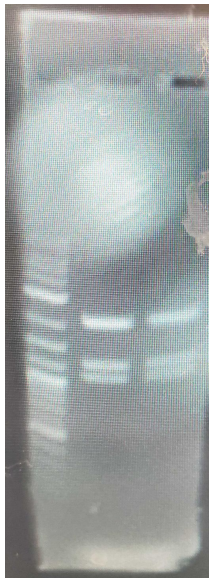
Transformation

Lab

- ✓ Inoculation Results:
    - Tubes (pIG_57 #1, #3) were very cloudy, inoculation appeared successful
    - pIG_57 #2 was clear, not successful
- ✓ Sequencing Results:
    - ✓ Failed: 107, 49_1, 105 (unsure about 105, won't recognize contigs in design…??)
    - ✓ Worked:
    - ✓ Questionable: 49_2, 99_1, 99_2 → will ask genewiz to try the forward again because the reverse worked great
- ✓ Miniprep 57 #1 & #3
    - ✓ Note: Buffer P2 had chunky salts -> put in 37C for 10 minutes to remove salts
    - ✓ Nanodrop:
        - ✓ pIG_057 #1: 304.2
        - ✓ pIG_057 #3: 288.7
    - ✓ Gel digest and Gel electrophoresis:
        - ✓ pIG_057 #1: 304.2
        - ✓ pIG_057 #3: 288.7
        - ✓ Results: No need to send pIG_057 to sequencing since gel cut sites are correct on the gel

Well #1	Well #2	Well #3
Ladder NEB 2log	pIG_057_1	pIG_057_3

ApaLI (1071 bp, 1250bp, 2158 bp)



- ✓ Retransform mKate2 pIG_100 (L1: hef1a_mKate2) and transform GG rxns (in -20 in thermocycler for other chemokines and also for 53 and 106)
    - Used stellar cells instead of DH5alpha because no more cells
    - Note: try with 2ul DNA, 10ul bacteria again; 100ul outgrowth
    - pIG_100, pIG_053, pIG_106 (Note: used 10 uL stellar cells)
    - GG rxns (Note: 5 uL stellar cells):

pIG_088 (possibly extra master mix)	pIG_089 (possibly extra master mix)	pIG_090	pIG_051
pIG_091	pIG_093	pIG_095	pIG_061
pIG_092	pIG_094	pIG_096	pIG_062
pIG_108	pIG_109	pIG_110	pIG_111

- ✓ Make TRE pL1 chemokine plasmid designs (in silico)
    - **<u>C5a-NeonGreen would not work?? - try digest and ligate -- need to ask Deepak (again)</u>**
- ✓ Inventory check for existing glycerol stocks (make new subsection) and confirm pL1s
- X Order primers with overhangs for IL8s with synthetic secretion tag (IL8-syntag, IL8-NeonGreen-syntag, IL8-Flag-syntag) -- if IL8-secretion test turns out bad
- X Make more LB agar plates with antibiotic!!

Friday 7/26/19

Lab

- ✓ Transformation Results:
  - ✓ Many Colonies: pIG_51, 53, 88, 89, 90, 92, 93, 94, 95, 96, 100, 106, 108, 109, 110, 111
  - ✓ Few colonies: 61, 62 (both more than 10, not going back in incubator)
  - ✓ No Colonies: 91 (back in incubator)
  - ✓ 51, 88, 89, 90 have tons of blue and some white
  - ✓ 53 and 100 have tons of red with some white
- ✓ MIT MUSEUM WORKSHOP

Monday 7/29/19

Protocol

[Making and pouring agarose gels]

Lab

- ✓ Inoculate transformations pIG_51, 53, 61, 62, 88, 89, 90, 92, 93, 94, 95, 96, 100, 106, 108, 109, 110, 111 - Ethan, Melody
  - GGs (pIG_53, 100, 106) + GGs with C5a and CCL5

pL0s	pIG_088 (possibly extra master mix)	pIG_089 (possibly extra master mix)	pIG_090	pIG_051
pL1s with hEF1a	**pIG_091**	pIG_093	pIG_095	pIG_061
pL1s with CMV	pIG_092	pIG_094	pIG_096	pIG_062
pL1s with TRE	pIG_108	pIG_109	pIG_110	pIG_111

- ✓ Made more gels - Melody
- X Transformation for 91, 100, 105, 107 (again; testing again!) - Melody (dependent on whether we can get cells… from Deepak)

Tomorrow:

- Double-check with mentors 49, 99, 100, 101, 102, 105-107 and see if GeneWiz got back to us on that? → if not, may need to make again; if good, update inventory with "verified" status! (sequencing analysis notes)
    - 49: reverse works, no forward; reverse does not go all the way through
    - 99-1: reverse works, forward works
    - 99-2: reverse works, forward works
    - 101:
    - 102:
    - 105:
    - 106:
    - 107:

Tuesday 7/30/19

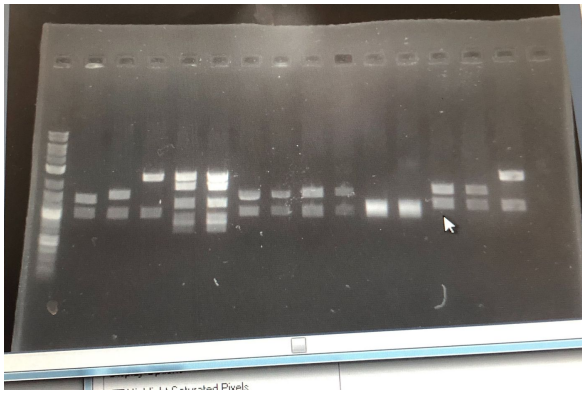
Protocol
Miniprep
Nanodrop
Gel digest
Transformation

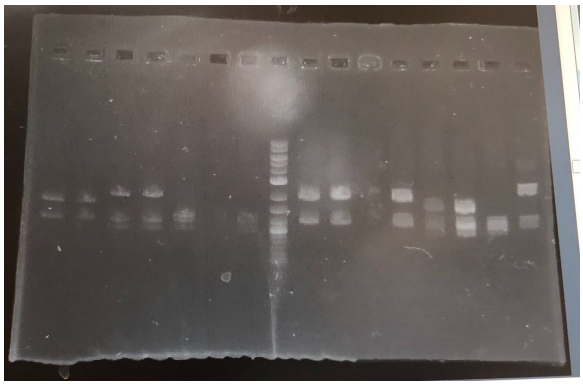
Lab

- ✓ Inoculation Results: pIG_51, 53, 61, 62, 88, 89, 90, 92, 93, 94, 95, 96, 100, 106, 108, 109, 110, 111
    - Most tubes were cloudy and beige: 51_1, 51_2, 53_2, 61_1, 61_2, 88_1, 88_2, 89_1, 89_2, 90_1, 90_2, 92_1, 92_2, 93_1, 93_2, 94_1, 94_2, 95_1, 95_2, 96_1, 96_2, 106_1, 108_1, 108_2, 109_1, 109_2, 110_1, 110_2, 111_1, 111_2
    - pIG_62 #1 and #2 were pink
    - 100 #2 had a slight pink tint
    - Failed: pIG_53 #1, 100 #1, 106 #2 -> Reinoculate
- ✓ Miniprep the inoculations - Ethan, Melody, Margaret
    - ✓ Nanodrop (see spreadsheet)
    - ✓ Gel Digest - Melody, Margaret

Ladder	51_1	51_2	53_2	61_1	61_2	88_1	88_2	89_1	89_2	90_1	90_2	92_1	92_2	93_1	93_2

94_1	94_2	95_1	95_2	96_1	96_2	106_1	Ladder	108_1	108_2	109_1	109_2	110_1	110_2	111_1	111_2





- ✓ Send to sequencing
  - ❏ 51_2, 53_2, 61_1, 61_2, 88_1, 89_2, 90_2, 92_1, 93_1, 94_2, 95_1, **96_1**, **106_1**, 108_2, 109_1, **110_2**, 111_2
- ✓ Reinoculate pIG_53, 100, and 106 (get from incubator tmrw morning) - Margaret
  - ✓ pIG_053 #3
  - ✓ pIG_100 #3
  - ✓ pIG_106 #3
- ✓ Retransform:
  - ✓ pIG_091, pIG_100, pIG_105, pIG_107, pIG_062

Wednesday 7/31/19

Protocol

Lab

- ✓ !! need DH5alpha cells from deepak !!
- ✓ !! need help with plasmid designs for 105, 106, 107, and 111
- ✓ Make new plates
  - ❏ Carb
- ✓ Inoculation Results:
  - ✓ All tubes cloudy and successful: pIG_53 3, 100 3, 106 3
- ✓ Transformation Results:
  - ✓ Many colonies: pIG_62, 91, 100, 105, 107

- ✓ No colonies (might be because used wrong antibiotic): pUC19

✓ Sequencing results/update (need to put this in cloning team progress doc):

- ❏ pL0s:
  - ✓ 88
  - ✓ 89
  - ✓ 90
  - ❏ 51 - high background; not sequenced?
- ❏ pL1s:
  - ❏ CCL5:
    - ❏ 91 (was not sequenced; was transformed again)
    - ❏ 92 → looks like 108
    - ❏ 108 → looks like 92
  - ❏ CCL5-Flag:
    - ❏ 93 → 91
    - ❏ 94 → 109
    - ❏ 109 → 94
  - ❏ CCL5-NeonGreen:
    - ✓ 95
    - ❏ 96 → 110
    - ❏ 110 → 96
  - ❏ C5a-NeonGreen:
    - ❏ 61
    - ❏ 62 (was not sequenced; was transformed again)
    - ❏ 111
- ❏ 53 → looks like pL0 hEF1a??!
- ❏ 106 →

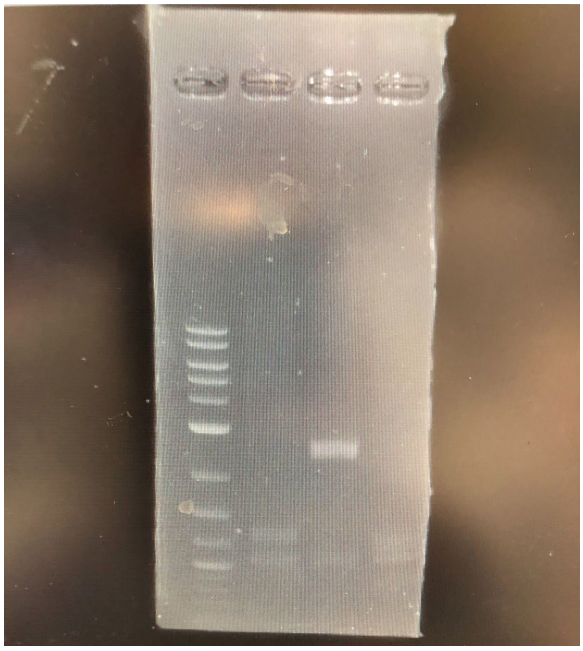
✓ Inoculate transformations: pIG_91, 100, 105, 107

- ✓ Note: pIG_062 did not have any white colonies twice therefore we did not inoculate it, must be GG again
- ❏ Note: did duplicates for the rest

✓ Miniprep inoculations for 53 #3, 100 #3, 106 #3

- ✓ Nanodrop
  - ❏ See plasmid inventory spreadsheet
- ✓ Gel digest

DNA Ladder	53 #3	100 #3	106 #3



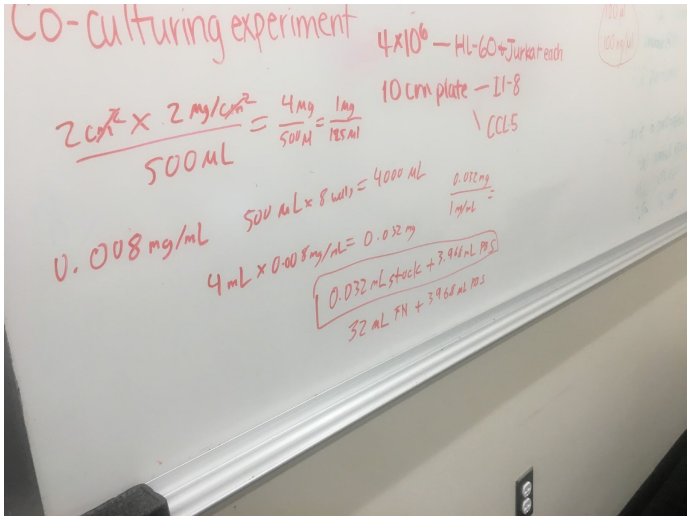
    - ❏ Send to sequencing
- X Send for sequencing again the mixed up ones?
    - ❏ See what happens… XD
- ✓ Made glycerol stock of 95 #1 and #2 (not verified by sequencing, but gel digest looks the same)
- ✓ Update Cloning Team Progress doc
- ✓ Note to ask tomorrow: Could we do a midi/maxi prep for major constructs that culture team will need?
- ● GG pIG_62 again, had no white colonies twice

Thursday 8/1/19

Protocol

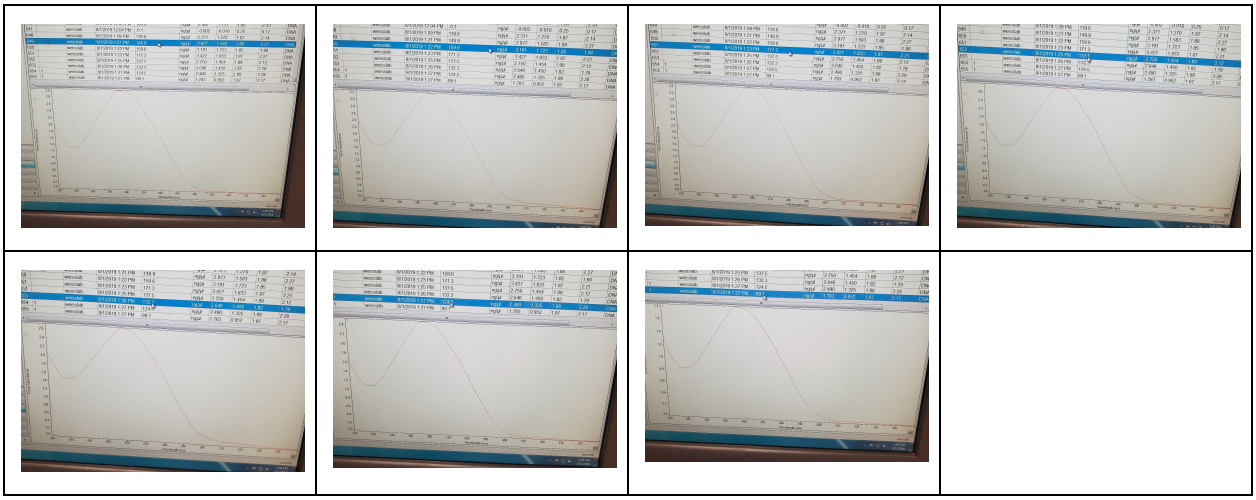
Fibronectin Coating

- Dilute Fibronectin with PBS to create 2 ug/cm^2
    - 32 ul of FN and 3968 ul of PBS
- 500 ul of FN-PBS solution in each well
- Incubate in 37 degrees for 60 minutes
- Wash with 600 ul of PBS
- Store with 500 ul of PBS/well in ziplock bag at 4 degrees Celsius
- Enough fibronectin for 250 24-wells



Lab

- ✓ Resuspend twist order gblocks (in 10uL NFW)
- ✓ Miniprep inoculations: pIG_91, 100, 105, 107 (Krissy)
  - ✓ Nanodrop (Krissy)
    - ✓ pIG_091_1 → 148.9
    - ✓ pIG_091_2 → 159.6
    - ✓ pIG_100_1 → 171.3
    - ✓ pIG_100_2 → 137.5
    - ✓ pIG_105_2 → 132.5
    - ✓ pIG_107_1 → 124.5
    - ✓ pIG_107_2 → 89.1



- ✓ Restriction Digest (Krissy)
- ✓ Gel (Krissy, Ethan)
- ✓ Send to sequencing (Krissy, Melody)

- Golden Gate pIG_062 again since no white colonies for inoculation (CMV C5a-NeonGreen) → table for Friday/next Monday because not needed urgently for experiments

✓ Inoculation Results:

- ✓ All tubes cloudy and successful:pIG_91, 100, 105, 107
- ✓ pIG_ 107 2 was cloudy, but not as cloudy as others, left in incubator for 1 hour extra
- ✓ pIG_105 was pink so we did not miniprep

✓ Send for sequencing again the mixed up ones and new ones

-

53 #3	100 #3	106 #3

- ✓ CCL5: 91 (miniprepped today), 92, 108
- ✓ CCL5-Flag: 93, 94, 109
- ✓ CCL5-NeonGreen: 96, 110
- ✓ C5a-NeonGreen: 62, 111
- ✓ 51? → may need to inoculate again?/send 51_1 miniprep
- ✓ 105, 107 (miniprepped today)

✓ Make glycerol stocks for:

- ✓ 99
- ✓ 49
- ✓ 88
- ✓ 89
- ✓ 90
- ✓ 61_1 and 61_2

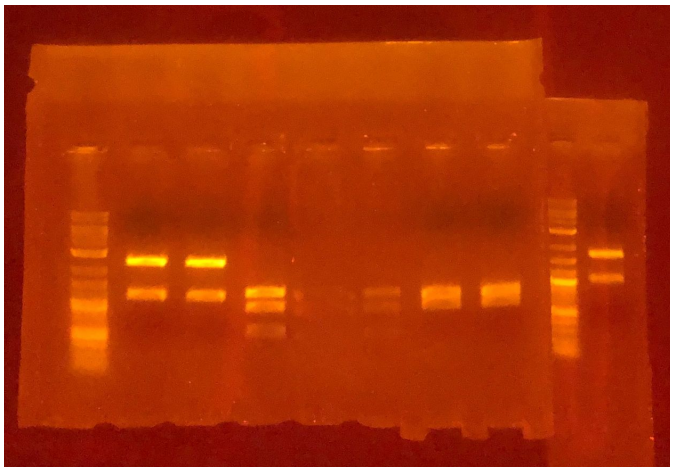
✓ **Check and plan midipreps (for culture team)**

- ✓ https://docs.google.com/spreadsheets/d/1_TaBUQNXNSmJhLGvloaUhXIGUE-xlHvyvirJxWuZ6sw/edit#gid=0
- ❏ IL8
  - ❏ pIG_053: hEF1a_IL-8 (still needs to be sequenced and confirmed)
  - ✓ pIG_054: CMV_IL-8
  - ✓ pIG_097: TRE_IL-8
- ❏ CCL5
  - ❏ pIG_091: hEF1a_CCL5 (still needs to be sequenced and confirmed)
  - ❏ pIG_092: CMV CCL5 (could be swapped with 108)
  - ❏ pIG_108: TRE CCL5 (could be swapped with 92)
- ❏ IL8-NeonGreen
  - ✓ pIG_057: hEF1a_IL-8-NeonGreen
  - ❏ pIG_099: TRE IL-8-NeonGreen
- ❏ CCL5-NeonGreen
  - ❏ pIG_095: hEF1a CCL5-Neon Green
  - ❏ pIG_110: TRE CCL5-NeonGreen (could be swapped with 96)
- ❏ NeonGreen

        - ❏ pIG_104: hEF1a NeonGreen (Grab more from Nika)
- ❏ Plate from glycerol stocks the above first/take from cultures^ & then begin midipreps tomorrow/Monday!
- ✓ **Note**: in the future, we won't be needing anything with Flag because we don't have enough TIME!!
    - ❏ Future GG: 105-107, 62, 111, 51, and basically anything we don't have glycerol stock or if we need it still
- ✓ Ran gel:

DNA Ladder	91 1	91 2	100 1	100 2	105 2	107 1

DNA Ladder	108 2



Friday 8/2/19

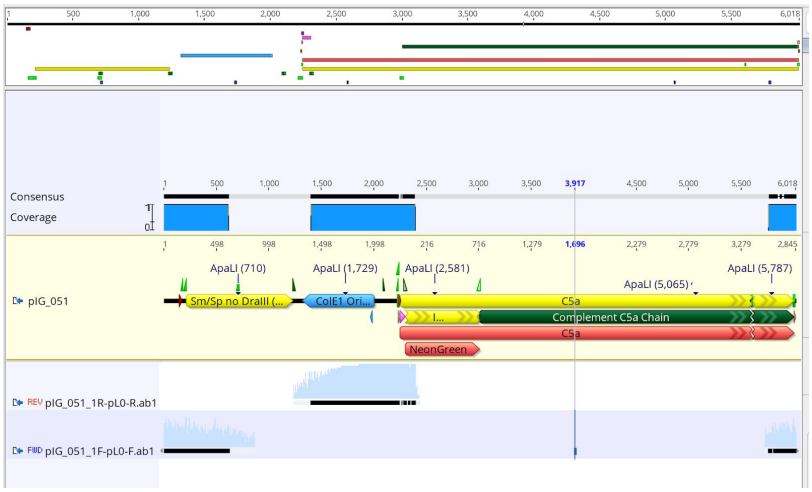
Protocol

Jurkat Stimulation

- Make 100 nM working stocks of CCL5 in serum free RPMI (1% BSA)
- Starved jurkat cells attached to tc flask
- Ran much longer timelapse of starved jurkats
- Used 900 ug of cells

Lab

- ✓ Transformation Results:
    - ✓ Many colonies: pIG_54, 57, 97, 95 1, 95 2
- ✓ Sequencing Results:
    - ~~51 - hard to tell if there's any completely correct parts?~~



- **53** - looks like hEF1a pL0 → need to GG again
- 91 → looks like 91 → make glycerol stock
- 92 → looks like 108 → make glycerol stock from 108 (label as 108; change miniprep labels from 108 to 92)
- ~~**93** → looks like 91~~
- ~~**94** → looks like 109~~
- 108 → looks like 92 → make glycerol stock from 92 (label as 108; change minipreps labels from 92 to 108)
- ~~109 → looks like 92~~
- 96 → looks like 110
- 110 → looks like 96
- ~~100 - only mKate2 gene matches~~
- ~~105 - didn't work~~
- ~~107 - didn't work (might be backbone, but didn't check)~~
- ~~106 - a mutation/sequencing is a bit bad but seems to relatively be matching? → nvm, seems to match 103 (pL0 NeonGreen)~~
- 111 → 62??/???

** all strike-throughs we are ignoring for now, but will probably need to be addressed if needed later on

- ❑ Check and plan midipreps:
  - ✓ https://docs.google.com/spreadsheets/d/1_TaBUQNXNSmJhLGvloaUhXIGUE-xlHvyvirJxWuZ6sw/edit#gid=0
  - ❑ IL8
    - ❑ pIG_053: hEF1a_IL-8 (still needs to be GG correctly)
    - ✓ pIG_054: CMV_IL-8
    - ✓ pIG_097: TRE_IL-8
  - ❑ CCL5
    - ✓ pIG_091: hEF1a_CCL5 → **make glycerol stock**
    - ✓ pIG_092: CMV CCL5 (swapped with 108) → **make glycerol stock**
    - ✓ pIG_108: TRE CCL5 (swapped with 92) → **make glycerol stock**

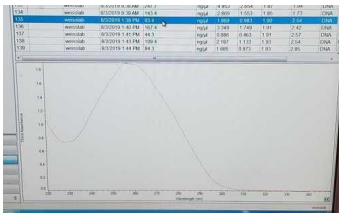
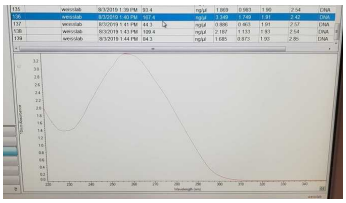
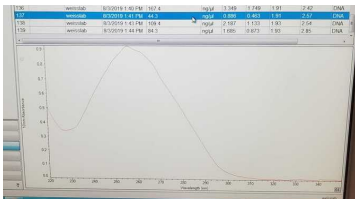
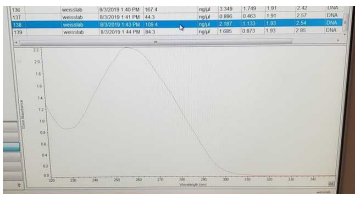
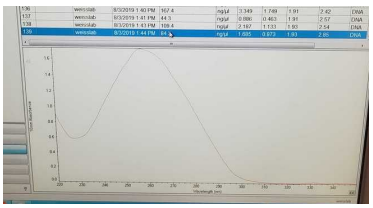
- ❏ IL8-NeonGreen
  - ✓ pIG_057: hEF1a_IL-8-NeonGreen
  - ✓ pIG_099: TRE IL-8-NeonGreen
- ❏ CCL5-NeonGreen
  - ✓ pIG_095: hEF1a CCL5-NeonGreen
  - ✓ pIG_096: CMV CCL5-NeonGreen → **make glycerol stock**
  - ✓ pIG_110: TRE CCL5-NeonGreen (swapped with 96) → **make glycerol stock**
- ❏ NeonGreen
  - ✓ pIG_104: hEF1a NeonGreen (Grab more from Nika)

Saturday 8/3/19

Protocol
Midiprep

Lab

- ✓ Midiprep + Nanodrop: - Krissy, Melody
  - ✓ 54 → 93.4 ng/ul
  - ✓ 57 → 167.4
  - ✓ 95_1 → 44.3
  - ✓ 95_2 →109.4
  - ✓ 97 → 84.3
- ✓ All were at/around 100ng/ul but in 150ul

Sunday 8/4/19

Lab

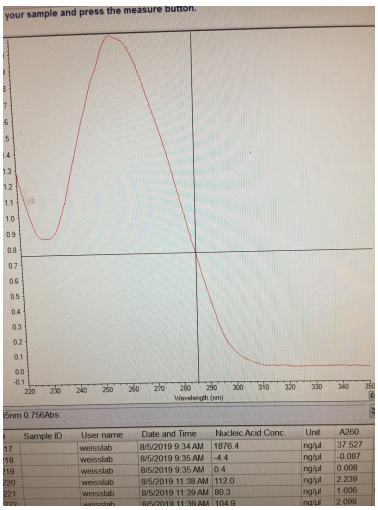
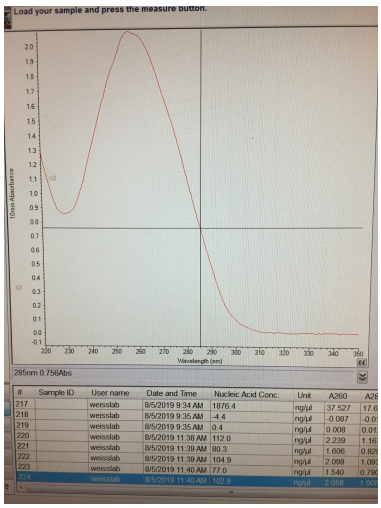
Came in and inoculated cultures to be miniprepped tomorrow - Melody (only ones really needed are 91 and 92, others are TRE versions if necessary)

- ✓ Make glycerol stocks for:
  - X 53 - looks like hEF1a pL0 → need to GG again
  - ✓ 91
  - ✓ 92
  - ✓ 108
  - ✓ 96
  - ✓ 110
- ❑ 53 - looks like hEF1a pL0 → need to GG again & transform
- ❑ Transform pIG_104 (from Nika)
  - ❑ Next week:
    - ❑ Inoculate
    - ❑ Miniprep
    - ❑ Nanodrop
    - ❑ Gel Digest

Monday (NEGEM) 8/5/19

Lab

- ✓ Midipreps + nanodropped (Krissy and Ethan)
  - 91
  - 92
  - 99
  - 108
  - 110

Tuesday 8/6/19

Lab

- ❏ 53 - looks like hEF1a pL0 → need to GG again & transform (save for next week)
- ✓ Transform SynNotch plasmids: pIG_112- pIG_116 - Shiva
- ✓ Transform pIG_104 (from Nika) - Margaret

Wednesday 8/7/19

Protocol

Lab

- ✓ Transformation Results: very successful, almost the entire dish is covered in colonies, but still some individual colonies good for inoculation
- ✓ Inoculate pIG_104 (from Nika) and pIG_112 - pIG_116 (Shiva's SynNotch plasmids) that she transformed (carb) (Krissy, Margaret, Melody)
- ✓ Plate from Glycerol Stock - to be midi-prepped (Margaret, Krissy, Melody)
    - ✓ pIG_029: inert_hEF1a_inert_EYFP_inert_SynthpA
    - ✓ pIG_033: inert_hEF1a_inert_TagBFP_inert_SynthpA
- ❏ Chemoattractant dilutions
    - ✓ IL8
        - ❏ Dissolve into 11.9 uL water for 100 uM solution
        - ❏ Incorrect dilution below, check thursday protocol
        - ❏ 9 uL of 100 uM into 991 water for 1000nM solution
        - ❏ 180 uL of 1000 nM into 1820 uL RPMI with 20% FBS for 100 nM solution
        - ❏ 18 uL of 1000 nM into 1982 uL RPMI 20% FBS for 10 nM solution
        - ❏ 9 uL of 1000 nM in 9991 uL RPMI 20% FBS for 1 nM solution

        - ❏ 180 ul of 1 nM into 1820 uL of RPMI with 20% FBS for 0.1 nM solution
    - ✓ Fmlp
        - ❏ Work with 100 uM solution
        - ❏ Desired concentration: 100 nM
        - ❏ 1/1000 dilution factor
        - ❏ 9 ul of 100 uM solution in 9991 ul of RPMI in 20% FBS

Thursday 8/8/19

Lab

- ✓ Inoculation Results: successful, all cloudy
- ✓ Inoculate for midiprep - Krissy, Margaret
    - ✓ pIG_029: inert_hEF1a_inert_EYFP_inert_SynthpA
    - ✓ pIG_033: inert_hEF1a_inert_TagBFP_inert_SynthpA

- ✓ Take from glycerol stock & plate following (in 37C!!): - Melody, Gabi

pIG_035	pL1	**inert_TRE_inert_EYFP_inert_SynthpA**
pIG_037	pL1	**inert_TRE_inert_mKO2mod_inert_SynthpA**
pIG_038	pL1	**inert_TRE_inert_TagBFP_inert_SynthpA**

- ✓ Miniprep - Krissy, Margaret
    - ✓ pIG_104 (from Nika) and pIG_112 - pIG_116 (Shiva's SynNotch plasmids) that she transformed (carb)
        - ✓ Nanodrop results
            - ✓ Very high for SynNotch parts! -- need to ask Shiva for her SynNotch plasmids); alright concentrations for 91, 92, 54, 57, 95, 97 (constitutive IL8 and CCL5 constructs as well as transformation protocol?
- ✓ Prepare for midiprep of pIG_104 (from Nika) and pIG_112 - pIG_116 (Shiva's one TRE IL8?) - Krissy, Margaret, Melody, Gabi

pIG_104		
pIG_112	pL1	TRE-tight-mkate (SRS07 175)
pIG_113	pL1	hEF1a-synCD19 (SRS05 27)
pIG_114	pL1	hEF1a-CD19 (SRS04 25)
pIG_115	pL1	hEF1a-EYFP (SRS02 10)
pIG_116	pL1	hEF1a-mKate (SRS03 174)

Note from Melody: Please beware that I may have mixed up 112 and 113 in a rush -- I don't know if it would be best to run a gel digest after midiprep on a few microliters of it… XD sorry--brainfart.

Made glycerol stocks of the above just in case as well - Melody, Gabi

Friday 8/9/19

Protocol

Annealing Oligos

1. Set up reaction in PCR tubes

Nuclease-free TE		7.5 ul
1M NaCl	0.5 ul	
Primer 1 (forward)		1 ul
Primer 2 (reverse)		1 ul
Nuclease-free TE		23.5 ul
3M NaCl	0.5 ul	
Primer 1 (forward)		3 ul
Primer 2 (reverse)		3 ul

2. Thermal cycler: ~20 min

Heat lid	110 C	
Temp	96 C	6 min
Temp	23 C (reduce 0.1 C/s)	1 sec
Store	23 C	

a. Run --> Browse through programs (bottom left) * This won't let you edit program

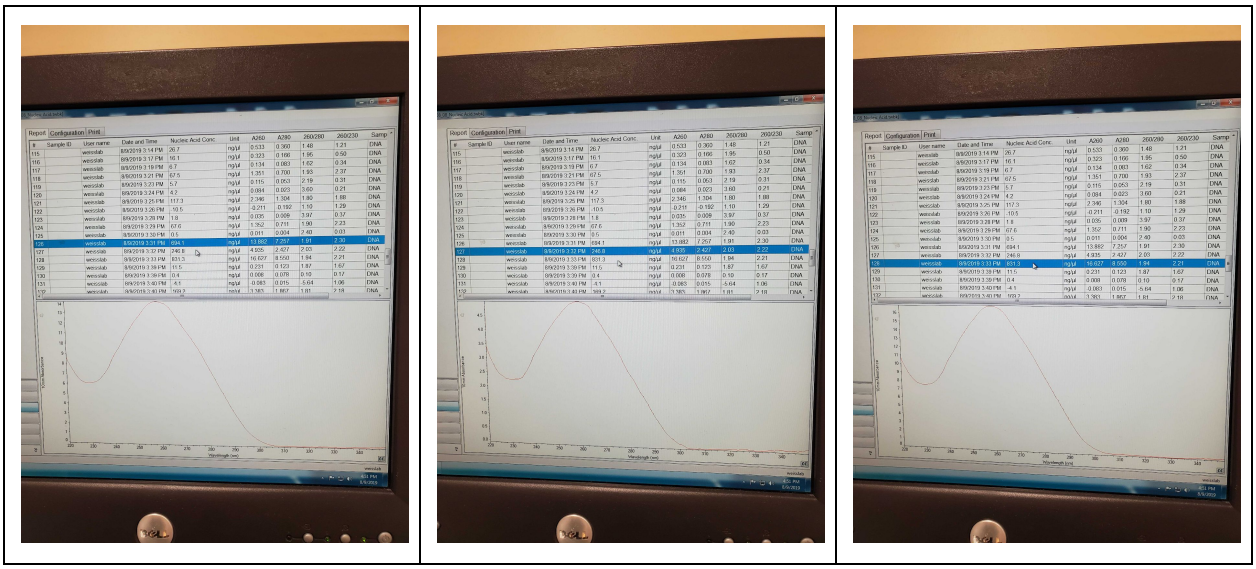
b. OR Programs --> Browse --> Open Ross' folder --> "anneal.js" --> Green save button --> Save then pick block (left or right) --> then it starts

c. Message will pop up to say it's done -- press "OK" and remove sample, leave lid open (pressing OK causes it to heat back up so make sure to remove sample)

3. Store primers and annealing products @ -20C

Lab

- ✓ Inoculation Results: successful, all cloudy
- ✓ Transformation Results: all three plates had many colonies
- ✓ Nanodrop
  - Low concentrations and irregular absorptions indicate the DNA should not be used, all plasmids should be reinoculated except pIG_114, 115, 116 (on Monday); potentially could skip all synNotch plasmids?
    - ✓ 114-694.1
    - ✓ 115-246.8
    - ✓ 116-831.3



- Next time:
  - Label all tubes beforehand CLEARLY
  - Make sure all reagents have necessary solutions added
  - Ensure that tubes are tightly on (be careful!) and that vacuum is at high pressure
    - Minimize # of midipreps done at the same time at vacuum
  - Ask if Qiagen vacuum could be used in the future?

✓ Obtain Gal4 primers/oligos from IDT, designed by Nika (if arriving today)

- Annealed oligos with Nika's protocol @ 11am (need to ask from her!) - Melody

Monday 8/12/19

Protocol

P.1	P.2
pIG_120 CMV **1 ul**	**pIG_121 2xGal4** **1 ul**

2. Mix the DNA parts (1ul each of 40 fmol/ul stocks) -- should be a total of 6 ul

3. Add the following reagents (should all be kept on ice/ice block)

Backbone pL0-P.2 (pIG_015)	0.5 ul
T4 ligase	0.5 ul
10x T4 buffer	2 ul
BsaI-HFv2 enzyme	1 ul

10x BSA buffer (NEB B9001)	2 ul
DEPC H20	12 uL
TOTAL (including DNA)	20 ul

Note: We can also make a master mix for multiple constructs (i.e. everything that's used*(however many constructs) with a little extra!!)

4. Thermal cycler (use one on my bench): ~2.5 hours for 10x cycles

iGEM folder --> "golden gate (BsaI, BsmbI)"

Heat lid	110 C	
Temp	37 C	20 min
Start cycle	10x	
	37 C	2 min
	16 C	5 min
Close cycle		
Temp	37 C	15 min
Temp	50 C	5 min
Temp	80 C	20 min
Store	8 C	

5. Transform bacteria as before and grow colonies on agar plates (Carb resistance), grow at 37C overnight

6. Miniprep

7. Test digest and run gel, send good clones to sequencing
NOW WE CAN USE THE PLASMID!!! :)

Lab

- ✓ Golden Gates: (Melody, Margaret, Krissy)
  - ✓ Done twice because first might have been using concentrated stock… XD
    - ✓ **Pick up second one in -20!!**
  - ❏ Tomorrow will need to transform!
- ✓ Inoculate for Midipreps - Krissy, Melody (from glycerol stock colonies which were plated 8/11)

- ✓ Prepare for midiprep of pIG_104 (from Nika) and pIG_112 - pIG_116 (from Shiva); as well as - Krissy, Margaret, Melody

pIG_104	pL1	2xcHS4insulator_inert_hEF1a_inert_NeonGreen_inert_SynthpA
pIG_112	pL1	TRE-tight-mkate (SRS07 175)
pIG_113	pL1	hEF1a-synCD19 (SRS05 27)

pIG_035	pL1	**inert_TRE_inert_EYFP_inert_SynthpA**
pIG_037	pL1	**inert_TRE_inert_mKO2mod_inert_SynthpA**
pIG_038	pL1	**inert_TRE_inert_TagBFP_inert_SynthpA**

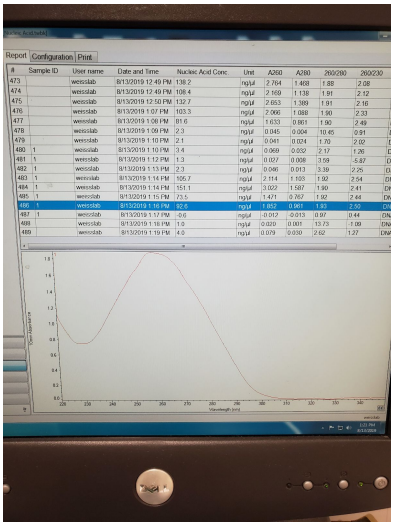
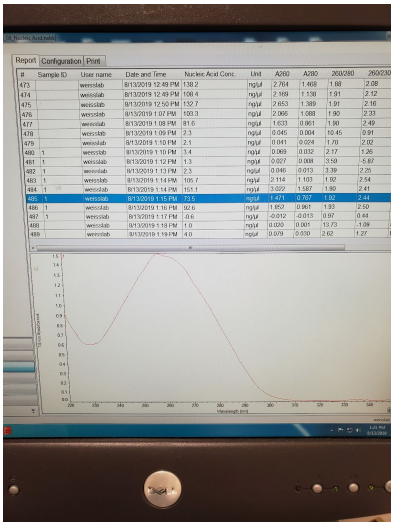
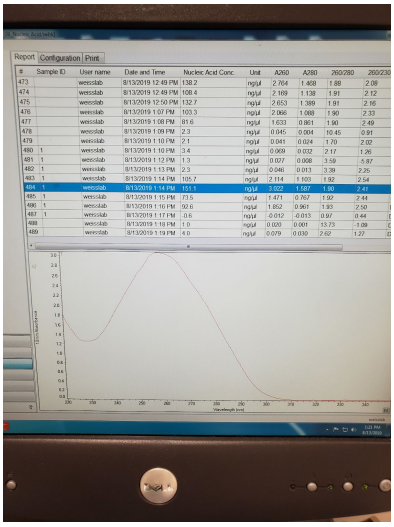
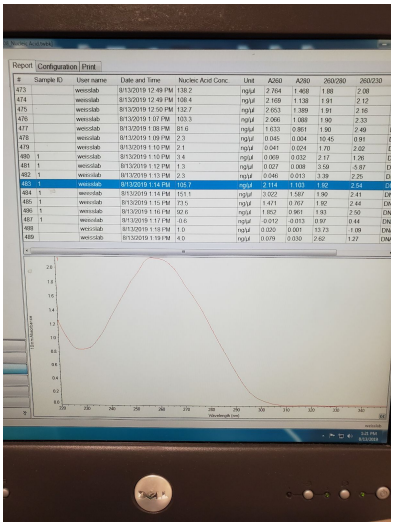
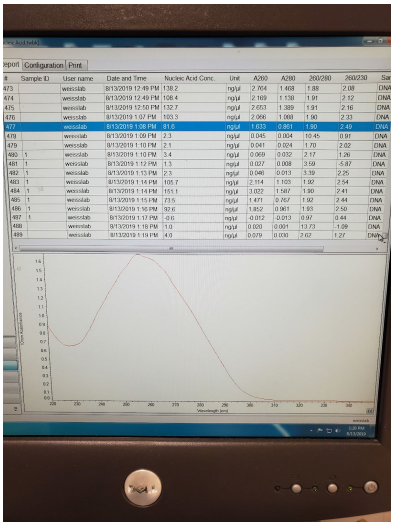
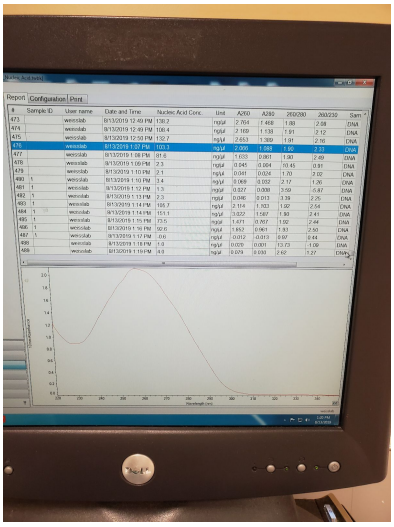
As well as:
54, 57, 91, 97, 29, 33

100ng/ul working stocks were made for pIG_33, pIG_115, pIG_116 - Melody

Tuesday 8/13/19

Lab

- ✓ Create 100ng/ul working stocks (use cloudy blue paper box that's labeled) for necessary constructs (hEF1a_Color and TRE_Color constructs)
- ✓ Nanodrop early pL1s (from wk1/2) to double check nanodrop curves
- ✓ Midiprep:
  - ✓ pIG_054: inert_CMV_inert_IL-8_inert_SynthpA - **105.7**
  - ✓ pIG_057: inert_hEF1a_inert_IL-8-NeonGreen_inert_SynthpA - **151.1**
  - ✓ pIG_091: inert_hEF1a_inert_CCL5_inert_SynthpA - **73.5**
  - ✓ pIG_097: inert_TRE_inert_IL-8 (CXCL-8)_inert_SynthpA - **92.6**
  - ✓ pIG_029: inert_hEF1a_inert_EYFP_inert_SynthpA - **103.3**
  - ✓ pIG_033: inert_hEF1a_inert_TagBFP_inert_SynthpA - **81.6**
  - ✓ pIG_035: inert_TRE_inert_EYFP_inert_SynthpA - X
  - ✓ pIG_037: inert_TRE_inert_mKO2mod_inert_SynthpA - X
  - ✓ pIG_038: inert_TRE_inert_TagBFP_inert_SynthpA - X
  - ✓ pIG_112: TRE-tight-mkate (SRS07 175) - X
  - ✓ pIG_113: hEF1a-synCD19 (SRS05 27) - X
  - ✓ pIG_104: 2xcHS4insulator_inert_hEF1a_inert_NeonGreen_inert_SynthpA - X

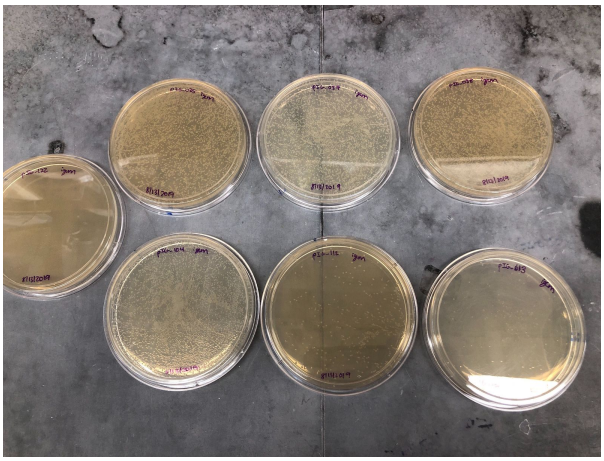







- ✓ Transformed with new protocol from Shiva - Shiva, Margaret, Krissy, Melody
  - ✓ pIG_104: 2xcHS4insulator_inert_hEF1a_inert_NeonGreen_inert_SynthpA
  - ✓ pIG_038: inert_TRE_inert_TagBFP_inert_SynthpA
  - ✓ pIG_122 GG: CMV-2xGal4 (pL0-P)
  - ✓ pIG_112: TRE-tight-mkate (SRS07 175)
  - ✓ pIG_113: hEF1a-synCD19 (SRS05 27)
  - ✓ pIG_035: inert_TRE_inert_EYFP_inert_SynthpA
  - ✓ pIG_037: inert_TRE_inert_mKO2mod_inert_SynthpA
  - ✓ **Melody's Hypothesis**: Shiva's plasmids are bigger → more nanograms/uL (potentially and not necessarily because of transformations…)
- ✓ Made working stock for transformations - Krissy, Melody
  - ✓ Need to write down which one

Wednesday 8/14/19

Lab

- ✓ Transformation Results:
  - ✓ pIG_104: 2xcHS4insulator_inert_hEF1a_inert_NeonGreen_inert_SynthpA
  - ✓ pIG_038: inert_TRE_inert_TagBFP_inert_SynthpA
  - ✓ pIG_112: TRE-tight-mkate (SRS07 175)
  - ✓ pIG_113: hEF1a-synCD19 (SRS05 27)
  - ✓ pIG_035: inert_TRE_inert_EYFP_inert_SynthpA
  - ✓ pIG_037: inert_TRE_inert_mKO2mod_inert_SynthpA
  - X pIG_122 GG: CMV-2xGal4 (pL0-P) - accidentally put on carb plate; GG rxn also needs to have T4 Ligase rxn with it



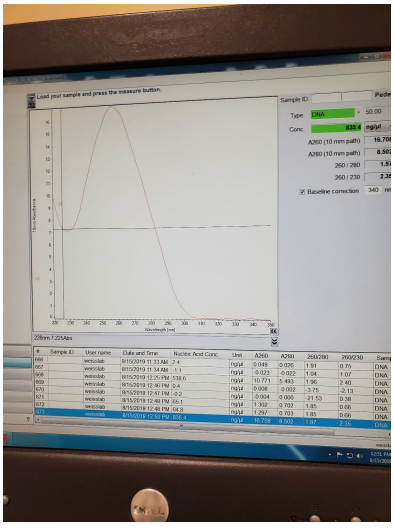
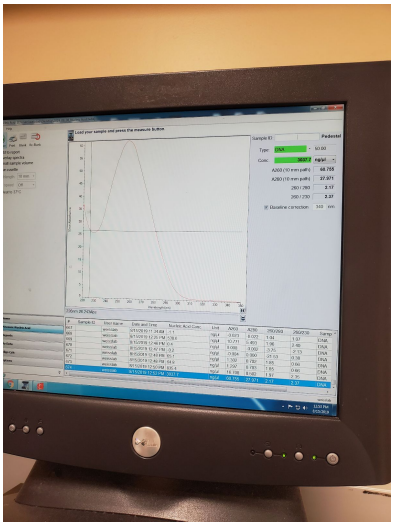
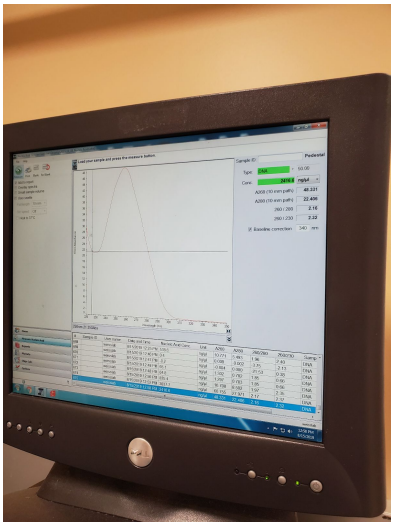
- ✓ Inoculate for midiprep: - Margaret, Krissy
  - ✓ pIG_104: 2xcHS4insulator_inert_hEF1a_inert_NeonGreen_inert_SynthpA
  - ✓ pIG_038: inert_TRE_inert_TagBFP_inert_SynthpA
  - ✓ pIG_112: TRE-tight-mkate (SRS07 175)
  - ✓ pIG_113: hEF1a-synCD19 (SRS05 27)
  - ✓ pIG_035: inert_TRE_inert_EYFP_inert_SynthpA
  - ✓ pIG_037: inert_TRE_inert_mKO2mod_inert_SynthpA
- ✓ Transform again pIG_122 - Margaret, Krissy
  - ✓ pIG_122 GG: CMV-2xGal4 (pL0-P)
    - ✓ Need to retransform and plate on Spec

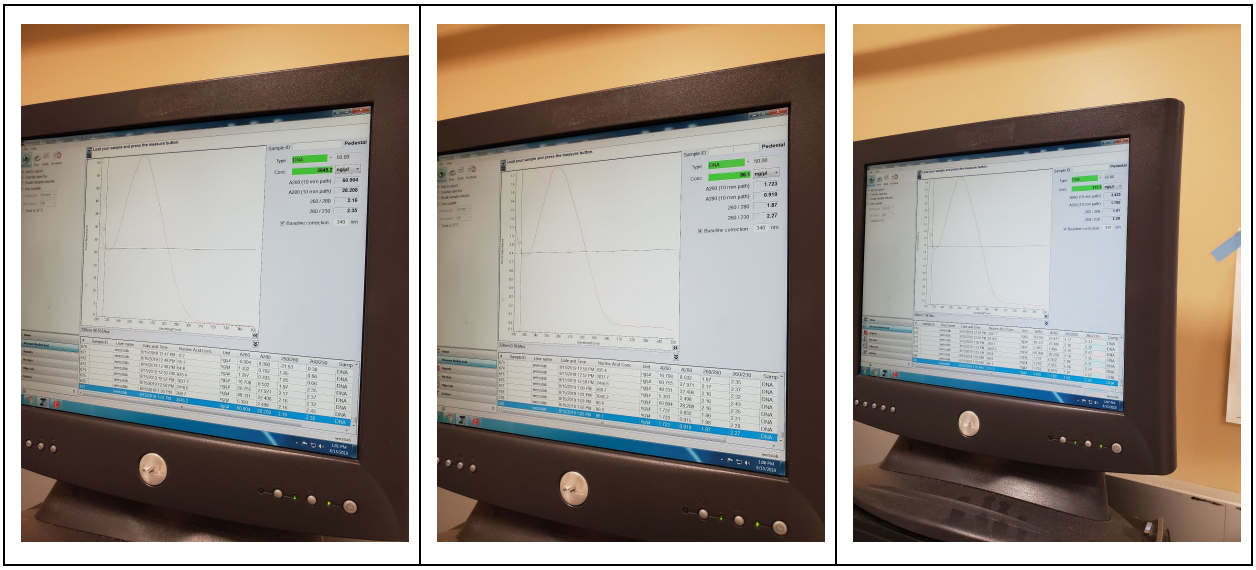
Thursday 8/15/19

Lab

- ✓ Transformation results:
  - pIG_122 GG: CMV-2xGal4 (pL0-P)
    - ✓ One white colony and four blue colonies → inoculate single white colony
- ✓ Inoculation results: All cloudy and ready for midiprep

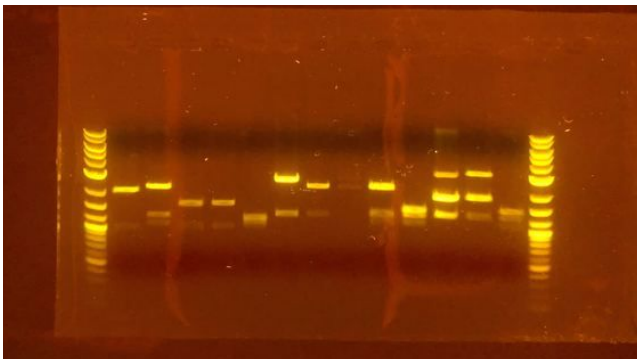
- ✓ Inoculate in mini tubes: (Margaret, Krissy)
  - ✓ pIG_104: 2xcHS4insulator_inert_hEF1a_inert_NeonGreen_inert_SynthpA
  - ✓ pIG_038: inert_TRE_inert_TagBFP_inert_SynthpA
  - ✓ pIG_112: TRE-tight-mkate (SRS07 175)
  - ✓ pIG_113: hEF1a-synCD19 (SRS05 27)
  - ✓ pIG_035: inert_TRE_inert_EYFP_inert_SynthpA
  - ✓ pIG_037: inert_TRE_inert_mKO2mod_inert_SynthpA
  - ✓ pIG_122
- ✓ Midiprep: (Krissy, Margaret)
  - ✓ pIG_104: 2xcHS4insulator_inert_hEF1a_inert_NeonGreen_inert_SynthpA
  - ✓ pIG_038: inert_TRE_inert_TagBFP_inert_SynthpA
  - ✓ pIG_112: TRE-tight-mkate (SRS07 175)
  - ✓ pIG_113: hEF1a-synCD19 (SRS05 27)
  - ✓ pIG_035: inert_TRE_inert_EYFP_inert_SynthpA
  - ✓ pIG_037: inert_TRE_inert_mKO2mod_inert_SynthpA
- ✓ Nanodrop results:
  - ✓ pIG_104: 2xcHS4insulator_inert_hEF1a_inert_NeonGreen_inert_SynthpA
    - ✓ 171.3
  - ✓ pIG_038: inert_TRE_inert_TagBFP_inert_SynthpA
    - ✓ 86.1
  - ✓ pIG_112: TRE-tight-mkate (SRS07 175)
    - ✓ 835.4
  - ✓ pIG_113: hEF1a-synCD19 (SRS05 27)
    - ✓ 3045.2
  - ✓ pIG_035: inert_TRE_inert_EYFP_inert_SynthpA
    - ✓ 3037.7
  - ✓ pIG_037: inert_TRE_inert_mKO2mod_inert_SynthpA
    - ✓ 2416.6



Friday 8/16/19

Lab

- ➢ Inoculation Results: All Cloudy ✓
- ✓ Miniprep: (Krissy)
  - ✓ pIG_122 nanodrop - 93 ng/ul
- ✓ Sent for sequencing pIG_122 -Melody
- ✓ Gel Digest pIG_122 and any minipreps/midipreps we were unsure about - Krissy, Melody
  - With ApaLI
  - 
  -

ladder	122	57	88	89	90	91	92	95_1	95_2	96_2	97	98	99	Ladder

  - Good:
    - 122 -
    - 57
    - 88
    - 89

        - 90
        - 91
        - 92
        - 99
    - We accidentally digested pL0s as well
    - Questionable:
        - 95_1 - really not there at all
        - 95_2 - check again looks like it is not it
        - 96_2
        - 97
        - 98

✓ Look through sequencing results and determine cause; email Genewiz to ask why so much error? -- all look pretty terrible…

- 104
    - ❏ Looks like it might be 113? Still a mutation even so though
- 35
    - ❏
- 37
    - ❏
- 38
    - ❏
- 112
    - ❏
- 113
    - ❏

Monday 8/19/19

Protocol

Lab (notes to Krissy from Melody)

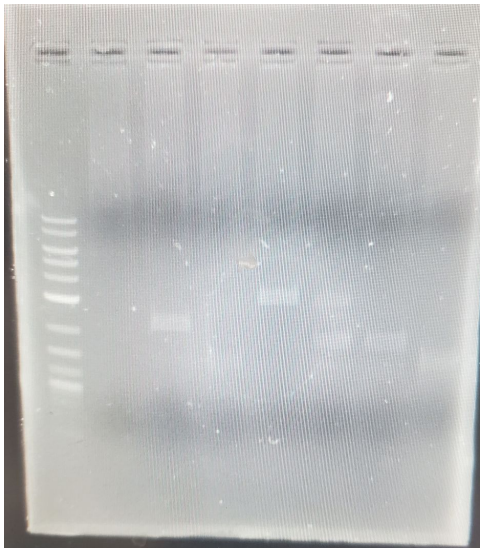
✓ Checked on pIG_122 sequencing results - Great!

- If it works -- yay! -- then go ahead and make glycerol stock if you still have the culture and if not you can transform with the pIG_122 that was purified and then inoculate again -- you'll have to figure out a good plan in terms of how to transform/when though

✓ Restriction Digest with ApaL1 + Gel(Krissy)

- 95_1
- 95_2
- 96_2
- 97
- 98_1

- 110



- ✓ Make Glycerol stock (Krissy)
  - 122
- ✓ Sent for sequencing - Krissy, Melody:
  - Midipreps
- ✓ Inoculate - depends if we want to midiprep them tomorrow (since these looked weird); whether you want to do this could depend on whether or not we have enough of it already; this means you'll need to check the inventory again :(sorry!
  - pIG_104: 2xcHS4insulator_inert_hEF1a_inert_NeonGreen_inert_SynthpA
  - pIG_038: inert_TRE_inert_TagBFP_inert_SynthpA
  - pIG_112: TRE-tight-mkate (SRS07 175)
  - pIG_113: hEF1a-synCD19 (SRS05 27)
  - pIG_035: inert_TRE_inert_EYFP_inert_SynthpA
  - pIG_037: inert_TRE_inert_mKO2mod_inert_SynthpA
  - pIG_122: CMV-2xGal4 pL0-P

Tuesday 8/20/19

Protocol

Lab

- ✓ **Midiprep Krissy, Melody**
  - ✓ pIG_104: 2xcHS4insulator_inert_hEF1a_inert_NeonGreen_inert_SynthpA
  - ✓ pIG_038: inert_TRE_inert_TagBFP_inert_SynthpA
  - ✓ pIG_112: TRE-tight-mkate (SRS07 175)
  - ✓ pIG_113: hEF1a-synCD19 (SRS05 27)
  - ✓ pIG_035: inert_TRE_inert_EYFP_inert_SynthpA
  - ✓ pIG_037: inert_TRE_inert_mKO2mod_inert_SynthpA
  - ✓ pIG_122: CMV-2xGal4 pL0-P

- ✓ Sent for sequencing - Krissy, Melody
    - Good:
        - ❏ pIG_054 - marked
        - ❏ pIG_091 - marked
        - ❏ pIG_097 - marked
    - Questionable:
        - ❏ pIG_029 - check again
        - ❏ pIG_033
        - ❏ pIG_057
- ✓ Plated pIG_122 from Glycerol Stock (Krissy)

Wednesday 8/21/19

Protocol

Lab

- ✓ Inoculated pIG_122 to 2mL culture for transfer to midiprep culture tomorrow and miniprepping tomorrow - Melody

Thursday 8/22/19

Protocol

~~**pIG_0001**~~ Inert	1ul	11ul
~~**pIG_005**~~ Inert	1ul	11ul
~~**pIG_012**~~ Inert	1 ul	11ul
~~**pIG_013**~~ Synthetic PA	1ul	11ul
~~pIG_122 - 61000~~ CMV-Gal4 promoter	1ul	11ul
Backbone pL1 (ex. ST1-2) ~~**pIG_020**~~	0.5 ul	5.5
~~T4 ligase~~	0.5 ul	5.5
~~10x T4 buffer~~	2 ul	22

~~BsaI-HFv2 enzyme~~	1 ul	11
~~10x BSA buffer (NEB B9001)~~	2 ul	22
~~DEPC H20~~	~~6 ul~~ 8 ul	88
TOTAL (including DNA above)	20 ul	220

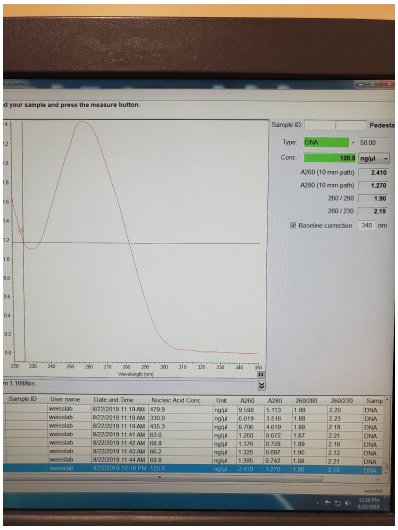
Lab

- ✓ Synthesis plasmids on geneious (Krissy)
- ✓ Golden Gate CMV-2xGal4 plasmids (Krissy)

pIG_117	pL1	pIG_047	inert_CMV-2xGal4_inert_IL-8_inert_SynthpA
pIG_118	pL1	pIG_048	inert_CMV-2xGal4_inert_IL-8-Flag_inert_SynthpA
~~pIG_119~~	~~pL1~~	~~pIG_049~~	~~inert_CMV-2xGal4_inert_IL-8-NeonGreen_inert_SynthpA~~

pIG_124	pL1	pIG_088	inert_CMV-2xGal4_inert_CCL5_inert_SynthpA
pIG_125	pL1	pIG_090	inert_CMV-2xGal4_inert_CCL5-NeonGreen_inert_SynthpA
pIG_126	pL1	**pIG_006**	inert_CMV-2xGal4_inert_mKate2_inert_SynthpA
pIG_127	pL1	**pIG_007**	inert_CMV-2xGal4_inert_EYFP_inert_SynthpA
pIG_128	pL1	**pIG_008**	inert_CMV-2xGal4_inert_TagBFP_inert_SynthpA
pIG_129	pL1	**pIG_009**	inert_CMV-2xGal4_inert_iRFP720_inert_SynthpA
~~pIG_130~~	~~pL1~~	~~pIG_103~~	~~inert_CMV-2xGal4_inert_NeonGreen_inert_SynthpA~~

- ✓ Miniprep pIG_122 (Krissy)
  Nanodrop-120.5



- ✓ Transfer 2ul miniprep culture to 25mL media for midiprep Friday

Friday 8/23/19

Protocol

Lab

- ❏ Midiprep pIG_122 (Krissy)
  - ❏ Nanodrop

Monday 8/26/19

Protocol

Lab

- ✓ Transform Golden Gates -Krissy

pIG_117	pL1	inert_CMV-2xGal4_inert_IL-8_inert_SynthpA
pIG_118	pL1	inert_CMV-2xGal4_inert_IL-8-Flag_inert_SynthpA
~~pIG_119~~	~~pL1~~	~~inert_CMV-2xGal4_inert_IL-8-NeonGreen_inert_SynthpA~~

pIG_124	pL1	inert_CMV-2xGal4_inert_CCL5_inert_SynthpA
pIG_125	pL1	inert_CMV-2xGal4_inert_CCL5-NeonGr

		een_inert_SynthpA
pIG_126	pL1	inert_CMV-2xGal4_inert_mKate2_inert_SynthpA
pIG_127	pL1	inert_CMV-2xGal4_inert_EYFP_inert_SynthpA
pIG_128	pL1	inert_CMV-2xGal4_inert_TagBFP_inert_SynthpA
pIG_129	pL1	inert_CMV-2xGal4_inert_iRFP720_inert_SynthpA
~~pIG_130~~	~~pL1~~	~~inert_CMV-2xGal4_inert_NeonGreen_inert_SynthpA~~

- ✓ Streak a plate of pIG_49 - Krissy
- ~ Transform the Neon Green from the L0 Box (Nika) - need more 103

Tuesday 8/27/19

Protocol

Lab

- ✓ Inoculate pIG_049 ~~and the NeonGreen from Nika (pL0 box)~~ - Melody
- ✓ Inoculate Golden Gates - Melody
  - ❏ Estimated number of colonies was put in plasmid inventory

pIG_117	pL1	inert_CMV-2xGal4_inert_IL-8_inert_SynthpA	
pIG_118	pL1	inert_CMV-2xGal4_inert_IL-8-Flag_inert_SynthpA	Might have put 2 colonies in 118_1 accidentally -- :) should be fine though if sequencing comes back ok
~~pIG_119~~	~~pL1~~	~~inert_CMV-2xGal4_inert_IL-8-NeonGreen_inert_SynthpA~~	

pIG_124	pL1	inert_CMV-2xGal4_inert_CCL5_inert_SynthpA
pIG_125	pL1	inert_CMV-2xGal4_inert_CCL5-NeonGreen_inert_SynthpA
pIG_126	pL1	inert_CMV-2xGal4_inert_mKate2_inert_SynthpA
pIG_127	pL1	inert_CMV-2xGal4_inert_EYFP_inert_SynthpA
pIG_128	pL1	inert_CMV-2xGal4_inert_TagBFP_inert

		_SynthpA
pIG_129	pL1	inert_CMV-2xGal4_inert_iRFP720_inert_SynthpA
~~pIG_130~~	~~pL1~~	~~inert_CMV-2xGal4_inert_NeonGreen_inert_SynthpA~~

Wednesday 8/28/19

Protocol

Lab

- ❏ Miniprep pIG_049 and the NeonGreen from Nika (pL0 box)
- ❏ Miniprep Golden Gates

pIG_117	pL1	inert_CMV-2xGal4_inert_IL-8_inert_SynthpA
pIG_118	pL1	inert_CMV-2xGal4_inert_IL-8-Flag_inert_SynthpA
~~pIG_119~~	~~pL1~~	~~inert_CMV-2xGal4_inert_IL-8-NeonGreen_inert_SynthpA~~

pIG_124	pL1	inert_CMV-2xGal4_inert_CCL5_inert_SynthpA
pIG_125	pL1	inert_CMV-2xGal4_inert_CCL5-NeonGreen_inert_SynthpA
pIG_126	pL1	inert_CMV-2xGal4_inert_mKate2_inert_SynthpA
pIG_127	pL1	inert_CMV-2xGal4_inert_EYFP_inert_SynthpA
pIG_128	pL1	inert_CMV-2xGal4_inert_TagBFP_inert_SynthpA
pIG_129	pL1	inert_CMV-2xGal4_inert_iRFP720_inert_SynthpA
~~pIG_130~~	~~pL1~~	~~inert_CMV-2xGal4_inert_NeonGreen_inert_SynthpA~~

- ❏ Nanodrop Golden Gates

- ❏ Restriction Digest and Gel golden gates
- ❏ Send Golden Gates to Sequencingftg,
- ❏ Inoculate for Midipreps of Golden Gates
    - ❏ Inoculate pIG_049 and the NeonGreen from Nika (pL0 box)
- ❏ Golden gate pIG_130 and pIG_119 with Neon Green

Thursday 8/29/19

Protocol

Lab

- ❏ Midiprep pIG_049 and the NeonGreen from Nika (pL0 box)
- ❏ Midiprep Golden Gates
    - ❏ Nanodrop Golden Gates
- ❏ Make Glycerol Stocks of Golden Gates

Friday 8/30/19

Protocol

Lab

Sequencing results (Krissy)

- Good: All of these are copy number #2; 117, 118, 124, 125, 126, 127, 128, 49_v2_2
- Questionable: 129 should be good king seq but all looks good