July 9, 2019: Inoculation and comp cell preparation

Project: 2019 iGEM
Authors: Claire Young

TUESDAY, 7/9/2019

Objective: Today, we will innoculate E. coli containing Shyam's plasmids into LB broth and after a growth period, we will mini prep the bacteria to obtain the plasmid DNA. Then, we will do a restriction digest and run the DNA on a gel to check the success of the mini prep. We will also prepare comp cells in a glycerol stock and make a seed culture. (Claire)

Materials for innoculation (done in Abercrombie lab):

- LB broth
- 1000X chloramphenicol
- E. coli containing the target plasmids
- 37 C shaking incubator (SN 06004817 VWR)
- Culture tubes (SN 07418023 Falcon)
- 50 mL centrifuge tubes (SN C-3394-3 ISC BioExpress)

Procedure for innoculation:

1. 11 14 mL polporpylene round-bottom tubes labeled with the plasmid number: 459, 413, 447, 451, 461, 617, 633, 658, 674, 501, 512
2. Pipetted 40 mL LB agar (from wetlab bootcamp) into 50 mL centrifuge tube using aseptic technique (Alicia)
3. Added 40 uL 1000X Chl (chloramphenicol) to the 40 mL LB (Samantha)
4. Aliquoted 3 mL of the LB broth + Chl into each 14 mL culture tube (Alicia)
    a. Note: as of this part, the ethanol burner wouldn't light anymore
5. Used a pipette tip to innoculate the bacteria into the prepared media (Alicia, Samantha, Stephanie, Claire)
6. Incubated in the 37 C shaker for 7 hr at 200 rpm
    a. Start time: 10:07 am
    b. End time: 8:12 pm
7. Stored the bacteria plate in the 4 C fridge
8. Pipetted 1.5 mL of each liquid culture into 2 1.5 mL tubes labeled with the plasmid number (see step 1) (Claire and Samantha)
9. Spun down at 6000 rcf for 3 min
10. Removed supernatent and stored in -20 C freezer in a box labeled "iGEM freezer box E. coli w/ plasmids" (Samantha)

Materials for comp cell preparation (done in Bennett Lab Keck 301):

- LB broth (from Bennett lab)
- TSS buffer (Transformation storage solution)
- Preculture grown in LB30 (from Shyam) E. coli DH10B-ALT
- 37 C shaking incubator (left incubator, New Brunswick scientific)
- Centrifuge (Beckman-Coulter Allegra X-30R Centrifuge)
- 250 uL thermocycler tubes
- Repeater pipette (HandyStep)

Procedure for comp cell preparation:

1. Note: 1/3 flask volume is max you want to fill for cultures
2. Using aseptic technique, transfered 100 mL LB broth to 250 mL flask
3. 133 uL preculture (which saturates at around OD = 15) added to flask
    a. Dilution based on getting a starting OD of 0.02
4. Added 10 mM magnesium sulfate

5. Left in 37 C shaking incubator for ~2 hr
    a. Start time: 11:49
    b. Time of first check: 2:08, OD = 0.266
    c. Check OD - final OD should be between 0.2-0.5, aim for 0.3
6. Checked the absorbance at 600 nm of the sample using LB to as the blank and a 700 uL sample (Alicia)
7. Placed flask in ice water bath
8. Ran the centrifuge @ 500 rpm to cool down the centifuge
9. On a cool brick system (a 1 C cold block), set out 96 250 uL thermocycler tubes (PCR tubes)
10. Aliquoted into 2 50 mL conical vials on ice
    a. balanced with autoclaved water
11. Centrifuged @2000 rcf at 3 C for 10 min
12. Poured excess media into the sink, stored pellets on ice
13. Resuspended a pellet into 5 mL TSS buffer on ice
14. Poured the resuspended pellet into the other pellet and used it to resuspend the second pellet on ice
15. Using the repeater pipette, aliquoted 50 uL into each PCR tube
    a. Only ended up with 85 filled tubes
16. Transported on ice back to Abercrombie, stored in -20 C freezer, switched to -80 C freezer in Bennett lab after approximately 5 hr at -20 C

Summary: Today, we innoculated our cultures and spun them down to store the pellet overnight. We also prepared TSS competent cells, which we stored in a -80 C freezer. Tomorrow, we will mini prep our pellets to extract the plasmid DNA and run a restriction digest to confirm that we got the intended plasmid. Due to uncertainty in the cloning method, we decided to mini prep tomorrow instead of today. (Claire)

July 10, 2019: Miniprep and DNA electrophoresis

Project: 2019 iGEM

Authors: Claire Young

WEDNESDAY, 7/10/2019

Objective: Today, we will do a miniprep to harvest the plasmid DNA from the liquid cultures pelleted yesterday. Afterward, we will do a restriction digest and run a DNA agarose gel to determine if we obtained the correct plasmids. (Claire)

Materials for Miniprep:

- QIAprep Spin Miniprep Kit (Cat. No. 27106 Qiagen)
- 100% ethanol (Cat. No. 111000200 Pharmoco-aaper)

Procedure for Miniprep:

1. Added 220 mL ethanol to Buffer PE (Alicia)
2. Added 200 uL RNase to Buffer P1
3. Resuspended the first pellet in 250 uL of Buffer P1, transfered the solution to the second tube since we pelleted into two tubes and resuspended the second pellet using the same 250 uL (Claire and Alicia)
    a. Stored Buffer P1 in the 4 C fridge
4. Added 250 uL Buffer P2 to each tube and inverted 4-6 times until the solution became clear (Alicia)
    a. Don't let sit for more than 5 min
5. Added 350 uL Buffer N3 to each tube and mixed by inverting 4-6 times (Claire)
6. Centrifuged for 10 min at 16,100 rcf
7. We ended up with two tubes labeled 459 after accidentally putting Buffer P1 and P2 into both origianl tubes
8. Pipetted 800 uL of the supernatent into a spin column (Tasneem and Samantha)
9. Centrifuged the spin columns for 1 min at 16,100 rcf
10. Discarded flowthrough and added 0.5 mL Buffer PB to each column (Tasneem and Samantha)
11. Centrifuged the columns for 1 min at 16,100 rcf
12. Discarded the flowthrough and added 0.75 mL Buffer PE to each column (Tasneem and Samantha)
13. Centrifuged for 1 min at 16,100 rcf
14. Placed 1 mL Buffer EB in a 36 C incubator (Alicia)
    a. Used the RT Buffer EB though
15. Removed the supernatent and centrifuged for 1 min at 16,100 rcf (Tasneem and Samantha)
16. Labeled 1.5 mL tubes with the plasmid numbers and transfered the columns to the 1.5 mL tubes
17. Dry spin the columns for 1 min at 16,100 rcf
18. Added 50 uL Buffer EB to each column and let stand for 5 min, then centrifuged for 1 min at 16,100 rcf (Tasneem and Samantha)
19. Removed the spin column from tubes
20. Used nanodrop spectrophotometer to check concentration (Alicia, Samantha, Tasneem, Claire)
21. Stored in iGEM freezer box labeled "iGEM freezer box miniprep w/ plasmid" in -20 C freezer

Materials for Making Gel

- 1X TBE buffer (5.3 M)
- Agarose (Apex BioResearch Products Cat# 20-102QD)
- Gel casting frame

Procedure for Making Gel (Claire)

1. Measured 0.5 g agarose and mixed with 50 mL TBE
2. Microwaved for 1 min until the solution was clear
3. Allowed to cool slightly before pouring into the gel casting frame
4. Set for at least 15 min

Materials for test digest

- diH20
- 10X CutSmart buffer (NEB Lot # 10018445)
- NotI restriction enzyme
- Template DNA from the miniprep

Procedure for test digest

1. Made a master mix according to the wetlab calculator - 128 uL di water, 19 uL of buffer, 3.12 uL of NotI and pipetted up and down to mix the buffer and water, when pipetting make sure to make contact with the surface of the solution - DO NOT release in air
2. Added 12 uL of the mastermix to each tube
3. Added 2-4 uL of the DNA to each tube(amount depends on the concentration)
4. Flicked to mix, and place tubes in a 37C incubator for 15 min
    a. Start time:
    b. End time:

Data for the nanodrop

Nanodrop Concentrations

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Plasmid	Blank	512	674 "624"	501	459 "559"	447	633	459	413	461	617	658	451 "452"
2	Concentration (ng/uL)	-0.4	35.1	35.0	47.1	22.8	40.9	215.7	98.1	49.9	108.7	60.2	126.2	82.9

Materials for Running Gel

- 1kb Plus DNA Ladder
- 6x DNA Loading Dye (Blue)
- 1X TBE buffer (used)

Procedure for Running Gel

1. Pipetted 3 uL of loading dye into each tube, and pipetted to mix.
2. Pipetted 18 uL of each sample into the wells, leaving a space in the middle well for the DNA ladder. We avoided the lanes on the side
3. Pipetted 7 uL of DNA ladder into the middle well.
4. Ran the gel for 57 min at 120V (switched to 130 V after 30 min)
   Start time: 8:06
   End time: 9:03
5. Imaged the gel using blue light

Gel Setup

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Lane #	1	2	3	4	5	6	7	8	9	10	11	12	13
2	Sample		674 "624"	501	459 "559"	447	633	ladder	459	413	461	617	451 "452"	658

Note: 512 is no longer usable due to PCR tube leakage during mixing.

Results of the Gel

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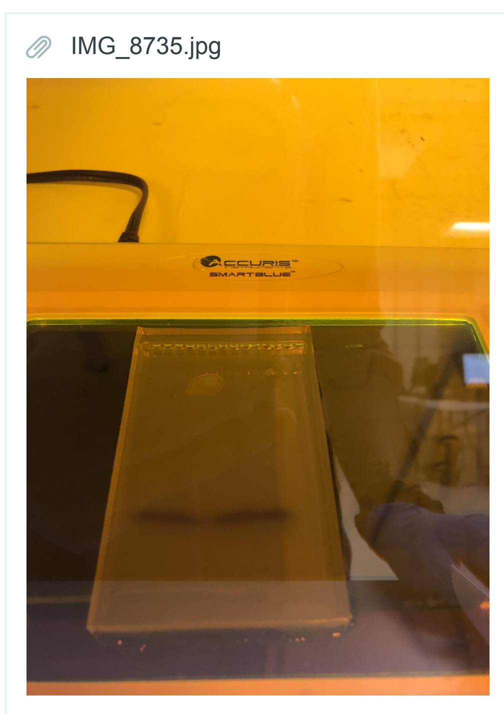

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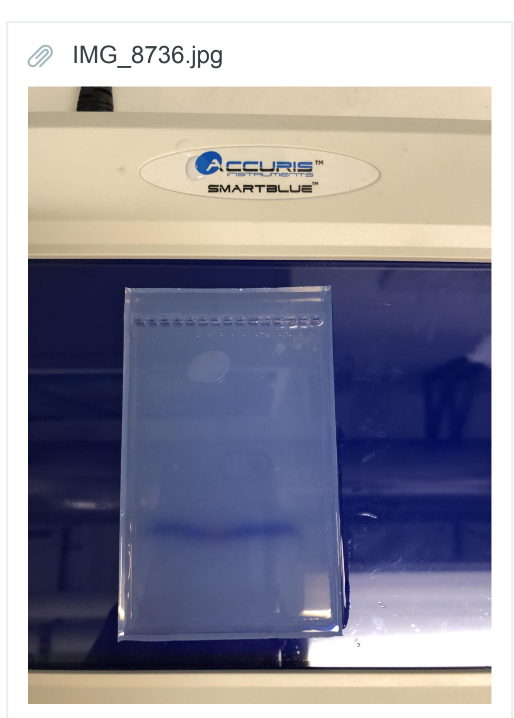


Summary: Today we miniprepped our pellets to extract the plasmid DNA, and we ran a restriction digest to confirm that we got the intended plasmid. Our gel did not yield any results, and we believe that this was caused by lack of DNA dye (since no binding to the gel occurred). Tomorrow we will redo the restriction digest and run it on a new gel. We plan to add dye to the loading buffer, which we will mix with each sample to dye it, and run it on a plain gel. (Samantha)

July 11, 2019: Inoculation, PCR, Restriction digest

Project: 2019 iGEM
Authors: Claire Young

THURSDAY, 7/11/2019

Objective: Today, we will inoculate E. coli containing Shyam's plasmids into LB broth. After a growth period, we will miniprep the bacteria to obtain the plasmid DNA. Then, we will do a restriction digest and run the DNA on a gel to check the success of the mini prep. We will also conduct a PCR reaction of araC part type 8 using the pBest template plasmid obtained from Shyam, and we will run the results on a gel. (Samantha)

Materials for Inoculation:

- LB broth
- 1000X chloramphenicol
- E. coli containing the target plasmids
- 37 C shaking incubator (SN 06004817 VWR)
- Culture tubes (SN 07418023 Falcon)
- 50 mL centrifuge tubes (SN C-3394-3 ISC BioExpress)

Procedure for Inoculation

1. 7 14 mL polporpylene round-bottom tubes labeled with the plasmid number: 469,480,671,677,406,430,436
2. Pipetted 30 mL LB agar (from wetlab bootcamp) into 50 mL centrifuge tube using aseptic technique (Alicia)
3. Added 30 uL 1000X Chl (chloramphenicol) to the 30 mL LB (Alicia)
4. Aliquoted 3 mL of the LB broth + Chl into each 14 mL culture tube (Alicia)
5. Used a pipette tip to inoculate the bacteria into the prepared media (Alicia, Samantha)
6. Incubated in the 37 C shaker for 7 hr at 200 rpm
    a. Start time: 1:05pm
    b. End time: pm
7. Stored the bacteria plate in the 4°C fridge

8. Pipetted 1.5 mL of each liquid culture into 2 1.5 mL tubes labeled with the plasmid number (see step 1) ()
9. Spun down at 6000 rcf for 3 min
10. Removed supernatent and stored in -20 C freezer in a box labeled "iGEM freezer box E. coli w/ plasmids" ()

Materials for PCR:

- oIG021 pCon-araC (8) P1
- oIG022 pCon-araC (8) P2
- Nuclease-free water (Lot#179161 Fisher BioReagents)
- NEB High Fidelity 2x PCR master mix (Lot# 10041218, NEB)
- pBest template plasmid (obtained from Shyam)
- Thermocycler (BioRad SN: CT026481)

Procedure for PCR:

Resuspending the primers:

1. Used centrifuge to spin down ordered primers for 30 sec. at 10,000 rcf
2. Added 325 uL of water to P1 (No: 215995438) to make a stock solution concentration of 100 uM (Claire)
3. Added 293 uL of water to P2 (No: 215995439) to make a stock solution concentration of 100 uM (Claire)
4. Vortexed for 10 sec.
5. Repeated step 1 centrifuge conditions
6. 10X dilution performed by combing 10 um stock solution with 90 um water (Claire)
    a. Labels from IDT were used for the working solution tubes, additionally labed with the concentration 10 uM

7. Repeated steps 4-5
8. Working and stock solutions of primers were stored in the -20 °C iGEM freezer box

PCR reaction:

1. Set up a 25 μL PCR reaction with 12.5 μL master mix, 1.25 μL of each primer, 1 μL of template, and 9 μL water (Samantha)
   a. PCR tube labeled "IG"
2. Cycling conditions:
   a. Initial denaturation: 98 C for 30 sec
   b. Denaturation: 98 C for 10 sec
   c. Annealing: 61 C for 30 sec
   d. Extension: 72 C for 30 sec, 35X
   e. Final extension: 72 C for 2 min
   f. Hold: 4 C
   g. Protocol labeled as NEBNext_HF
   h. Should take about 1:10 hours
3. Stored the PCR product at 4 C.

Materials for test digest

- diH20
- 10X CutSmart buffer (NEB Lot # 10018445)
- NotI restriction enzyme
- Template DNA from the miniprep

Procedure for test digest (Samantha)

1. Made a master mix according to the wetlab calculator - 96 uL di water, 14 uL of buffer, 2.34 uL of NotI and pipetted up and down to mix the buffer and water.
2. Added 12 uL of the mastermix to each tube
3. Added 2-4 uL of the DNA to each tube amount depends on the concentration)
   a. Don't need to digest plasmid # 447, 658, and 674
4. Flicked to mix, and place tubes in a 37C incubator for 15 min
   a. Start time: 3:17 pm
   b. End time: 3:34 pm

Materials for Making Gel

- 1X TBE buffer (5.3 M)
- Agarose (Apex BioResearch Products Cat# 20-102QD)
- Gel casting frame

Procedure for Making Gel (Claire)

1. Measured 0.5 g agarose and mixed with 50 mL TBE
2. Microwaved for 1 min until the solution was clear
3. Allowed to cool slightly before pouring into the gel casting frame
4. Set for at least 15 min

Materials for Running Gel

- 1kb Plus DNA Ladder
- 6x DNA Loading Dye (Blue)
- 1X TBE buffer (used)
- DNA Gel Stain, 20,000x in water. 1.0mL/Unit (Apex, Genesee#20-278)

Procedure for Running Gel

1. Pipetted 1mL of 6x loading dye into a tube. (Claire)

2. Pipetted 0.6 uL of gel stain into the tube and pipetted to mix. (Tasneem)
3. Pipetted 5 uL DNA ladder and 1 uL loading dye into a PCR tube, and pipetted to mix. (Claire)
4. Pipetted 3 uL of loading dye into each restriction digest tube, and pipetted to mix. (Claire)
5. Combined 2.5 uL PCR product with 0.5 uL loading dye into a new PCR tube. (Alicia and Claire)
6. Loaded 18 uL of each miniprep sample, 6 uL ladder, and 3.0 uL PCR product into the gel wells. We skipped lanes 1 and 2 and avoided the lanes on the side.
7. Ran the gel for 30 min at 120V
   Start time: 4:13 pm
   End time: 4:46
8. Imaged the gel using blue light

Procedure for Runnin Gel w/ Gel Green

Gel Preparation

1. Measured 0.5 g agarose and mixed with 50 mL TBE (tasneem)
2. With a loosened cap, microwave agarose suspension or solid until completely clear and homogeneous liquid (≈1 min per 100 mL on high). Watch as it boils to stop the microwave before it can overflow. Swirl, handling with a silicone mit. Continue microwaving in smaller increments if necessary. (alicia)
3. added 5µl of 10000x gelgreen to molten agarose (alicia)
4. Poured in cast and set for 15 min

Electrophoresis (Alicia)

1. prepare 10µl of miniprep samples and 2.5 µl of pcr product. Mix in 2µl and .5µl of 6x loading dye into the samples
2. load in 5 µl dna ladder
3. Fill electrophoresis chamber with 1× TAE (running buffer) until it's a bit under the gel+tray height.
4. Remove comb from cast gel and submerge gel+tray in the electrophoresis chamber, oriented with wells at the top and the negative (black) terminals of the chamber at the top. If TAE doesn't cover the gel, add sufficient TAE.
5. Secure the lid onto the chamber, and plug in lid into power supply. Match terminal colors. Ensure negative black terminals are at the top of the chamber and lid and positive red at the bottom. Thus anionic nucleic acids run to the red anode. Run to red. Ran at 120V for 30 min
6. 8. Inspect/cut the gel under blue light with orange glasses or UV with UV shield. Image the gel with the blue light (or UV) imager: Open Canon EOS utility on the computer to awaken the connected camera and use Remote Live View to adjust the gel position and autofocus before taking a picture. The camera should typically be in the No Flash setting (auto-set exposure, ISO, and aperture). Exit EOS Utility when done. Blue light is safer to work with and less damaging to DNA than UV light. Requires GelGreen, SYBR green, SYBR Safe, etc.

Gel Setup (Gel Green)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	Sample	633	674	452	512	459	461	ladder	PCR	559	617	501	413	658	447

Materials for Miniprep:

- QIAprep Spin Miniprep Kit (Cat. No. 27106 Qiagen)
- 100% ethanol (Cat. No. 111000200 Pharmoco-aaper)

Procedure for Miniprep: (Tasneem and Samantha)

1. Added 220 mL ethanol to Buffer PE
2. Added 200 uL RNase to Buffer P1
3. Resuspended the first pellet in 250 uL of Buffer P1, transfered the solution to the second tube since we pelleted into two tubes and resuspended the second pellet using the same 250 uL
   a. Stored Buffer P1 in the 4 C fridge

4. Added 250 uL Buffer P2 to each tube and inverted 4-6 times until the solution became clear
    a. Don't let sit for more than 5 min
5. Added 350 uL Buffer N3 to each tube and mixed by inverting 4-6 times
6. Centrifuged for 10 min at 16,100 rcf
7. We ended up with two tubes labeled 459 after accidentally putting Buffer P1 and P2 into both origianl tubes
8. Pipetted 800 uL of the supernatent into a spin column
9. Centrifuged the spin columns for 1 min at 16,100 rcf
10. Discarded flowthrough and added 0.5 mL Buffer PB to each column
11. Centrifuged the columns for 1 min at 16,100 rcf
12. Discarded the flowthrough and added 0.75 mL Buffer PE to each column
13. Centrifuged for 1 min at 16,100 rcf
14. Placed 1 mL Buffer EB in a 36 C incubator
    a. Used the RT Buffer EB though
15. Removed the supernatent and centrifuged for 1 min at 16,100 rcf
16. Labeled 1.5 mL tubes with the plasmid numbers and transfered the columns to the 1.5 mL tubes
17. Dry spin the columns for 1 min at 16,100 rcf
18. Added 50 uL Buffer EB to each column and let stand for 5 min, then centrifuged for 1 min at 16,100 rcf
19. Removed the spin column from tubes
20. Used nanodrop spectrophotometer to check concentration
21. Stored in iGEM freezer box labeled "iGEM freezer box miniprep w/ plasmid" in -20 C freezer

Nanodrop Data

	A	B	C	D	E	F	G	H	I
1	Plasmid #	Blank	480	469	436	430	406	671	677
2	Concentration (ng/uL)	-0.1	48.9	26.5	46.0	48.1	31.6	27.1	25.8

July 12, 2019: Restriction digest, gel electrophoresis

Project: 2019 iGEM
Authors: Claire Young

FRIDAY, 7/12/2019

Objective: Today, we will run a restriction digest on all miniprepped plasmids and use gel electrophoresis to check the sucess of the miniprep. We will also do a golden gate reaction to make a part vector for the part 8 araC gene and transform that plasmid into our comp cells. (Claire)

Restriction digest

Materials for restriction digest:

- diH20
- 10X CutSmart buffer (NEB Lot # 10018445)
- NotI-HF restriction enzyme
- Template DNA from the miniprep from July 10 and July 11

Procedure for restriction digest (Claire)**:**

Note about the last gel electrophoresis attempt from July 11: A restriction digest was not done before running the samples on the gel, so we are repeating the gel for those samples again, this time doing a restriction digest.

1. Made a master mix according to the wetlab calculator - 171 uL di water, 25 uL of buffer, 4.16 uL of NotI and pipetted up and down to mix the buffer and water.
2. Added 12 uL of the mastermix to each tube
3. Added 2-4 uL of the DNA to each tube amount depends on the concentration)
    a. Don't need to digest plasmid # 447, 658, and 674
    b. 3 uL per tube except for 459, which got 4 µL, and 633, which got 2 µL
4. Flicked to mix, and place tubes in a 37C incubator for 15 min
    a. Start time: 10:37 pm
    b. End time: 10:53 pm

Running Gel w/ Gel Green

Materials

1. agarose
2. TBE
3. gel green
4. loading buffer (NEB 6x)
5.

Gel Preparation

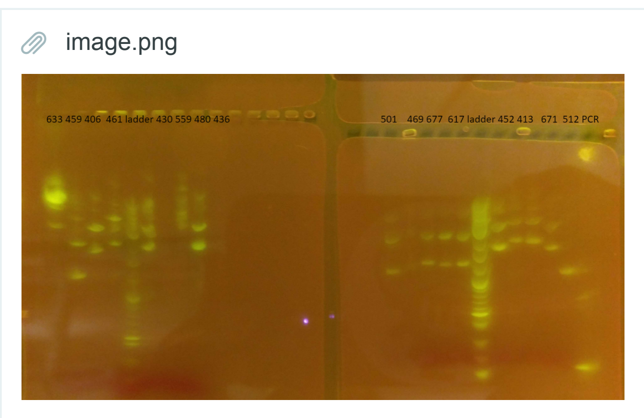
1. Measured 1 g agarose and mixed with 100 mL TBE (Alicia)
2. With a loosened cap, microwave agarose suspension or solid until completely clear and homogeneous liquid (≈1 min per 100 mL on high). Watch as it boils to stop the microwave before it can overflow. Swirl, handling with a silicone mit. Continue microwaving in smaller increments if necessary. (alicia)
3. added 10µl of 10000x gelgreen to molten agarose (alicia)
4. Poured in cast and set for 15 min
    a. start 10;17

Electrophoresis

1. Mixed 3 µL of loading dye with 15 µL digest reactions
2. Mixed 10 µL ladder with 2 µL loading dye and loaded 6 µL into each gel
3. Mixed 2.5 µL pcr product with 0.5 µL loading dye
4. Loaded samples into gel in order described in table "Gel Setup (Gel Green)"

5. Fill electrophoresis chamber with 1× TAE (running buffer) until it's a bit under the gel+tray height.
6. Remove comb from cast gel and submerge gel+tray in the electrophoresis chamber, oriented with wells at the top and the negative (black) terminals of the chamber at the top. If TAE doesn't cover the gel, add sufficient TAE.
7. Secure the lid onto the chamber, and plug in lid into power supply. Match terminal colors. Ensure negative black terminals are at the top of the chamber and lid and positive red at the bottom. Thus anionic nucleic acids run to the red anode. Run to red. Ran at 120V for Rmin
8. Inspect the gel under blue light with orange UV shield.
9.

image.png



Gel Setup (Gel Green)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	4	1	2	1	2	3	4	5	6	7	8	9	10	11	14
2	Sample (gel 1)			633	459	406	461	ladder	430	559	480	436			
3	Sample (gel 2)		501	blank	469	677	617	ladder	452	413	blank	671	512	pcr product	
4				fail	fail	fail	fail		pass	fail	possible pass, genomic contamination?				
5			possiible pass? need more DNA		possible pass? need more DNA, possible uncut	possible pass? need more DNA, possible uncut	possible pass? need more DNA, possible uncut					confusion, band sizes weird, possibly uncut	possibly uncut	unusable, primer dimer	

LB30

materials

- LB
- yeast extract (EMD 1.03753.0500)

Procedure

- autoclaved using liquid 15 min cycle

PCR

Procedure for PCR:

Resuspending the primers:

1. Used centrifuge to spin down ordered primers for 30 sec. at 10,000 rcf
2. Added 325 uL of water to P1 (No: 215995438) to make a stock solution concentration of 100 uM
3. Added 293 uL of water to P2 (No: 215995439) to make a stock solution concentration of 100 uM
4. Vortexed for 10 sec.
5. Repeated step 1 centrifuge conditions
6. 10X dilution performed by combing 10 µm stock solution with 90 µL water
   a. Labels from IDT were used for the working solution tubes, additionally labed with the concentration 10 uM
7. Repeated steps 4-5
8. Working and stock solutions of primers were stored in the -20 °C iGEM freezer box

PCR reaction:

1. Set up a 25 μL PCR reaction with 12.5 μL master mix, 1.25 μL of each primer, 2 μL of template, and 6.75 μL water, 1.25 μL DMSO
    a. PCR tube labeled "IG"
2. Cycling conditions:
    a. Initial denaturation: 98 C for 30 sec
    b. Denaturation: 98 C for 10 sec
    c. Annealing: 61 C for 30 sec
    d. Extension: 72 C for 30 sec, 35X
    e. Final extension: 72 C for 2 min
    f. Protocol labeled as NEBNext_HF
    g. Should take about 1:10 hours
3. Stored the PCR product at 4 C.

Notes

- step six of electrophoresis on the lab notebook entry from July 11, 2019 should be ammended to say "Inspect the gel under blue light with orange UV shield."
- For oIG021, the homodimer min delta G is -10.1 kcal and the monomer min delta G is -0.3 kcal
- For oIG022, the homodimer min delta G is -8.9 kcal and the monomer min delta G is -0.9 kcal
- For oIG021 and 22 heterodimer min delta G is -7.2 kcal

July 14, 2019: Preparing Media and inoculation

Project: 2019 iGEM
Authors: Alicia Selvera

SUNDAY, 7/14/2019

LB 8.5 Preparation (alicia)

materials

- LB Broth Miller L322-250
- yeast extract EMD 1.03753.0500

Procedure

1. added in 25 g of LB broth to 1 L water
2. added in 3.5 g of yeast extract
3. dissolve by heating and stiring
4. autoclave using liquid 20 min cycle

Innoculation of Shyam's plasmids

Materials

- LB 30
- 13 culture tubes
- 2 plates of plasmids
- shaking incubator
- ethanol burner
- spray bottle of ethanol
- lighter
- Ampiciliin

Procedure

1. using aseptic technique, Prepare 50 mL of Amp-LB30
2. Clean Pippette w/ ethanol
3. using pippette scoop up bacteria from middle of culture
4. eject pippette tip into liquid Amp-LB30

Summary

I failed to make LB30. LB30 is equal part yeast extract to millers LB broth. Instead, Shyam's LB30 was used instead to innoculate the plasmids.

July 15, 2019: Inoculation, miniprep

Project: 2019 iGEM
Authors: Alicia Selvera

MONDAY, 7/15/2019

Innoculation, continued

1. Labeled 2 mL centrifuge tubes, pSPB406, pSPB413, pSPB430, pSPB451, pSPB459, pSPB469, pSPB480, pSPB501, pSPB512, pSPB617, pSPB633, pSPB671, pSPB677 (Shyam)
2. Poured 2 mL of each liquid culture into 2 mL tubes labeled with the plasmid number (see step 1) (Shyam and Alicia)
3. Spun down at 6000 rcf for 2 min
4. Removed supernatent by pouring and stored the pellets in -20 C freezer in Bennett lab

Miniprep

Materials for Miniprep:

- QIAprep Spin Miniprep Kit (Cat. No. 27106 Qiagen)

Procedure for Miniprep:

1. Resuspended the first pellet in 250 uL of Buffer P1
    a. Stored Buffer P1 in the 4 C fridge
    b. vortexed for 30 seconds
2. Added 250 uL Buffer P2 to each tube and inverted 4-6 times until the solution became clear
    a. Don't let sit for more than 2 min
3. Added 350 uL Buffer N3 to each tube and mixed by inverting 4-6 times
4. Centrifuged for 10 min at 16,100 rcf
5. We ended up with two tubes labeled 459 after accidentally putting Buffer P1 and P2 into both origianl tubes
6. Pipetted 800 uL of the supernatent into a spin column
7. Centrifuged the spin columns for 1 min at 16,100 rcf
8. Discarded flowthrough and added 0.5 mL Buffer PB to each column
9. Centrifuged the columns for 1 min at 16,100 rcf
10. Discarded the flowthrough and added 0.75 mL Buffer PE to each column
11. Centrifuged for 1 min at 16,100 rcf
12. Placed 1 mL Buffer EB in a 36 C incubator
    a. Used the RT Buffer EB though
13. Removed the supernatent and centrifuged for 1 min at 16,100 rcf
14. Labeled 1.5 mL tubes with the plasmid numbers and transfered the columns to the 1.5 mL tubes
15. Dry spin the columns for 1 min at 16,100 rcf
16. Added 50 uL Buffer EB to each column and let stand for 5 min, then centrifuged for 1 min at 16,100 rcf
17. Removed the spin column from tubes
18. Used nanodrop spectrophotometer to check concentration
19. Stored in iGEM freezer box labeled "iGEM freezer box miniprep w/ plasmid" in -20 C freezer

Nanodrop Concentrations

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Plasmid	Concentration												
2	677	78.8												
3	633	49.3												
4	671	181.3												
5	617	162.2												
6	451	211.1												
7	459	120.5												
8	469	87.9												
9	406	144.3												
10	413	145.0												
11	430	31.3												
12	480	276.1												
13	501	103.9												
14	512	99.5												

Conclusion

We miniprepped shyam's innoculated part plasmids

July 16, 2019: PCR, oligo anneals, and minipreps on a gel

Project: 2019 iGEM
Authors: Claire Young

TUESDAY, 7/16/2019

Objective: Today, we will prepare our linear DNA pieces by either PCR or oligo annealing. Then, we will use Golden Gate to put those pieces into the part vector and transform the part vectors into E. coli for overnight growth. (Claire)

Materials for PCR:

- Primers (listed in the table below)
- Nuclease free water
- NEB High Fidelity 2x PCR master mix (Lot# 10041218, NEB)
- Thermocycler

Procedure for PCR:

Resuspending the primers:

1. Used centrifuge to spin down ordered primers for 30 sec. at 10,000 rcf
2. Added the amount of water in the table below to each primer (Claire, Stephanie)
3. Pipetted up and down to mix
4. 10X dilution performed by combing 10 um stock solution with 90 um water (Samantha, Tasneem)
    a. Labels from IDT were used for the working solution tubes, additionally labed with the concentration 10 uM
5. Working and stock solutions of primers were stored in the -20 °C iGEM freezer box

PCR reaction:

1. Set up a 25 µL PCR reaction with 12.5 µL master mix, 1.25 µL of each primer, 1 µL of template, and 9 µL water (Samantha)
    a. for master mix, 118.75 uL master mix, 85.5 uL of water
2. For getting parts from the well: pierced the foil covering each well with a pipette tip, took 15 µL of nuclease-free water and added to each well, pulled out the liquid and transfered to a
3. Cycling conditions for otsB and otsA:
    a. Initial denaturation: 98 C for 1 min
    b. Denaturation: 98 C for 10 sec
    c. Annealing: 61 C for 30 sec
    d. Extension: 72 C for 45 sec, 35X
    e. Final extension: 72 C for 2 min
    f. Hold: 12 C
    g. Protocol labeled as PCR_45
4. Cycling conditions for RNAT7-9 and iaaHM 1, 2:
    I. Initial denaturation: 98 C for 1 min
    II. Denaturation: 98 C for 10 sec
    III. Annealing: 62 C for 30 sec
    IV. Extension: 72 C for 15 sec, 35X
    V. Final extension: 72 C for 2 min
    VI. Hold: 12 C
    VII. Protocol labeled as PCR_15
    VIII. Should take about 1:10 hours
5. Cycling conditions for iaaHM 3:
    1. Initial denaturation: 98 C for 1 min
    2. Denaturation: 98 C for 10 sec
    3. Annealing: 59 C for 30 sec

4. Extension: 72 C for 90 sec, 35X
5. Final extension: 72 C for 2 min
6. Hold: 12 C
7. Protocol labeled as PCR_15
8. Should take about 1:10 hours

Materials for oligo anneals:

- Primers for RNAT1-4 and Para Marionette
- Nuclease-free water
- Thermocycler

Procedure for oligo anneals:

1. Resuspended oligos (For RNAT 1-4 and Para Marionette) as described above
2. For a 50 µL reaction, added 5 µL of each oligo to a PCR tube and 40 µL nuclease-free water (Claire)
   a. For Para Marionette, since it has 4 oligos, add 5 µL of each and 30 µL water
3. Placed in thermocycler with the following conditions:
   a. Heat to 95 °C for 2 min
   b. Gradual cool from 95 °C to 25 °C in 20 min in 0.5 °C intervals 140 times (adjust with the ramp option)
   c. Infinite hold at 4 °C

Materials for test digest

- diH20
- 10X CutSmart buffer (NEB Lot # 10018445)
- NotI restriction enzyme
- Template DNA from the miniprep

Materials for phosphorylation:

Procedure for phosphorylation:

Procedure for test digest (Claire)

1. Made a master mix according to the wetlab calculator - 147 uL di water, 22 uL of buffer, 3.57 uL of NotI and pipetted up and down to mix the buffer and water.
2. Added 12 uL of the mastermix to each tube
3. Added 2 µL template for all plasmids except for 633, 469, and 430 which had 3 µL
4. Flicked to mix, and place tubes in a 37C incubator for 30 min
   a. Start time: 7:16
   b. End time: 8:15

Materials for making TAE Buffer:

- Water
- DNA Type Grade 50X TAE Buffer

Procedure for making buffer:

1. Measured 20 mL of 50X TAE buffer in a graduated cylinder
2. Poured 20 mL of 50X TAE buffer into a 1 L bottle
3. Added 980 mL of diH20 to the 1 L bottle

Materials for Gel

1. agarose
2. TBE

3. gel green
4. loading buffer TAE

Procedure for Gel Preparation

1. Measured 1 g agarose and mixed with 100 mL TAE (Stephanie)
2. With a loosened cap, microwave agarose suspension or solid until completely clear and homogeneous liquid (≈1 min per 100 mL on high). Watch as it boils to stop the microwave before it can overflow. Swirl, handling with a silicone mit. Continue microwaving in smaller increments if necessary.
3. added 10µl of 10000x gelgreen to molten agarose
4. Poured 100 mL in cast and set for 15 min
5. Because the 100 mL gel was too large, we remelted the gels and remade them as 50 µL gels, which is what we should target for

Procedure for Electrophoresis

1. Mixed 5 µL of loading dye with 25 µL PCR reactions (Tasneem)
2. Mixed 2 µL of loading dye with 10 µL oligo anneal reactions
3. Mixed 3 µL of loading dye with 15 µL test digest reactions
4. Mixed 10 µL ladder with 2 µL loading dye and loaded 6 µL into each gel
5. Loaded samples into gel in order described in table "Gel 1 for cleanup"
6. Fill electrophoresis chamber with 1× TAE (running buffer) until it's a bit under the gel+tray height.
7. Remove comb from cast gel and submerge gel+tray in the electrophoresis chamber, oriented with wells at the top and the negative (black) terminals of the chamber at the top. If TAE doesn't cover the gel, add sufficient TAE.
8. Secure the lid onto the chamber, and plug in lid into power supply. Match terminal colors. Ensure negative black terminals are at the top of the chamber and lid and positive red at the bottom. Thus anionic nucleic acids run to the red anode. Run to red. Ran at 120V for Rmin
9. Inspect the gel under blue light with orange UV shield.

Note loading dye was added to the oligo anneals, so now we need to remove the dye because of the SDS in it*

Materials for oligo anneal cleanup:

- DNA clean and concentrator-5 kit (Zymo research cat # 11302)
- oligo anneals with loading dye

Procedure for oligo anneal cleanup:

1. Added 60 µL binding buffer to each tube with oligo anneal
2. Centrifuged at max speed for 30 s
3. Added 200 µL wash buffer, centrifuged for 30 s
4. Repeated wash step
5. *note for future, run a dry spin to remove exccess ethanol*
6. Added 15 µL elution buffer, let sit for 1 min, eluted using centrifuge into 1.5 µL tubes
7. Checked concentrations with nanodrop

Materials for tranformation:

-

Procedure for tranformation:

1.

Concentrations of oligo anneals

	A	B
1	Oligo anneal	Concentration (ng/µL)
2	IG006	9.2
3	IG007	28.9
4	IG008	14.4
5	IG009	10.3
6	IG012a	18.2
7	IG012b	10.0

1.

Gel 1 for gel cleanup

	A	B	C	D	E	F	G	H
1	Lane 1	2	3	4	5	6	7	8
2	Blank	pIG002	pIG001	pIG003a	ladder	pIG003b	pIG003c	Blank

Gel 2 for gel cleanup

	A	B	C	D	E	F	G	H
1	Lane 1	2	3	4	5	6	7	8
2	Blank	pIG013	pIG014	pIG015	ladder	pIG016T9	pIG005	Blank

Gel for test digests

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Lane 1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	406	413	430	439	451	469	480	ladder	501	512	612	633	671	677

Water added to bring primers to 100 µM solution

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	**Part**	RNAT1	RNAT2	RNAT3	RNAT4	Para Marionette	otsB	otsA	iaaHM	accd	Pcon araC (not used)	RNAT6	RNAT7	RNAT8	RNAT9
2	**P1**	243 µL	236	245	240	(27) 393	321	314	399	237	398	453	364	295	320
3	**P2**	266	305	264	326	(28) 325	263	236	373	261	288	303	252	330	312
4	**P3**					(29) 275			**299**						
5	**P4**					(30) 271			295						
6	**P5**								250						
7	**P6**								279						

Summary: pIG005 PCR Failed, need new template + new primers

Dna Gel recovery

Procedure

1. Weigh gels

Table1

	A	B	C	D	E	F	G	H	I	J
1		pcr002	pcr001	pcr003a	pcr003b	pcr003c	pcr013	pcr014	pcr015	pcr016
2	Weight (mg)	115.9	135.2	147.3	127.0	155.3	105.3	117.4	132.0	179.5
3	Weight X 3	347.7	405.6	441.9	381	465.9	315.9	352.2	396	538.5

2. multiply weight by 3
3. add in that much µL agarose disolving buffer
4. incubate at 50•C for 10 minutes shaking
5. Transfer melting agarose to spin collems
6.Centrifuge at max speed 60 seconds
7. discard flow through by pipetting it out
8. Add in 200 µl of DNA wash buffer
9. Centrifuge at max fo r 30 seconds, discard flow through via pipetting, repeat
10. dry spin for 30
10. elute w/ 20 µl of elution buffer, transfer to 15 ml, spin for 30

Transformation

July 17, 2019: Golden gate, tranformation

Project: 2019 iGEM
Authors: Alicia Selvera

WEDNESDAY, 7/17/2019

objective:

Today, we will tranform the results of the golden gate reaction into comp cells, let those grow, and select colonies for miniprepping tonight. (Claire)

Nanodrop results

Table1

	A	B	C	D	E	F	G	H	I	J
1	Blank	pIG001	PIG002	pIG003a	pIG003b	pIG003c	pIG013	pIG014	pIG015	pIG016
2	ng/ul	28.2	50.5	32.1	27.5	36.1	32.7	15.5	30.3	28.6

Golden Gate

Materials

- Esp3I
- T_4 DNA Ligase
  - 0.5–1 µL range, 2000 CEU/µL cligase ∝ misligation
- 10x T_4 Ligase Buffer
  - Titurate/vortex to dissolve solids. Limit freeze/thaws to ~5-10x
- 10x BSA + PEG
  - 10x: 1mg/mL BSA, 10% PEG-3350
  - BSA: Enables full BsaI activity at 37°C, absorbs contaminants
  - PEG: reduces diffusion, enhancing ligation efficiency
- DNAs
  - 10–40 fmol equimolar
  - 2–5-fold less vector to reduce background
- Deionized Water
  - 10–20 µL range
- custom stabilizing buffer
- cool brick (1°C)

Procedure

1. first diluted samples to 100nm
2. Prepare a mastermix by adding together enzyme, T 4 DNA Ligase, 10X T 4 Ligase Buffer, 10X BSA, and water
3.

Table2

	A	B	C	D	E	F
1	Golden Gate Assembly 1					
2	***Reactions***	13		***BSA?***	yes	
3	***# Unique Parts***	1			*1*	
4	***# Common Parts/Vector***	1			*1*	
5	***# Others (PCR pdts, oligo pts)***	0			*1*	
6	***Excess***	2%	0%	0%	-	
7						
8	**Reactions**	**13.26**	13	1	*1 ref*	
9						< Warnings
10	**diH2O**	**132.6**	130	10	**10**	μL
11	10× Ligase Buffer	**19.89**	19.5	1.5	**1.5**	μL
12	**10× BSA**	**19.89**	19.5	1.5	**1.5**	μL
13	**T4 DNA Ligase**	**6.63**	6.5	0.5	0.5	μL
14	Type IIs endonuclease	**6.63**	6.5	0.5	0.5	μL
15		***Total:***			***Each:***	
16	Unique Parts	**6.63**	6.5	0.5	0.5	μL
17	**Common Parts/Vector**	**6.63**	6.5	0.5	0.5	μL
18		**#N/A**	#N/A	#N/A		
19	Others	0	0	0	0.5	μL
20						
21	***Total:***	**198.9**	195	**15**	*15*	μL
22	***Total – DNA:***	**185.64**	182	**14**	**14**	μL
23	***Total – Uniques & Others:***	**192.27**	188.5	**14.5**	-	μL

Materials for tranformation:

- NEB turbo comp cells (from Shyam)
- The iGEM comp cells
- Plasmids from the golden gate reaction (labeled 1, 2, 3, 4, 6, 7, 8, 9, 12, 13, 14, 15, 16 for the plasmid number)
- Recovery media (from Shyam)

Procedure for tranformation: (Claire + Shyam)

1. Pipetted 5 µL of each plasmid into a tube containing the comp cells (on ice)
2. Left the tubes on ice for 30 min
    a. Start time: 10:51 am
    b. End time: 11:20 am
3. Heat shocked tubes for 30 s at 42 °C
4. Placed on ice for 2 min
5. Added 150 µL recovery media to each tube, shook to mix
6. Placed in 37 °C incubator for 1 hr
    a. Start time: 11:30 am
    b. End time: 12:30 pm
7. To test the efficiency of the iGEM comp cells, also transformed plasmids oIG001 and oIG013 into our comp cells, tubes labeled 1b and 13 b
    a. Start time for 30 min on ice: 11:40 am
    b. End time: 12:10 pm
    c. Start time for 1 hr in 37 °C incubator: 12:20 pm
    d. End time: 1:20 pm
8. Placed LB + Chl plates in 37 °C incubator to pre-warm
    a. Two of the iGEM plates had contamination and were replaced with plates from Shyam's lab
9. Used glass beads to plate ~200 µL of each tube onto the warm LB plates labeled with the plasmid number
    a. Tubes 1 and 13 were plated on the plates from Shyam's lab
10. Placed plates in 37 °C incubator for ~9 hr
11. Plated using the same method above and stored the 1b and 13b plates were stored in a 30 °C incubator starting at 1:30 pm
    a. Will check on those plates tomorrow morning
12. Oligo anneals and golden gate reactions were stored in the 4 °C fridge in the Bennett lab
13. Around 10, we checked the plates to see if there were colonies
    a. We did not find colonies on 2 and 7

Materials for Inoculation:

- LB 30+ broth
- 1000X chloramphenicol
- E. coli containing the target plasmids
- 37 C shaking incubator (SN 06004817 VWR)
- Culture tubes (SN 07418023 Falcon)
- 50 mL centrifuge tubes (SN C-3394-3 ISC BioExpress)

Procedure for Inoculation

1. 11 14 mL polporpylene round-bottom tubes labeled with the plasmid number:
2. Pipetted 29 mL LB agar (from wetlab bootcamp) into 50 mL centrifuge tube using aseptic technique
3. Added 29 uL 1000X Chl (chloramphenicol) to the 29 mL LB
4. Aliquoted 2 mL of the LB broth + Chl into each 14 mL culture tube
5. Used a pipette tip to inoculate the bacteria into the prepared media
    a. Make sure to not touch the pippette tip
6. Incubated in the 37 C shaker for 7 hr at 200 rpm
    a. Start time: ~ 1 am
    b. End time: when we come back in the morning

Summary

Today in the morning we carried out Golden Gate and then transformed those into comp cells. Our transformations do not look that promising, we were not able to see any gfp flourscence. For the plates that formed colonies, we prepared them for overnight inoculation

July 18, 2019: Transformation Results, Miniprep, Plate Preparation, Test Digest

Project: 2019 iGEM
Authors: Claire Young

THURSDAY, 7/18/2019

Objective: Today, we will reexamine our plates from yesterday to see if more colonies have grown. We will also remove our overnight cultures and potentially miniprep them depending on the amount of bacterial growth. We also might redo the part assemblies and retransform to obtain better results. (Claire)

Procedure for Inoculation (continued from last night)
1. Pipetted 1.5 mL of each liquid culture into 2 1.5 mL tubes labeled with the plasmid number (see step 1)
2. Spun down at 6000 rcf for 3 min
3. Removed supernatent and stored in -20 C freezer

Transformation results have been recorded in the cloning log

Materials for Plate Preparation
1. Agar
2. Chlor

Procedure for Plate Preparation
1. Melted down the LB agar broth and removed stirring rods
2. Added chlor(antibiotic) to media, 1000x
3. Poured out plates
4.

Miniprep

Materials for Miniprep:
- QIAprep Spin Miniprep Kit (Cat. No. 27106 Qiagen)

Procedure for Miniprep:
1. Resuspended the first pellet in 250 uL of Buffer P1
    a. Stored Buffer P1 in the 4 C fridge
    b. vortexed for 30 seconds
2. Added 250 uL Buffer P2 to each tube and inverted 4-6 times until the solution became clear
    a. Don't let sit for more than 2 min
3. Added 350 uL Buffer N3 to each tube and mixed by inverting 4-6 times
4. Centrifuged for 10 min at 16,100 rcf
5. We ended up with two tubes labeled 459 after accidentally putting Buffer P1 and P2 into both origianl tubes
6. Pipetted 800 uL of the supernatent into a spin column
7. Centrifuged the spin columns for 1 min at 16,100 rcf
8. Discarded flowthrough and added 0.5 mL Buffer PB to each column
9. Centrifuged the columns for 1 min at 16,100 rcf
10. Discarded the flowthrough and added 0.75 mL Buffer PE to each column
11. Centrifuged for 1 min at 16,100 rcf
12. Placed 1 mL Buffer EB in a 36 C incubator
    a. Used the RT Buffer EB though
13. Removed the supernatent and centrifuged for 1 min at 16,100 rcf

14. Labeled 1.5 mL tubes with the plasmid numbers and transfered the columns to the 1.5 mL tubes
15. Dry spin the columns for 1 min at 16,100 rcf
16. Added 50 uL Buffer EB to each column and let stand for 5 min, then centrifuged for 1 min at 16,100 rcf
17. Removed the spin column from tubes
18. Used nanodrop spectrophotometer to check concentration
19. Stored in iGEM freezer box labeled in -20 C freezer
20. For nanodrop: use 1.5 µL

Nanodrop for Miniprep

	A	B
1	Plasmid	Concentration (ng/µL)
2	pIG001	78.2
3	pIG003	152.0
4	pIG004	83.8
5	pIG006	48.1
6	pIG008	56.0
7	pIG009	51.7
8	pIG014	69.0
9	pIG015	32.9
10	pIG016	108.7

Restriction Digest

Materials

Procedure

1. Digested all parts and the vector with BsaI by making a master mix with 113 µL diH20, 16.5 µL cutsmart buffer, and 2.75 µL BsaI
   a. Aliquoted 12 µL into labeled PCR tubes and added 3 µL template for all plasmids except for 430 and 015, which got 4 µL, and 003, which got 2 µL
   b. Tubes labeled in plasmid number order, followed by 430B for the BsaI reaction, then 430E for the Esp3I reaction
2. For Esp3I reaction for pIG430, added 20.5 µL diH20, 3 µL buffer, and 0.5 µL Esp31. Aliquoted 12 µL of that into a PCR tube and added 4 µL of pIG430
3. For the test digest reactions for pSPB413, pSPB469, pSPB480, added 34 µL diH20, 5 µL buffer, 0.83 µL BsaI. For pSPB413 and pSPB480, added 2 µL of DNA. For pSPB469, added 3 µL of DNA.
4. Time running
   a. Start: 7:04
   b. End: 7: 47
   c. Start: 7:22
   d. End: 7: 52
5. Added 3 µL of loading dye into each of the PCR tubes

Electrophoresis

Materials

- 2 log ladder
- Loading dye

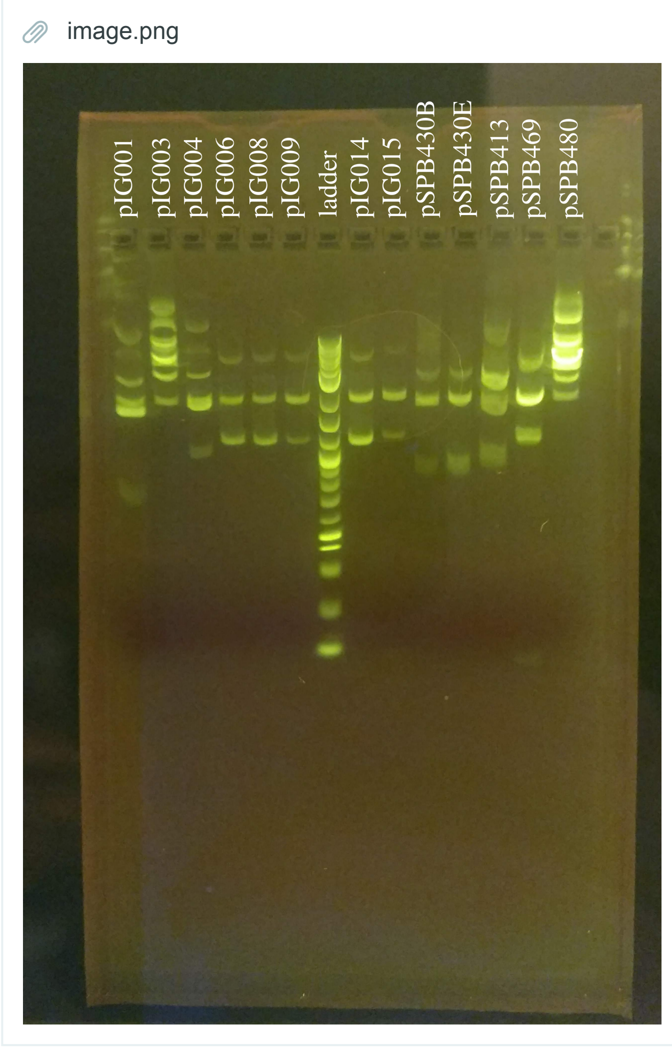
Procedure

1. Mixed 2 log ladder with 80 µL water and added 20 µL Gel Loading Dye Purple 6X (B7024S) loading dye
2. Added 5 µL of 2 log DNA ladder
3. Loaded 18 µL of each restriction digest onto the gel
4. Ran gel at 120 V
    a. Start time: 8:08 pm
    b. End time: 8:45 pm

Restriction digest gel

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	pIG001 BSAI	pIG003 BSAI	pIG004 BSAI	pIG006 BSAI	pIG008 BSAI	pIG009 BSAI	Ladder	pIG014	pIG015	pSPB430 BSAI	pSPB430 ESP3I	pSPB413	pSPB469	pSPB480
2	805, 1898	3063, 1856	1063, 1856	78, 1856	72, 1856	79, 1856		56,1898		1024, 1856	1024, 1856	1132, 1856	94, 1856	1856, 2524
3	fail	fail	pass	fail	fail	fail							pass	

image.png



notes:

July 19, 2019: Golden gate of pBEST araC and pSPB440, transformation, plating

Project: 2019 iGEM
Authors: Tasneem Mustafa

FRIDAY, 7/19/2019

Golden Gate (Alicia + Shyam)

Materials

- Esp3I
- T_4 DNA Ligase
  - 0.5–1 µL range, 2000 CEU/µL cligase ∝ misligation
- 10x T_4 Ligase Buffer
  - Titurate/vortex to dissolve solids. Limit freeze/thaws to ~5-10x
- 10x BSA + PEG
  - 10x: 1mg/mL BSA, 10% PEG-3350
  - BSA: Enables full BsaI activity at 37°C, absorbs contaminants
  - PEG: reduces diffusion, enhancing ligation efficiency
- DNAs
  - 10–40 fmol equimolar
  - 2–5-fold less vector to reduce background
- Deionized Water
  - 10–20 µL range
- custom stabilizing buffer
- cool brick (1°C)

Procedure

  - used pBEST araC and pSPB440
  - ran for 2 hours

Table1

	A	B	C	D	E
1	Golden Gate Assembly 3				
2	***Reactions***	2		***BSA?***	no
3	***# Parts***	1			*1*
4	***# Common Parts/Vector***	1			*1*
5	***# Others (PCR pdts, oligo pts)***	0			*1*
6	***Excess***	0%	0%	0%	-
7					
8	**Reactions**	**2**	2	1	*1 ref*
9					
10	**diH2O**	**20**	20	10	**10**
11	10× Ligase Buffer	**3**	3	1.5	**1.5**
12	**10× BSA**	0	0	0	0
13	**T4 DNA Ligase**	**1**	1	0.5	0.5
14	Type IIs endonuclease	**1**	1	0.5	0.5
15			***Total:***		***Each:***
16	Unique Parts	**4**	4	2	2
17	**Common Parts/Vector**	**1**	1	0.5	0.5
18		**#N/A**	#N/A	#N/A	
19	Others	0	0	0	0.5
20					
21	***Total:***	**30**	30	**15**	*15*
22	***Total – DNA:***	**25**	25	**12.5**	**12.5**
23	***Total – Uniques & Others:***	**26**	26	**13**	-
24					

Materials for tranformation:

- NEB turbo comp cells (from Shyam)
- The iGEM comp cells
- Plasmids from the golden gate reaction (labeled 1, 2, 3, 4, 6, 7, 8, 9, 12, 13, 14, 15, 16 for the plasmid number)
- Recovery media (from Shyam)

Procedure for tranformation: (Alicia + Shyam)

a. Pipetted 5 µL of each plasmid into a tube containing the comp cells (on ice)
b. Left the tubes on ice for 30 min
    I. Start time: 10:51 am
    II. End time: 11:20 am
c. Heat shocked tubes for 30 s at 42 °C
d. Placed on ice for 2 min
e. Added 150 µL recovery media to each tube, shook to mix
f. Placed in 37 °C incubator for 1 hr
    I. Start time: 11:30 am
    II. End time: 12:30 pm
g. To test the efficiency of the iGEM comp cells, also transformed plasmids oIG001 and oIG013 into our comp cells, tubes labeled 1b and 13 b
    I. Start time for 30 min on ice: 11:40 am
    II. End time: 12:10 pm
    III. Start time for 1 hr in 37 °C incubator: 12:20 pm
    IV. End time: 1:20 pm
h. Placed LB + Chl plates in 37 °C incubator to pre-warm
    I. Two of the iGEM plates had contamination and were replaced with plates from Shyam's lab
i. Used glass beads to plate ~200 µL of each tube onto the warm LB plates labeled with the plasmid number
    I. Tubes 1 and 13 were plated on the plates from Shyam's lab
j. Placed plates in 37 °C incubator for ~9 hr
k. Plated using the same method above and stored the 1b and 13b plates were stored in a 30 °C incubator starting at 1:30 pm
    I. Will check on those plates tomorrow morning
l. Oligo anneals and golden gate reactions were stored in the 4 °C fridge in the Bennett lab
m. Around 10, we checked the plates to see if there were colonies
    I. We did not find colonies on 2 and 7

Materials for Inoculation:

- LB 30+ broth
- 1000X chloramphenicol
- E. coli containing the target plasmids
- 37 C shaking incubator (SN 06004817 VWR)
- Culture tubes (SN 07418023 Falcon)
- 50 mL centrifuge tubes (SN C-3394-3 ISC BioExpress)

Procedure for Inoculation

a. 11 14 mL polporpylene round-bottom tubes labeled with the plasmid number:
b. Pipetted 29 mL LB agar (from wetlab bootcamp) into 50 mL centrifuge tube using aseptic technique
c. Added 29 uL 1000X Chl (chloramphenicol) to the 29 mL LB
d. Aliquoted 2 mL of the LB broth + Chl into each 14 mL culture tube
e. Used a pipette tip to inoculate the bacteria into the prepared media
    I. Make sure to not touch the pippette tip
f. Incubated in the 37 C shaker for 7 hr at 200 rpm
    I. Start time: ~ 1 am
    II. End time: when we come back in the morning

Golden Gate (Alicia + Shyam)

Materials

- Esp3I

- T_4 DNA Ligase
    - 0.5–1 µL range, 2000 CEU/µL cligase ∝ misligation
- 10x T_4 Ligase Buffer
    - Titurate/vortex to dissolve solids. Limit freeze/thaws to ~5-10x
- 10x BSA + PEG
    - 10x: 1mg/mL BSA, 10% PEG-3350
    - BSA: Enables full BsaI activity at 37°C, absorbs contaminants
    - PEG: reduces diffusion, enhancing ligation efficiency
- DNAs
    - 10–40 fmol equimolar
    - 2–5-fold less vector to reduce background
- Deionized Water
    - 10–20 µL range
- custom stabilizing buffer
- cool brick (1°C)

Procedure

- ran for 2 hr and 44 minutes
- Used pBEST araC and pSPB440

Table2

	A	B	C	D	E	F
1	Golden Gate Assembly 3					
2	***Reactions***	1		***BSA?***	yes	
3	***# Parts***	1			*1*	
4	***# Common Parts/Vector***	1			*1*	
5	***# Others (PCR pdts, oligo pts)***	0			*1*	
6	***Excess***	0%	0%	0%	-	
7						
8	**Reactions**	**1**	1	1	*1 ref*	
9						< Warnings
10	**diH2O**	**7.5**	7.5	7.5	**7.5**	µL
11	10× Ligase Buffer	**1.5**	1.5	1.5	**1.5**	µL
12	**10× BSA**	**1.5**	1.5	1.5	**1.5**	µL
13	**T4 DNA Ligase**	**0.5**	0.5	0.5	0.5	µL
14	Type IIs endonuclease	**0.5**	0.5	0.5	0.5	µL
15		***Total:***			***Each:***	
16	Unique Parts	**3**	3	3	3	µL
17	**Common Parts/Vector**	**0.5**	0.5	0.5	0.5	µL
18		**#N/A**	#N/A	#N/A		
19	Others	0	0	0	0.5	µL
20						
21	***Total:***	**15**	15	**15**	*15*	µL
22	***Total – DNA:***	**11.5**	11.5	**11.5**	**11.5**	µL
23	***Total – Uniques & Others:***	**12**	12	**12**	-	µL

July 21, 2019: Inoculation of pIG005

Project: 2019 iGEM

Authors: Tasneem Mustafa

SUNDAY, 7/21/2019

Today we checked our plates for colony growth and then picked colonies for overnight inoculation

Materials for Inoculation:

- LB 30 broth
- 1000X chloramphenicol
- E. coli containing the target plasmids
- 37 C shaking incubator (SN 06004817 VWR)
- Culture tubes (SN 07418023 Falcon)
- 50 mL centrifuge tubes (SN C-3394-3 ISC BioExpress)

Procedure for Inoculation

1. 2 14 mL polporpylene round-bottom tubes labeled with the plasmid number: pIG005
2. Aliquoted 2 mL of the LB broth + Chl into each 14 mL culture tube because this is Shyam's ultra comp cells
3. Used a pipette tip to inoculate the bacteria into the prepared media
    a. Make sure to not touch the pippette tip
4. Incubated in the 34 C shaker for 7 hr at 200 rpm
    a. Start time: ~4 PM
    b. End time: around 8 AM 7/22

July 22, 2019: Inoculation of pIG005, Miniprep, restriction digest of pSPB, transformation of pIG101 and 102

Project: 2019 iGEM

Authors: Samantha

MONDAY, 7/22/2019

Objectives: Today, we will miniprep the overnight culture started yesterday and create the cassette vector.

note: the overnight cultures for pIG005 from yesterday fluoresced green under a blue light, so the colonies picked had not been sucessfully transformed. We need to redo the inoculation.
note 2 apparently there's strong yellow autofluoresence and the colonies picked were actually fine.

Materials for Inoculation:

- LB 30 broth
- 1000X chloramphenicol
- E. coli containing the target plasmids
- 37 C shaking incubator (SN 06004817 VWR)
- Culture tubes (SN 07418023 Falcon)
- 50 mL centrifuge tubes (SN C-3394-3 ISC BioExpress)

Procedure for Inoculation

1. 3 14 mL polporpylene round-bottom tubes labeled with the plasmid number: pIG005
2. Aliquoted 2 mL of the LB broth + Chl
3. Used a pipette tip to inoculate the bacteria into the prepared media
    a. Make sure to not touch the pippette tip
4. Incubated in the 37 C shaker for 7 hr at 200 rpm
    a. Start time: 9:58 am
    b. End time:

Materials for Miniprep:

- QIAprep Spin Miniprep Kit (Cat. No. 27106 Qiagen)

Procedure for Miniprep:

1. Resuspended the first pellet in 250 uL of Buffer P1
    a. Stored Buffer P1 in the 4 C fridge
    b. vortexed for 30 seconds
2. Added 250 uL Buffer P2 to each tube and inverted 4-6 times until the solution became clear
    a. Don't let sit for more than 2 min
3. Added 350 uL Buffer N3 to each tube and mixed by inverting 4-6 times
4. Centrifuged for 10 min at 16,100 rcf
5. We ended up with two tubes labeled 459 after accidentally putting Buffer P1 and P2 into both origianl tubes
6. Pipetted 800 uL of the supernatent into a spin column
7. Centrifuged the spin columns for 1 min at 16,100 rcf
8. Discarded flowthrough and added 0.5 mL Buffer PB to each column
9. Centrifuged the columns for 1 min at 16,100 rcf
10. Discarded the flowthrough and added 0.75 mL Buffer PE to each column

11. Centrifuged for 1 min at 16,100 rcf
12. Placed 1 mL Buffer EB in a 36 C incubator
    a. Used the RT Buffer EB though
13. Removed the supernatent and centrifuged for 1 min at 16,100 rcf
14. Labeled 1.5 mL tubes with the plasmid numbers and transfered the columns to the 1.5 mL tubes
15. Dry spin the columns for 1 min at 16,100 rcf
16. Added 50 uL Buffer EB to each column and let stand for 5 min, then centrifuged for 1 min at 16,100 rcf
17. Removed the spin column from tubes
18. Used nanodrop spectrophotometer to check concentration
    a. pIG005a concentration: 23.5 ng/μL
    b. pIG005b concentration: 30.6 ng/μL
19. Stored in iGEM freezer box labeled in -20 C freezer
20. For nanodrop: use 1.5 μL

Materials for restriction digest:

Procedure for restriction digest:

1. Digested all parts and the vector with BsaI by making a master mix with 144, μL diH20, 21 μL cutsmart buffer, and 3.5 μL BsaI
    a. For pSPB406, 413, 451, 459, 480, 501, 512, 617, 633, 671, 677, pIG005a and pIG005b
    b. Aliquoted 12 μL into labeled PCR tubes and added 2 μL template for all plasmids except for 633, which got 3 μL, and pIG005a and pIG005b, which got 4 μL
    c. Tubes labeled in plasmid number order
2. Incubated at 37 C for 30 min
    a. Start time: 12:58 pm
    b. End time: 1:28 pm

Gel Preparation

1. Measured 0.5 g agarose and mixed with 50 mL TBE
2. With a loosened cap, microwave agarose suspension or solid until completely clear and homogeneous liquid (≈1 min per 100 mL on high). Watch as it boils to stop the microwave before it can overflow. Swirl, handling with a silicone mit. Continue microwaving in smaller increments if necessary. (alicia)
3. added 5μl of 10000x gelgreen to molten agarose (alicia)
4. Poured in cast and set for 15 min
    a. start: 1:11 pm
    b. end: 1:40 pm

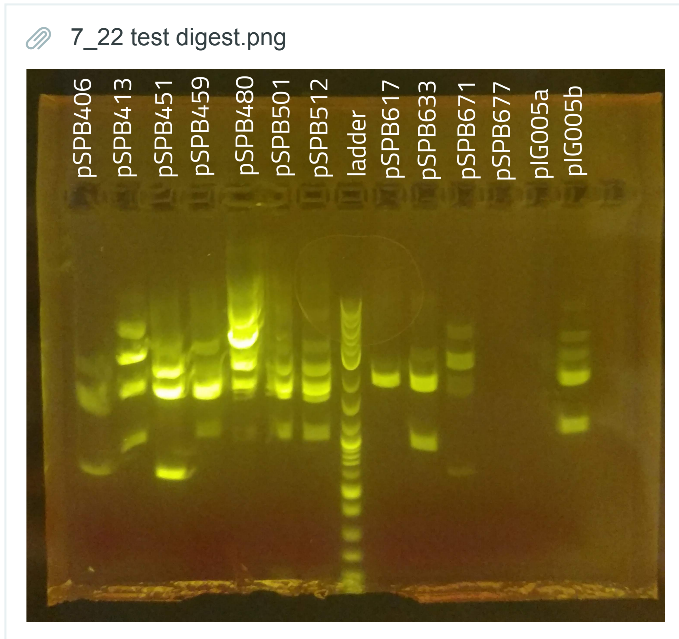
Gel loading

1. Mixed 3 μL 6X loading buffer with each restriction digest reaction
2. Made a 2x dilution of the 2 log loading ladder and loaded 5 μL
3. Loaded the gel according to the table below
4. Gel run times
    a. start: 2:00 pm
    b. end: 2:31 pm

Table1

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Lane 1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	pSPB406	413	451	459	480	501	512	ladder	617	633	671	677	pIG005a	pIG005b

7_22 test digest.png



Phosphorylation

pIG006, 7, 8, 9, and 12

Table2

	A	B	C	D	E	F	G	H
1	DNA Phosphorylation							
2	*Notes*	**Reactions**	6	**ATP?**	no		PNK buffer needs ATP	
3		**Excess**	3%	0%				
4		**Reactions**	**6.18**	**6**	***1 ref***			Combine n 10 µL rxns. Anneal.
5						< Warnings		Dilute (100/n)-fold to give 50 nM product.
6		**diH2O**	**46.35**	45	**7.5**	µL		OR combine ≤ten 10 µL rxns in 100 µL final, and dilute tenfold to give 50 nM product.
7		**10× Ligase/PNK buffer**	**6.18**	6	**1**	µL		
8		10 mM ATP	0	0	0	µL		
9		**T4 PNK**	**3.09**	3	0.5	µL	< 1 µL total	
10		**Oligo(s)**	**6.18**	6	1	µL	No recessed/blunt ends	
11								
12		**Total:**	**61.8**	**60**	**10**	µL		
13		***Total–DNA:***	**55.62**	**54**	**9**	µL		
14		***Total–Enz:***	**58.71**	**57**	**9.5**	µL		
15		***Total–DNA/Enz:***	**52.53**	**51**	**8.5**	µL		

Golden gate

Esp3I ChlR::pcr005, pSPB440

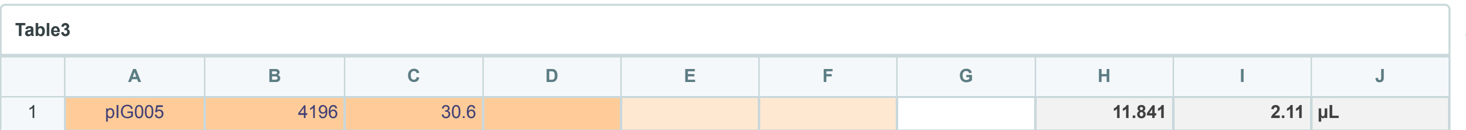


Table3

	A	B	C	D	E	F	G	H	I	J
1	pIG005	4196	30.6					**11.841**	**2.11**	**µL**

Table4

	A	B	C	D	E	F
1	Golden Gate Assembly 1					
2	Reactions	2		BSA?	yes	
3	# Unique Parts	0			1	
4	# Common Parts/Vector	4			1	
5	# Others (PCR pdts, oligo pts)	0			1	
6	Excess	2%	0%	0%	-	
7						
8	Reactions	2.04	2	1	1 ref	
9						< Warnings
10	diH2O	18.36	18	9	9	µL
11	10× Ligase Buffer	3.06	3	1.5	1.5	µL
12	10× BSA	3.06	3	1.5	1.5	µL
13	T4 DNA Ligase	1.02	1	0.5	0.5	µL
14	Type IIs endonuclease	1.02	1	0.5	0.5	µL
15		Total:			Each:	
16	Unique Parts	0	0	0	0.5	µL
17	Common Parts/Vector	4.08	4	2	0.5	µL
18		#N/A	#N/A	#N/A		
19	Others	0	0	0	0.5	µL
20						
21	Total:	30.6	30	15	15	µL
22	Total – DNA:	26.52	26	13	13	µL
23	Total – Uniques & Others:	30.6	30	15	-	µL
24						

Materials for PCR:

- Primers oIG001 and oIG004
- Nuclease free water
- NEB High Fidelity 2x PCR master mix (Lot# 10041218, NEB)
- Thermocycler

PCR reaction:

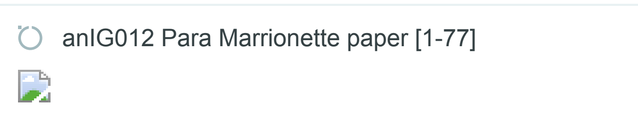
1. Set up a 25 μL PCR reaction with 12.5 μL master mix, 1.25 μL of each primer, 3 μL of template, and 7 μL water (Alicia)
2. Cycling conditions for otsBA:
    a. Initial denaturation: 98 C for 1 min
    b. Denaturation: 98 C for 10 sec
    c. Annealing: 60 C for 30 sec
    d. Extension: 72 C for 45 sec, 35X
    e. Final extension: 72 C for 2 min
    f. Protocol labeled as PCR_45

Innoculation, continued

1. Labeled 2 mL centrifuge tubes pIG005
2. Poured 3 2 mL of each liquid culture into 2 mL tubes labeled pIG005 (Samantha)
3. Spun down at 6000 rcf for 2 min
4. Removed supernatant and stored in -20 C freezer

Transformation

Transformed pIG101 and 102 into shyams cells using the CCMB80 protocol



July 23, 2019: Remaking part plasmids and cassette assembly

Project: 2019 iGEM
Authors: Claire Young

TUESDAY, 7/23/2019

Objectives: Today, we will remake the part plasmids for pIG003, 006, 007, 008, 009, 010, 012, 014, 015 with a golden gate reaction. We will also purify the otsBA PCR product from yesterday.

Gel Preparation

1. Measured 0.5 g agarose and mixed with 50 mL TBE
2. With a loosened cap, microwave agarose suspension or solid until completely clear and homogeneous liquid (≈1 min per 100 mL on high). Watch as it boils to stop the microwave before it can overflow. Swirl, handling with a silicone mit. Continue microwaving in smaller increments if necessary. (alicia)
3. added 5µl of 10000x gelgreen to molten agarose (alicia)
4. Poured in cast and set for 15 min
   a. start: 1:13 pm
   b. end: 1:35 pm

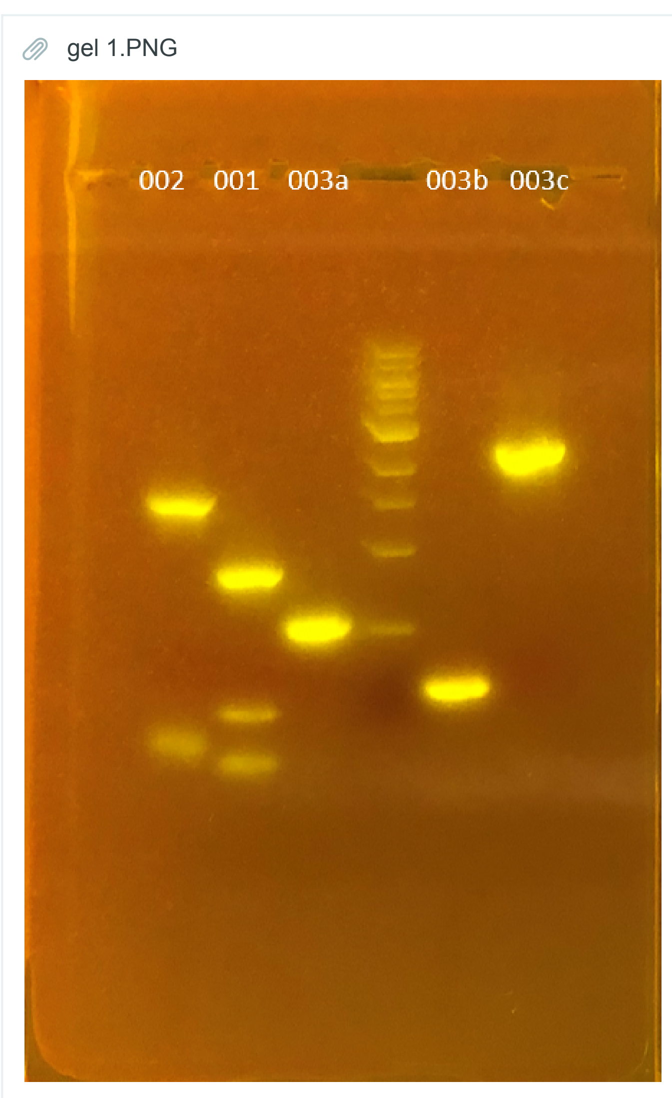
Gel loading

1. Mixed 10 µL 6X loading buffer with PCR sample (S for standard, T for touchdown)
   a. both are otsBA PCR reactions off of E. coli genome using oIG001 and oIG004
2. Loaded 30 µL 2x of each sample
3. Made a 2x dilution of the 2 log loading ladder and loaded 10 µL
4. Loaded the gel according to the table below
5. Gel run times at 120V
   a. start: 1:42 pm
   b. end: 2:12 pm

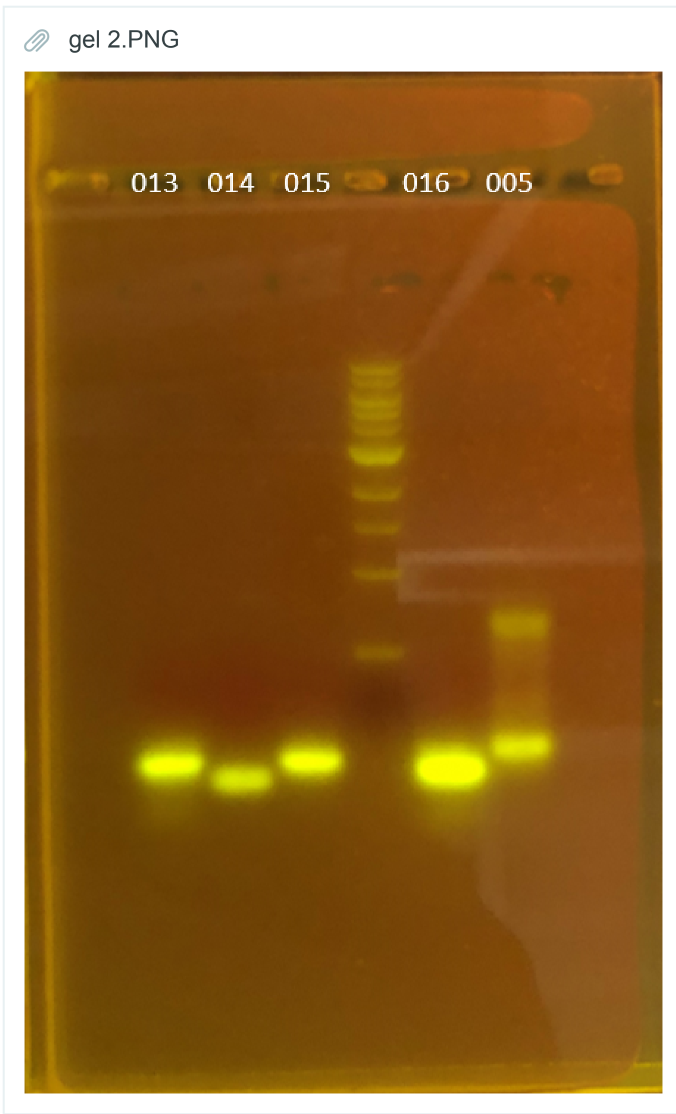
Gel Loading Diagram

	A	B	C	D	E	F	G	H
1	Lane 1	2	3	4	5	6	7	8
2	Blank	S1	S2	Ladder	T1	T2	Blank	Blank

gel 1.PNG



gel 2.PNG



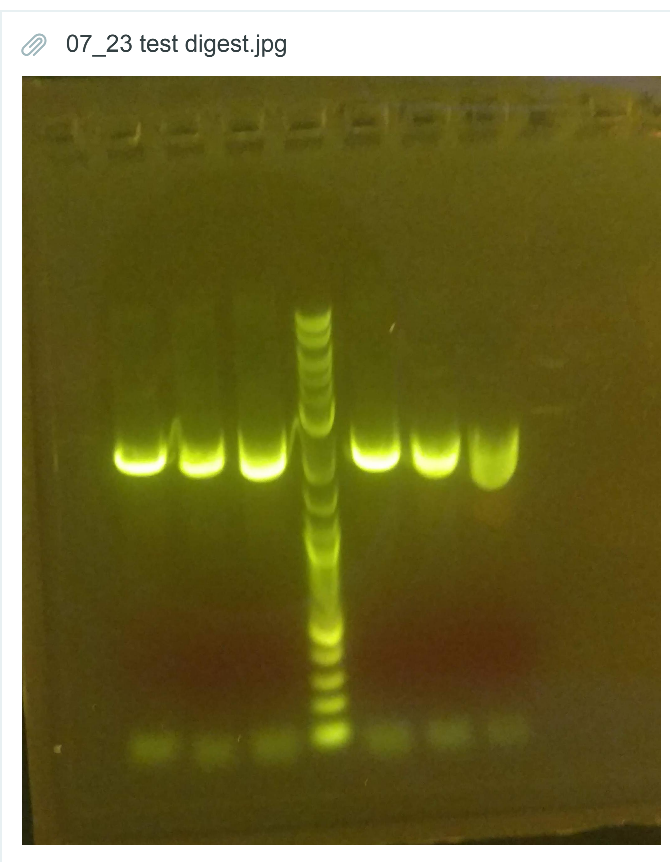
Images above (from July 16, 2019): PCR and oligo anneal products. pIG005 failed and was repeated.

Restriction digest conditions test:

BsaI restriction digest of pSPB469, incubated in the 37 °C incubator, the heat block, and the thermocycler to see which is more effective.

1. Digested pSPB469 with BsaI-HF v2 by making a master mix with 67.5 µL diH20, 9.9 µL cutsmart buffer, 1.65 µL BsaI, and 18 µL pSPB469
2. Alliquoted into 15 µL into 6 tubes (tube 6 only got 10 µL)
3. 2 tubes were placed in the 37 °C incubator (tubes 5,6), heatblock (tubes 3,4), and thermocycler (tubes 1,2) for 30 min
    a. Start time: 3:00 pm
    b. End time: 3:30 pm
4. Mixed 3 µL 6x loading dye with each sample and loaded in order : blank, 1, 2, 3, ladder, 4, 5, 6 (used other half of gel made on July 22)
    a. Start time: 3:43 pm
    b. End time: 4:14 pm

07_23 test digest.jpg



Gel results: the samples incubated in the 37 °C incubator resulted in slightly less clear bands, so it might be in the future to use the heat block or thermocycler. All samples yielded two bands of the predicted size, which calls into question why this test digest worked without having any other bands and past ones did not.

Procedure for making 1000x amp stock: (Stephanie)

1. Added 2 mL water, 2 mL ethanol, 500 mg of ampicillan sodium salt, then added water to 5 mL total volume
    a. Note: doesn't need to be sterile because the ethanol will take care of the bacteria

Innoculation:

Notes: the plate with pIG101 has a lot of green colonies, while pIG102 is under control of a Para so it needs to be grown in media with arabinose to check for successful transformations (so after growing in arabinose media, miniprep the ones that are green)

Use amp with the LB-30

Materials for Inoculation:

- LB 30 broth
- 1000X amp
- E. coli containing the target plasmids
- 37 C shaking incubator (SN 06004817 VWR)
- Culture tubes (SN 07418023 Falcon)
- 50 mL centrifuge tubes (SN C-3394-3 ISC BioExpress)

Procedure for Inoculation

1. 8 14 mL polporpylene round-bottom tubes labeled with the plasmid number: pIG101 and pIG102
    a. using pIG009 as negative control
2. Aliquoted 1 mL of the LB broth + amp for the arabinose testing, 2 mL for the miniprep cultures
    a. pIG101 g ara (green), pIG101 white ara, pIG102 a (white colony), pIG102 b (white colony), con (pIG009)
    b. added 18 μL arabinose to the 1 mL cultures for arabinose testing

3. Used a pipette tip to inoculate the bacteria into the prepared media
    a. Make sure to not touch the pippette tip
4. Incubated in the 37 C shaker for 7 hr at 200 rpm
    a. Start time: 4:02 pm
    b. End time:

Golden Gate

Table2

	A	B	C	D	E	F	G	H	I	J	K
1	pIG003a	pIG003b	pIG006	pIG007	pIG008	pIG009	pIG012	pIG013	pIG014		
2	is made from pcrIG003a, pcrIG003b, and pcrIG003c, and pSPB430	uses the low copy plasmid pSPB440					is made from pcrIG012a and pcrIG012b				

Table1

	A	B	C	D	E	F
1	Golden Gate Assembly 1					
2	***Reactions***	9		***BSA?***	yes	
3	***# Unique Parts***	0			*1*	
4	***# Common Parts/Vector***	4			*1*	
5	***# Others (PCR pdts, oligo pts)***	0			*1*	
6	***Excess***	2%	0%	0%	-	
7						
8	**Reactions**	**9.18**	9	1	*1 ref*	
9						< Warnings
10	**diH2O**	**82.62**	81	9	**9**	μL
11	10× Ligase Buffer	**13.77**	13.5	1.5	**1.5**	μL
12	**10× BSA**	**13.77**	13.5	1.5	**1.5**	μL
13	**T4 DNA Ligase**	**4.59**	4.5	0.5	0.5	μL
14	Type IIs endonuclease	**4.59**	4.5	0.5	0.5	μL
15		***Total:***			***Each:***	
16	Unique Parts	**0**	0	0	0.5	μL
17	**Common Parts/Vector**	**18.36**	18	2	0.5	μL
18		**#N/A**	#N/A	#N/A		
19	**Others**	0	0	0	0.5	μL
20						
21	***Total:***	**137.7**	135	**15**	*15*	μL
22	***Total – DNA:***	**119.34**	117	**13**	**13**	μL
23	***Total – Uniques & Others:***	**137.7**	135	**15**	-	μL

Materials for tranformation:

- The iGEM comp cells
- Plasmids from the golden gate reaction (labeled 3a, 3b, 6,7,8,9,12,13,14 for the plasmid number)
- Recovery media (from Shyam)
- Chl-LB plates

Procedure for tranformation:

1. Thaw comp cells on ice for 5 min
2. Pipetted 5 µL of each plasmid into a tube containing the comp cells (on ice)
    a. flick to mix, DO NOT pippette or vortex
3. Left the tubes on ice for 30 min
    a. Start time: 8:10 pm
    b. End time: 8:40 pm
4. Heat shocked tubes for 45 s at 42 °C
5. Immediately placed on ice for 2 min
6. Added 150 µL of SOC to each tube, flick to mix
7. Placed in 37 °C incubator for 1 hr
    a. Start time: 8:51 pm
    b. End time: 9:51 pm
8. Placed LB chl plates in 37 °C incubator to pre-warm
9. Used streaking method to plate ~200 µL of each tube onto the warm LB plates labeled with the plasmid number
10. Placed plates in 37 °C incubator

Redo Innoculation:

Redid the inoculation for pIG101

Start time:~11 pm

Special restriction digest

Procedure

1. Digested pIG102 with 8 µL diH20, 1.5 µL 3.1 buffer, 5µL DNA and 2 µL SwaI
2. Time running
    a. start time:11:30 pm
    b. end tomorrow morning

July 24, 2019: Miniprep, transformation of pIG102 (SwaI), and inoculation

Project: 2019 iGEM

Authors: Claire Young

WEDNESDAY, 7/24/2019

Objectives: Today, we will miniprep the overnight cultures of pIG101 and pIG102 from yesterday, transform the SwaI digest of pIG102, and innculate the cultures transformed yesterday (pIG003a, 003b, 006, 007, 008, 009, 012, 013, 014). (Claire)

Materials for Miniprep:

- QIAprep Spin Miniprep Kit (Cat. No. 27106 Qiagen)

Procedure for Miniprep: (Claire)

1. Miniprepped the overnight cultures of pIG101 and 102 (pIG101 white, pIG101 green, pIG101b, pIG102b, pIG102a, pIG101b)
2. Had green fluorescence: pIG101 green, pIG101 g ara
3. Had no fluorescence: pIG101 white, control, pIG101b, pIG102 a ara, pIG102 b ara, pIG 102 b, pIG102 a, pIG101 a, pIG101 ara
4. Control showed no growth
5. Note: 101b formed a very small pellet
6. Resuspended the first pellet in 250 uL of Buffer P1
    a. Stored Buffer P1 in the 4 C fridge
    b. vortexed for 30 seconds
7. Added 250 uL Buffer P2 to each tube and inverted 4-6 times until the solution became clear
    a. Don't let sit for more than 2 min
8. Added 350 uL Buffer N3 to each tube and mixed by inverting 4-6 times
9. Centrifuged for 10 min at 16,100 rcf
10. We ended up with two tubes labeled 459 after accidentally putting Buffer P1 and P2 into both origianl tubes
11. Pipetted 800 uL of the supernatent into a spin column
12. Centrifuged the spin columns for 1 min at 16,100 rcf
13. Discarded flowthrough and added 0.5 mL Buffer PB to each column
14. Centrifuged the columns for 1 min at 16,100 rcf
15. Discarded the flowthrough and added 0.75 mL Buffer PE to each column
16. Centrifuged for 1 min at 16,100 rcf
17. Placed 1 mL Buffer EB in a 36 C incubator
    a. Used the RT Buffer EB though
18. Removed the supernatent and centrifuged for 1 min at 16,100 rcf
19. Labeled 1.5 mL tubes with the plasmid numbers and transfered the columns to the 1.5 mL tubes
20. Dry spin the columns for 1 min at 16,100 rcf
21. Added 50 uL Buffer EB to each column and let stand for 5 min, then centrifuged for 1 min at 16,100 rcf
22. Removed the spin column from tubes
23. Used nanodrop spectrophotometer to check concentration
    a. pIG005a concentration: 23.5 ng/µL
    b. pIG005b concentration: 30.6 ng/µL
24. Stored in iGEM freezer box labeled in -20 C freezer
25. For nanodrop: use 1.5 µL

Nanodrop concentrations

	A	B
1	plasmid	concentration (ng/µL)
2	pIG101G	86.3
3	pIG101W	161.0
4	pIG101A	37.5
5	pIG101B	20.5
6	pIG102A	123.8
7	pIG102B	107.4

Transformation of SwaI restriction digest of pIG102

Materials for tranformation:

- Shyam's DH10B comp cells
- SwaI pIG102
- SOC Recovery media (from Shyam)
- arabinose Amp-LB plates

Procedure for tranformation: (Alicia)

1. Thaw comp cells on ice for 5 min
2. Pipetted 7.5 µL of each plasmid into a tube containing the comp cells (on ice)
    a. flick to mix, DO NOT pippette or vortex
3. Left the tubes on ice for 30 min
    a. Start time: pm
    b. End time: pm
4. Heat shocked tubes for 45 s at 42 °C
5. Immediately placed on ice for 2 min
6. Added 150 µL of SOC to each tube, flick to mix
7. Placed in 37 °C incubator for 1 hr
    a. Start time: 10:54 pm
    b. End time: pm
8. Placed LB Amp plates in 37 °C incubator to pre-warm
9. Used streaking method to plate ~200 µL of each tube onto the warm LB plates labeled with the plasmid number
    a. Plate half of the recovery on a regular LB amp plate and the rest on the +ara one. If you need my amp plates, they're in Fridge 0 with the blue stripe. Arabinose is in a bag in the Freezer 0 door labeled _Sara - arabinose_
10. Placed plates in 37 °C incubator
    a. start time: 12:03 pm

Meanwhile preparation of LB30- Amp plates

1. Meanwhile, add 20% arabinose to a final 0.5% in one LB-amp plate, assuming 20 mL agar in a plate. Do this by mixing the arabinose in a final 400 µL SOC and spreading it evenly over the agar surface using a P1000 tip.
    a. 10 µl of arabinose and 390 µL
2. Dry this plate without a lid in incubator 1 during the ice incubation of the transformation, until 1.5 hr later when you plate (assuming it's dry by then).

Notes

- SwaI digest from yesterday was performed using a SwaI digest protocol in the thermocycler in the Bennett lab

Materials for Inoculation:

- LB 30 broth
- 1000X chl
- E. coli containing the target plasmids
- 37 C shaking incubator (SN 06004817 VWR)
- Culture tubes (SN 07418023 Falcon)
- 50 mL centrifuge tubes (SN C-3394-3 ISC BioExpress)

Procedure for Inoculation (Claire, Stephanie, Samantha, Tasneem)

1. Removed cultures from the 37 °C incubator at 12:58 pm
    a. Except for pIG003b, which had no visible colonies and was placed back in the incubator
2. 16 14 mL polporpylene round-bottom tubes labeled with the plasmid number: pIG003a, 006, 007, 008, 009, 012, 013, 014, (for each plasmid, picked two cultures and labeled either a or b)
3. Aliquoted 2 mL of LB-30 + 1000x Chl into 16 tubes
4. Used a pipette tip to inoculate the bacteria into the prepared media
    a. Make sure to not touch the pippette tip
    b. Don't want to pick the green colonies
5. Incubated in the 37 C shaker for 7 hr at 200 rpm
    a. Start time: 1:38 pm
    b. End time: 9:42 pm
6. Poured 2 mL of each liquid culture into 2 mL tubes labeled with the plasmid number (see step 1)
7. Spun down at 6000 rcf for 2 min
8. Removed supernatent by pouring and stored the pellets in -20 C freezer
9. Rexamined pIG003b, had 1 visible colony
    a. Will wait until the morning, was placed back in the incubator

Untitled

Introduction

Get started by giving your protocol a name and editing this introduction.

Materials

›

Procedure

1.

July 25, 2019: Transformation of pIG102 (SwaI digest), miniprep, and test digest, Golden gate to make pIG102 using linIG102 and pIG101

Project: 2019 iGEM
Authors: Alicia Selvera

THURSDAY, 7/25/2019

Objectives: Since the cultures las night showed no growth, today we will retransform and try again. We will also miniprep the pellets spun down from last night and do a restriction digest and gel electrophoresis to check their success.

Notes: The plates left overnight that were plated with cells transformed with pIG102 showed no growth.

Procedure for tranformation: (Alicia)

1. Thaw comp cells on ice for 5 min
   a. using pIG102 SwaI digest and undigested
   b. our cell and shyams
2. Pipetted 3.0 µL of each plasmid into a tube containing the comp cells (on ice)
   a. flick to mix, DO NOT pippette or vortex
3. Left the tubes on ice for 30 min
   a. Start time: 9:36 am
   b. End time: 10:06am
4. Heat shocked tubes for 45 s at 42 °C
5. Immediately placed on ice for 2 min
6. Added 150 µL of SOC to each tube, flick to mix
7. Placed in 37 °C incubator for 1 hr
   a. Start time: 10:13 pm
   b. End time: 11:13 pm
8. Placed LB Amp plates in 37 °C incubator to pre-warm
9. Used streaking method to plate ~200 µL of each tube onto the warm LB plates labeled with the plasmid number
   a. Plate half of the recovery on a regular LB amp plate and the rest on the +ara one. If you need my amp plates, they're in Fridge 0 with the blue stripe. Arabinose is in a bag in the Freezer 0 door labeled _Sara - arabinose_
10. Placed plates in 37 °C incubator
    a. start time: 11:30am

Meanwhile preparation of LB30- Amp plates

1. Meanwhile, add 20% arabinose to a final 0.5% in one LB-amp plate, assuming 20 mL agar in a plate. Do this by mixing the arabinose in a final 400 µL SOC and spreading it evenly over the agar surface using a P1000 tip.
   a. 10 µl of arabinose and 390 µL
2. Dry this plate without a lid in incubator 1 during the ice incubation of the transformation, until 1.5 hr later when you plate (assuming it's dry by then).

Notes

- SwaI digest is 25 °C for 2hrs
- and 65°C for 20 min

Materials for Miniprep:

- QIAprep Spin Miniprep Kit (Cat. No. 27106 Qiagen)

Procedure for Miniprep: (Clairez+Alicia)

1. Miniprepped pIG003aa, 006, 007, 008, 009, 012, 013, 014
2. Resuspended the first pellet in 250 uL of Buffer P1
    a. Stored Buffer P1 in the 4 C fridge
    b. vortexed for 30 seconds
3. Added 250 uL Buffer P2 to each tube and inverted 4-6 times until the solution became clear
    a. Don't let sit for more than 2 min
4. Added 350 uL Buffer N3 to each tube and mixed by inverting 4-6 times
5. Centrifuged for 10 min at 16,100 rcf
6. Pipetted 800 uL of the supernatent into a spin column
7. Centrifuged the spin columns for 1 min at 16,100 rcf
8. Discarded flowthrough and added 0.5 mL Buffer PB to each column
9. Centrifuged the columns for 1 min at 16,100 rcf
10. Discarded the flowthrough and added 0.75 mL Buffer PE to each column
11. Centrifuged for 1 min at 16,100 rcf
12. Placed 1 mL Buffer EB in a 36 C incubator
    a. Used the RT Buffer EB though
13. Removed the supernatent and centrifuged for 1 min at 16,100 rcf
14. Labeled 1.5 mL tubes with the plasmid numbers and transfered the columns to the 1.5 mL tubes
15. Dry spin the columns for 1 min at 16,100 rcf
16. Added 50 uL Buffer EB to each column and let stand for 5 min, then centrifuged for 1 min at 16,100 rcf
17. Removed the spin column from tubes
18. Used nanodrop spectrophotometer to check concentration
    a. pIG005a concentration: 23.5 ng/µL
    b. pIG005b concentration: 30.6 ng/µL
19. Stored in iGEM freezer box labeled in -20 C freezer
20. For nanodrop: use 1.5 µL

Nanodrop concentrations

	A	B
1	Plasmid	Concentration (ng/µL)
2	pIG003a	15.3
3	pIG006	40.3
4	pIG007	21.9
5	pIG008	26.6
6	pIG009	20.6
7	pIG012	43.4
8	pIG013	19.1
9	pIG014	25.9

Test Digest:

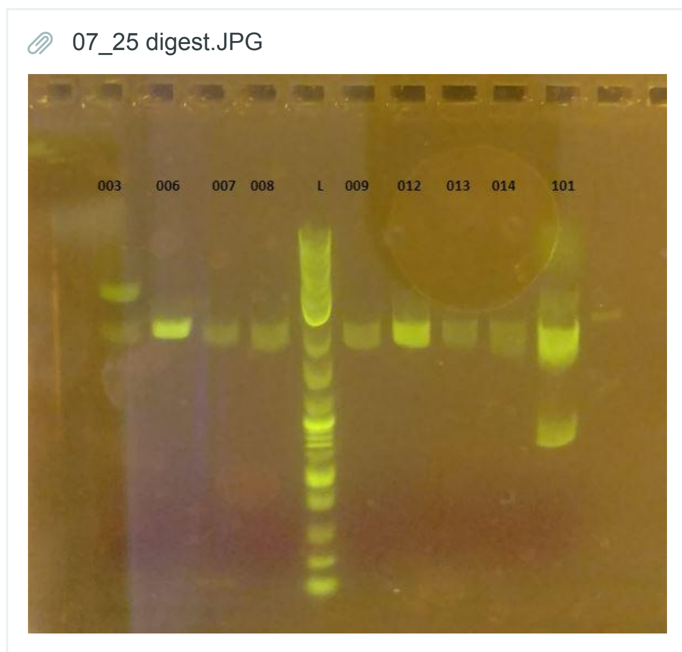
1. Digested pIG003a, 006, 007, 008, 009, 012, 013, 014, and 101g with BsaI-HF v2 by making a master mix with 91.5 µL diH20, 14.85 µL cutsmart buffer, 2.47µL BsaI
2. Alliquoted into 11 µL into 9 tubes
3. Added 4 µL of each plasmid into a tube

4. Tubes were incubated in a 37 °C incubator
    a. Start time: 1:31 pm
    b. End time: 2:07

Electrophoresis

1. 50 ml TAE + .50g of agarose + 5µl of 10,000x gel green
2. heated in microwave for 1 min
3. cast with two wells
4. Mixed 3 µL 6x loading dye with each sample and loaded in order : blank, pIG003, 006, 007, 008, ladder (5 µL), 009, 012, 013, 014, 101
    I. Start time: 2:24 pm
    II. End time: 2:55 pm

07_25 digest.JPG



pIG003, 012, and 101 had all bands that were supposed to be there, but it's possible the others had a smaller band as well that's too dim to see.

Golden gate to make pIG102 using linIG102 and pIG101

Steps

Prepare a mastermix by adding together enzyme, T 4 DNA Ligase, 10X T 4 Ligase Buffer, 10X BSA, and water

Use the material notes to determine abouts for components in the mastermix, table can be used as an example

Table1

	A	B	C	D	E	F
1	Golden Gate Assembly 1					
2	***Reactions***	1		***BSA?***	no	
3	***# Unique Parts***	2			*1*	
4	***# Common Parts/Vector***				*1*	
5	***# Others (PCR pdts, oligo pts)***	0			*1*	
6	***Excess***	2%	0%	0%	-	
7						
8	**Reactions**	**1.02**	1	1	*1 ref*	
9						< Warnings
10	**diH2O**	**11.73**	11.5	11.5	**11.5**	µL
11	10× Ligase Buffer	**1.53**	1.5	1.5	**1.5**	µL
12	10× BSA	0	0	0	0	µL
13	**T4 DNA Ligase**	**0.51**	0.5	0.5	0.5	µL
14	Type IIs endonuclease	**0.51**	0.5	0.5	0.5	µL
15			***Total:***		***Each:***	
16	Unique Parts	**1.02**	1	1	0.5	µL
17	Common Parts/Vector	0	0	0	0.5	µL
18						
19	Others	0	0	0	0.5	µL
20						
21	***Total:***	**15.3**	15	**15**	*15*	µL
22	***Total – DNA:***	**14.28**	14	**14**	**14**	µL
23	***Total – Uniques & Others:***	**14.28**	14	**14**	-	µL

Assemble reactions in PCR tubes on ice or cold block

Pipette up and down to mix

Distribute mastermix into labelled pcr tubes

Add DNA components for each reaction to the concentrations specified

Flick a few times to mix

Centrifuge a few seconds to recollect the liquid

Place the reaction tubes into the thermocycler and adjust the settings for a given enzyme as shown in the following tables

Table2

	A	B	C	D	E	F	G	H
1		Initial Digestion (opt.)	37°C	10 min			37°C	20 min
2	Repeat 25x/15x	Digestion	37°C	1.5 min		Repeat 5-10x	37°C	1.5 min
3	Repeat 25x/15x	Annealing & Ligation	16°C	3 min		Repeat 5-10x	16°C	3 min
4		Digestion & Ligase Inact.	50°C	10 min			50°C	10 min
5		Inactivation	80°C	10 min			80°C	10 min
6		Storage	12°C	∞			12°C	∞

Note: lid temp should be 5-10 C higher than the highest temperature

Miniprep of part plasmids (B)

Materials for Miniprep:

- QIAprep Spin Miniprep Kit (Cat. No. 27106 Qiagen)

Procedure for Miniprep: (Samantha + Tasneem)

1. Parts: pIG003ab, 006b, 007b, 008b, 009b, 012b, 014b
    a. Shyam did 003ab, 012b
    b. discarded 013b
2. Resuspended the first pellet in 250 uL of Buffer P1 (P1 stored in 4 C fridge)
    a. vortexed for 30 seconds until no pellet remained
3. Added 250 uL Buffer P2 to each tube and inverted 4-6 times until the solution became clear
    a. Let sit for at least 1 min, but not more than 2 min
4. Added 350 uL Buffer N3 to each tube and mixed by inverting 4-6 times
    a. Centrifuged for 10 min at 16,100 rcf
5. Poured supernatent into a spin column
    a. Centrifuged the spin columns for 30 sec at 16,100 rcf
    b. Discarded the flowthrough **by pipetting out**
6. Added 750 uL Buffer PE to each column
    a. Centrifuged for 30 sec at 16,100 rcf
    b. Discarded the flowthrough **by pipetting out**
    c. Centrifuged for 2 min at 16,100 rcf (dry spin)
7. Labeled 1.5 mL tubes with the plasmid numbers and transfered the columns to the 1.5 mL tubes
8. Added 30 uL Buffer EB to each column and let stand for 5 min, then centrifuged for 1 min at 16,100 rcf
9. Removed the spin column from tubes
10. Used **nanodrop** spectrophotometer to check concentration (1.5 uL of each sample)
    a. pIG003aa concentration: 25.2 ng/μL
    b. pIG003ab concentration: 294.3 ng/μL
    c. pIG006b concentration: 52.9 ng/μL
    d. pIG007b concentration: 117.1 ng/μL
    e. pIG008b concentration: 49.8 ng/μL
    f. pIG009b concentration: 60.6 ng/μL
    g. pIG012a concentration: 60.6 ng/μL
    h. pIG012b concentration: 79.5 ng/μL
    i. pIG014b concentration: 23.1 ng/μL
    j. pIG101 G concentration: 121.8 ng/μL

Transformation:

1. Thaw comp cells on ice for 5 min
    a. Using pIG102
    b. our cell and shyams
2. Pipetted 5.0 µL of plasmid into a tube containing the comp cells (on ice)
    a. Flick to mix, DO NOT pipette or vortex
3. Left the tubes on ice for 30 min
    a. Start time: 9:15 pm
    b. End time: 9:45 pm
4. Heat shocked tube for 45 s at 42 °C
5. Immediately placed on ice for 2 min
6. Added 600 µL of SOC to tube, flick to mix
7. Placed in 37 °C incubator for 1 hr
    a. Start time: 9:49 pm
    b. End time: 10:45 pm
8. Placed LB Amp plate in 37 °C incubator to pre-warm
9. Used streaking method to plate ~600 µL of tube onto the warm LB plate labeled with the plasmid number
10. Placed plate in 37 °C incubator
    a. start time: 10:47 pm

July 26, 2019: Inoculation of pIG102, electrophoresis, sequencing of pIG005 and pIG012, transformation of pIG005

Project: 2019 iGEM

Authors: Claire Young

FRIDAY, 7/26/2019

Objectives:

Inoculation of pIG102:

1. Labeled two 14 mL culture tubes with pIG102 and added 2 mL of LB-30 and 2 µL of 1000x amp
2. Picked two green colonies made with Shyam's comp cells off the pIG102 plate w/ arabinose and put in each culture tube
3. Incubated at 37 °C for ~8 hr
   a. Start time: 10:37 am

Restriction digest

Table2

	A	B	C	D	E	F	G
1	DNA Restriction Digest 2						
2	*Notes*	**Reactions**	1	**BSA?**	no		
3		**Excess**	2%	0%			
4		**Reactions**	**1.02**	**1**	***1 ref***		
5						< Warnings	
6		**diH2O**	**7.9764**	7.82	**7.82**	µL	
7		**10× rxn buffer**	**1.02**	1	**1**	µL	
8		10× BSA	0	0	0	µL	
9		**Enzyme 1**	**0.51**	0.5	0.5	µL	< 1 µL total
10		**Enzyme 2**	**0**	0	0	µL	
11		**Enzyme 3**	**0**	0	0	µL	
12		**DNA**	**0.6936**	0.68	0.68	µL	Consider copy#
13							
14		**Total:**	**10.2**	**10**	10	µL	
15		***Total–DNA:***	**9.5064**	**9.32**	**9.32**	µL	
16		***Total–Enz:***	**9.69**	**9.5**	**9.5**	µL	
17		***Total–DNA/Enz:***	**8.9964**	**8.82**	**8.82**	µL	

Table1

	A	B	C	D	E	F	G
1	DNA Restriction Digest 2						
2	*Notes*	**Reactions**	15	**BSA?**	no		
3		**Excess**	2%	0%			
4		**Reactions**	**15.3**	**15**	***1 ref***		
5						< Warnings	
6		**diH2O**	**84.15**	**82.5**	**5.5**	µL	
7		**10× rxn buffer**	**15.3**	**15**	**1**	µL	
8		**10× BSA**	**0**	**0**	**0**	µL	
9		**Enzyme 1**	**7.65**	**7.5**	0.5	µL	
10		**Enzyme 2**	**0**	**0**	0	µL	< 1 µL total
11		**Enzyme 3**	**0**	**0**	0	µL	
12		**DNA**	**45.9**	**45**	3	µL	Consider copy#
13							
14		**Total:**	**153**	**150**	10	µL	
15		***Total–DNA:***	**107.1**	**105**	**7**	µL	
16		***Total–Enz:***	**145.35**	**142.5**	**9.5**	µL	
17		***Total–DNA/Enz:***	**99.45**	**97.5**	**6.5**	µL	

For a 10 uL reaction

Mix, by pipetting up and down, 1µL 10X buffer, and 7.5 µL water
add in 0.5 µL of enzyme, and mix by pipetting up and down
mix in 200 ng of template DNA
3: 0.7 µL
3A: 4 µL
6: 4 µL
7: 4 µL
8: 4 µL
9: 4 µL
12: 3.3 µL
14: 4 µL
101G: 1.64 µL
6B: 3.77 µL
7B: 1.71 µL
8B: 4.0 µL
9B: 3.33 µL
12B: 2.5 µL
14B: 4.0 µL
101W: 3.0 µL
Incubate samples for 30 min - 1 hr at 37 C.
NotI is suggested to run for 30 min.
Start time: 11:55 am

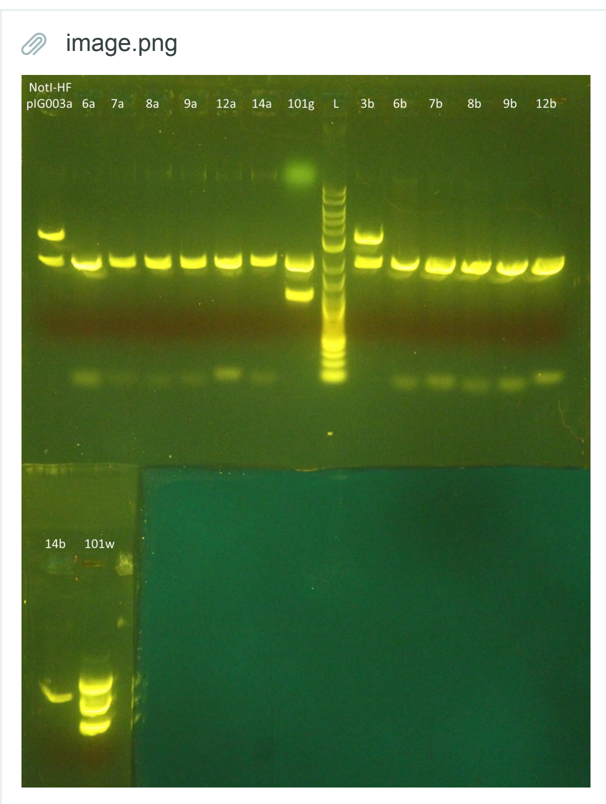
End time: 1:30 pm

Store samples at -20 C or use for gel electrophoresis

Gel electrophoresis

1. Added 2 µL 6x loading buffer to each restriction digest
2. Used agarose gels from Shyam
3. Loaded gel in order 3, 6, 7, 8, 9, 12, 14, 101g, 3a, 6b, 7b, 8b, 9b, 12b, 14b, 101w
4. Ran for 20 min at 110 V
    a. Start time: 1:54 pm
    b.

image.png



Sequecing

1. Sequenced pIG005 and pIG012 (4,5,6,7,8,9,12)
2. For pIG005, added 12.5 µL AB17 primer, pIG005

(put off until the weekend, leaving here for notes) Golden gate

remaking pIG101 w/ RSF1010 mut origin (pSBP449) (and pMB1 pSBP417)

Table3

	A	B	C	D	E	F
1	Golden Gate Assembly 2					
2	***Reactions***	3		***BSA?***	yes	
3	***# Unique Parts***	0			*1*	
4	***# Common Parts/Vector***	0			*1*	
5	***# Others (PCR pdts, oligo pts)***	0			*1*	
6	***Excess***	2%	0%	0%	-	
7						
8	**Reactions**	**3.06**	3	1	*1 ref*	
9						< Warnings
10	**diH2O**	**33.66**	33	11	**11**	µL
11	10× Ligase Buffer	**4.59**	4.5	1.5	**1.5**	µL
12	**10× BSA**	**4.59**	4.5	1.5	**1.5**	µL
13	**T4 DNA Ligase**	**1.53**	1.5	0.5	0.5	µL
14	Type IIs endonuclease	**1.53**	1.5	0.5	0.5	µL
15		***Total:***			***Each:***	
16	Unique Parts	**0**	0	0	0.5	µL
17	Common Parts/Vector	0	0	0	0.5	µL
18						
19	Others	0	0	0	0.5	µL
20						
21	***Total:***	**45.9**	45	**15**	*15*	µL
22	***Total – DNA:***	**45.9**	45	**15**	**15**	µL
23	***Total – Uniques & Others:***	**45.9**	45	**15**	-	µL

Procedure for tranformation: (Alicia)

1. Thaw comp cells on ice for 5 min
    a. using pIG005 SwaI digest and undigested
    b. our cell and shyams
2. Pipetted 3.0 µL of each plasmid into a tube containing the comp cells (on ice)
    a. flick to mix, DO NOT pippette or vortex

3. Left the tubes on ice for 15 min
4. Heat shocked tubes for 45 s at 42 °C
5. Immediately placed on ice for 2 min
6. Added 150 μL of SOC to each tube, flick to mix
7. recovered att 37C for an hour
8. Placed LB Amp plates in 37 °C incubator to pre-warm
9. Used streaking method to plate ~200 μL of each tube onto the warm LB plates labeled with the plasmid number
10. Placed plates in 37 °C incubator
    a. start time: 6:53 pm

Conclusion

The cassettes were entirely wrong on the gel. Trouble shooting wasn't easy, so we decided to sequence the parts that made them (plus some extra) just so we can problem solve moving forward. Once sequencing comes back, we'll have a better idea of how to move forward.

July 28, 2019: Inoculation of pIG005 (failed) cont.

Project: 2019 iGEM
Authors: Stephanie Trejo

SUNDAY, 7/28/2019

Inoculation of pIG005 (cont.)

1. Incubated in the 37 C shaker for __ at 275 rpm
    a. Start time:
    b. End time: 9:43 am

Notes: The tubes showed no growth, and the pIG005 plate wrapped in parafilm left to grow overnight did not show any colonies, only a few air bubbes. The tubes were discarded, and the plate was wrapped in parafilm and set out on the bench again until further troubleshooting.

Notes (2): The protocol for the transformation for pIG005 done July 27th was not updates into the lab notebook, but followed the same TSS heat shock protocol that we have been using. Shyam's turbo E coli cells were used

July 31, 2019: Preparation of P. putida comp cells, inoculation, pcrIG010 otsBA made

Project: 2019 iGEM
Authors: Claire Young

WEDNESDAY, 7/31/2019

Objectives:

Preparation of P. putida comp cells procedure:

1. Moved plates of P. putida from the 30 °C incubator in the Sillberg lab to the 4 °C fridge in Abercrombie at 9 am
    a. The LB plate showed significant growth
    b. The two PNS plates with varying levels of sucrose showed no growth

Inoculation

Materials for Inoculation:

- LB 30 broth
- P. putida
- 30 C shaking incubator (SN 06004817 VWR)
- Culture tubes (SN 07418023 Falcon)
- 50 mL centrifuge tubes (SN C-3394-3 ISC BioExpress)

Procedure for Inoculation

1. 4 14 mL polporpylene round-bottom tube labeled as *P. putida* + LB30
2. Aliquoted 4 mL of LB-30 into 2 tubes and 7 mL into the other 2 tubes
3. Used a pipette tip to inoculate the bacteria from *P. putida* LB plate (7-30-19) into the prepared media
4. Incubated in the 30 C shaker for 7 hr at 275 rpm
    a. Start time for 4 mL: 5:30 pm
    b. Start time for 7 mL: 5:43 pm
    c. End time:

Materials for PCR:

- Primers (listed in the table below)
- Nuclease free water
- NEB High Fidelity 2x PCR master mix (Lot# 10041218, NEB)
- Thermocycler

Procedure for PCR of otsBA: (alicia)

Resuspending the primers:

I. Used centrifuge to spin down ordered primers for 30 sec. at 10,000 rcf
II. Added the amount of water in the table below to each primer
III. Pipetted up and down to mix
IV. 10X dilution performed by combing 10 um stock solution with 90 um water
    1. Labels from IDT were used for the working solution tubes, additionally labed with the concentration 10 uM
V. Working and stock solutions of primers were stored in the -20 °C iGEM freezer box

PCR reaction:

I. Set up a 25 µL PCR reaction with 12.5 µL master mix, 1.25 µL of each primer, 1 µL of template, and 9 µL water
    1. for master mix, 118.75 uL master mix, 85.5 uL of water

II. For getting parts from the well: pierced the foil covering each well with a pipette tip, took 15 µL of nuclease-free water and added to each well, pulled out the liquid and transfered to a

III. Cycling conditions for otsB and otsA:

1. Initial denaturation: 98 C for 1 min
2. Denaturation: 98 C for 10 sec
3. Annealing: 70 C for 30 sec
4. Extension: 72 C for 45 sec, 35X
5. Final extension: 72 C for 2 min
6. Hold: 12 C

Two tubes of otsBA: one labeled otsBA and other otsBA (good)

context: otsBA tube had some pipette problems so it was done again in another tube

August 1, 2019: Preparation of P. putida comp cells

Project: 2019 iGEM
Authors: Claire Young

THURSDAY, 8/1/2019

Objectives: Today, we will prepare electrocompetent P. putida cells, as well as analyze the autofluorescent spectrum of P. putida and prepare a glycerol-cell stock for storage. (Claire)

Procedure for inoculation (continued from July 31):

1. Removed P. putida cultures from 30 °C incubator at 8:28 am
2. Added 2 mL overnight culture to 13 mL of LB-30 into 3 15 mL conical tubes, placed in 30 °C shaking incubator in Bennett lab
3. Start time: 9:15 am
    a. OD: 0.415 at 600 nm
    b. 11:08 am OD: 0.593
    c. 1:12 pm OD: 0.799

Measurement of the flourescence of p putida cultures in relation to the emmission and excitation of sfgfp

Materials:

- 96-well plate
- Overnight P. putida culture

Procedure:

1. Pipetted 150 µL of overnight culture into well A1 of a 96-well plate
2. Used the SEA plate reader in the Chappell lab to measure the emission and excitation wavelengths of the culture

Making Glycerol Stock
Materials

- 100% glycerol
- DNA grade water

Procedure for 50% glycerol stock

1. 11 mL of 100% glycerol
2. 11 mL of water
3. Shake until homogeneous
4. Pipette 0.5 mL of overnight culture and 0.5 mL 50% glycerol into an epi tube
5. Chilled on ice

Procedure for 10% glycerol stock

1. 22 mL 50% glycerol
    a. split into 2 50 mL tubes of 11 mL 50% glycerol
2. Added 44 mL of DNA grade water to each tube
3. Shake until homogeneous
4. Chilled on ice

Making Electrocompetent Cells

Materials:

- overnight *P. putida* culture
- 10% glycerol washing solution
- Allegra X-30R Centrifuge (Beckman Coulter)

Procedure:

1. Inoculate 50 mL standard-1 medium with 7 mL of a fresh overnight culture of *P. putida*. Grow cells at 30 deg C to a density of O.D. of 0.8 (see procedure from yesterday and continued today)
2. Poured 21 mL of *P. putida* culture into 2 50 mL tubes
3. Harvested by centrifugation
   a. 3000 g for 7 min at 7 deg C
4. Washed twice with 25 mL of 10% ice-cold glycerol in each tube, centrifuged
   a. 300 g for 7 min at 7 deg C
5. Resuspended cells in 0.8 mL of 10% ice-cold glycerol, kept on ice
6. Aliquoted 40 uL of the cells into 28 PCR tubes, kept on ice
   a. they were all labeled "p"
   b. did not use liquid nitrogen to flash freeze the tubes because we could not get any
7. Placed in Silberg lab -80 deg C freezer in P. putida EC box and the glycerol stock paced in Jordan's glycerol stock box

August 5, 2019: PCR of pcr005, gel purification of pcr005 and pcr010

Project: 2019 iGEM

Authors: Claire Young

MONDAY, 8/5/2019

Objectives: Today, we will amplify the Pcon-araC part out of the pBEST plasmid using PCR. We will then use gel electrophoresis to verify that the part is correct then do a gel purification to obtain the product. (Claire)

Materials for PCR of Pcon-araC:

- Primers oIG044 and oIG045
- 4578g pBEST template from Kshitij
- E. coli genome from Li Chieh
- Nuclease free water
- NEB High Fidelity 2x PCR master mix (Lot# 10041218, NEB)
- Thermocycler

Procedure for PCR of Pcon-araC + otsBA:

Resuspending the primers: (Claire)

1. Used centrifuge to spin down ordered primers for 30 sec. at 10,000 rcf
2. Added the 318 µL of water to oIG044 and 301 µL of water to oIG045 to get to 100 µM
3. Pipetted up and down to mix
4. 10X dilution performed by combing 10 um stock solution with 90 um water
    a. Labels from IDT were used for the working solution tubes, additionally labed with the concentration 10 uM
5. Working and stock solutions of primers were stored in the -20 °C iGEM freezer box

PCR reaction: (Claire + Alicia)

1. Set up a 25 µL PCR reaction with 12.5 µL master mix, 1.25 µL of each primer, 1 µL of template, and 9 µL water
    a. Also set up a PCR reaction for pcr010
2. Cycling conditions for pcr005:
    a. Initial denaturation: 98 C for 30 sec
    b. Denaturation: 98 C for 10 sec
    c. Annealing: 66 C for 30 sec
    d. Extension: 72 C for 30 sec, 35X
    e. Final extension: 72 C for 2 min
    f. Hold: 12 C
3. Cycling conditions for pcr010:
    I. Initial denaturation: 98 C for 30 sec
    II. Denaturation: 98 C for 10 sec
    III. Annealing: 69 C for 30 sec
    IV. Extension: 72 C for 1 min, 35X
    V. Final extension: 72 C for 2 min
    VI. Hold: 12 C

note: otsBA most likely had too much primer added to the last PCR rxn that we did

Gel Preparation (Claire)

1. Measured 0.5 g agarose and mixed with 50 mL TBE

2. With a loosened cap, microwave agarose suspension or solid until completely clear and homogeneous liquid (≈1 min per 100 mL on high). Watch as it boils to stop the microwave before it can overflow. Swirl, handling with a silicone mit. Continue microwaving in smaller increments if necessary.
3. added 5µl of 10000x gelgreen to molten agarose (alicia)
4. Poured in cast and set for at least 15 min
5. Because two combs were put in, the other half of the gel was stored in cling wrap in a tupperware in the fridge
   a. Note: don't use lane 1 on that gel because a sample was run in it to make sure the gel green was still okay after accidentally being put in the fridge

Gel Electrophoresis (Claire)

1. Mixed 5 µL 6X DNA loading dye with each pcr product to make the total volume 30 µL
2. Loaded pcr005 in lane 3, the ladder in lane 4, pcr010 from 8/5 in lane 5, and pcr010 from 7/31 in lane 6
3. Ran gel at 120 V
   a. Start time: 3:29 pm
   b. End time: 3:57 pm

Gel Purification (Claire)

1. Cut out target bands and weigh gels in 1.5 mL centrifuge tubes

Gel weight and nanodrop concentrations

	A	B	C	D
1		pcr005	pcr010 8/5 (prc010a)	pcr010 7/31 (pcr010b)
2	Weight (mg)	137.0	142.8	212.6
3	Weight X 3	411.0	428.4	637.8
4	[ng/µl]	16.7	33.1	23.6

2. multiplied weight by 3
3. added in that much µL agarose disolving buffer
4. incubated at 50•C for 10 minutes shaking
5. Transfered melting agarose to spin columns
6.Centrifuged at max speed 60 seconds
7. discarded flow through
8. Added in 200 µl of DNA wash buffer
9. Centrifuged at max fo r 30 seconds, discard flow through via pipetting, repeat
10. dry spun for 30
10. eluted w/ 20 µl of elution buffer, transfered to 1.5 ml centrifuge tube, spun for 30 sec at max speed
    Note for future: elute in 6 µL instead of 20 µL
11. Stored in freezer

Summary: The gel showed bands at around the correct sizes, and because we eluted in 20 µL instead of 6 µL, the concentrations aren't as bad as they seem so we don't need to redo the reactions soley based on the concentrations. Tomorrow, we will use the pcr005 in a golden gate reaction to remake pIG101. (Claire)

August 6, 2019: Golden gate of pIG101 and transformation of pIG101

Project: 2019 iGEM

Authors: Claire Young

TUESDAY, 8/6/2019

Objectives: Today, we will do a golden gate reaction using pSPB437, pSPB413, pcrIG005, and either pSPB480, pSPB449, or pSPB417 to obtain pIG101. (Claire)

Materials for golden gate of pIG101

- BsaI
- T_4 DNA Ligase
    - 0.5–1 μL range, 2000 CEU/μL cligase ∝ misligation
- 10x T_4 Ligase Buffer
    - Titurate/vortex to dissolve solids. Limit freeze/thaws to ~5-10x
- 10x BSA + PEG
    - 10x: 1mg/mL BSA, 10% PEG-3350
    - BSA: Enables full BsaI activity at 37°C, absorbs contaminants
    - PEG: reduces diffusion, enhancing ligation efficiency
- DNAs
    - 10–40 fmol equimolar
    - 2–5-fold less vector to reduce background
- Deionized Water
    - 10–20 μL range
- custom stabilizing buffer
- cool brick (1°C)
- pSPB437, 413, pcrIG005

Procedure for golden gate of pIG101

1. For each reaction, 8.5 μL of water, 1.5 μL 10X ligase buffer, 0.5 μL T4 ligase, 0.5 μL BsaI, 1.5 μL BSA, 0.5 μL of all pSPB parts, and 1 μL of pcrIG005
    a. For master mix: 26.5 μL water, 4.59 μL 10X ligase buffer, 1.53 μL T4 ligase, 1.53 μL BsaI, 4.59 μL BSA, 1.53 μL pSPB437, 413, 3.06 μL pcrIG005,
    b. Added either 0.5 μL pSPB480, 449, or 447 to each reaction

Procedure for tranformation: (Alicia)

1. Thaw comp cells on ice for 5 min
    a. A = pIG101a:BsaI EOL AmpR:: pSPB437, pSPB413, pcrIG005, pSPB480
    b. B = pIG101b:BsaI EOL AmpR:: pSPB437, pSPB413,pcrIG005, pSPB449
    c. C = pIG101c:BsaI EOL AmpR:: pSPB437, pSPB413, pctIG005, pSPB417
2. Pipetted 3.0 μL of each plasmid into a tube containing the comp cells (on ice)
    a. flick to mix, DO NOT pippette or vortex
3. Left the tubes on ice for 30 min
4. Heat shocked tubes for 45 s at 42 °C
5. Immediately placed on ice for 2 min
6. Added 150 μL of SOC to each tube, flick to mix
7. recovered att 37C for an hour
8. Placed LB Amp plates in 37 °C incubator to pre-warm

9. Used streaking method to plate ~200 µL of each tube onto the warm LB plates labeled with the plasmid number
10. Placed plates in 37 °C incubator
    a. start time: 6:53 pm

August 7, 2019: pIG101 - miniprep, restriction digest, gel

Project: 2019 iGEM
Authors: Claire Young

WEDNESDAY, 8/7/2019

Objectives: Today, we will pick pIG101 colonies grown overnight and inoculate for ~8 hrs. Then, we will spin down and miniprep the cultures and test the results using a restriction digest. (Claire)

Inoculation of pIG101

Procedure for inoculation of pIG101

1. Removed plates from 37 °C incubator at 12:15 pm
2. Prepared 20 mL of LB-30 with 1000X amp and aliquoted into 6 14-mL conical vials labeled pIG101ax, pIG101ay, pIG101bx, pIG101by, pIG101cx, pIG101cy
    a. The x and y are because we picked two colonies from each plate, but didn't want to get the labeling confused with a, b, and c
3. Inoculated a colony into each tube and left in 37 °C shaking incubator
    a. Start time: 12:45 pm
    b. End time: 9:00 pm
4. Spun down cultures in 2 1.5 mL tubes for 2.5 min at 6000 rcf
5. pIG101a had a few colonies, pIG101b had ~25, pIG101c ~15

Miniprep pIG101

Materials for Miniprep:

- QIAprep Spin Miniprep Kit (Cat. No. 27106 Qiagen)

Procedure for Miniprep:

1. Parts: pIG101a, pIG101b, pIG101c (x for all cultures)
2. Resuspended the first pellet in 250 uL of Buffer P1 (P1 stored in 4 C fridge)
    a. vortexed for 30 seconds until no pellet remained
3. Added 250 uL Buffer P2 to each tube and inverted 4-6 times until the solution became clear
    a. Let sit for at least 1 min, but not more than 2 min
4. Added 350 uL Buffer N3 to each tube and mixed by inverting 4-6 times
    a. Centrifuged for 10 min at 16,100 rcf
5. Poured supernatent into a spin column
    a. Centrifuged the spin columns for 30 sec at 16,100 rcf
    b. Discarded the flowthrough **by pipetting out**
6. Added 750 uL Buffer PE to each column
    a. Centrifuged for 30 sec at 16,100 rcf
    b. Discarded the flowthrough **by pipetting out**
    c. Centrifuged for 2 min at 16,100 rcf (dry spin)
7. Labeled 1.5 mL tubes with the plasmid numbers and transfered the columns to the 1.5 mL tubes
8. Added 30 uL Buffer EB to each column and let stand for 5 min, then centrifuged for 1 min at 16,100 rcf
9. Removed the spin column from tubes
10. Used **nanodrop** spectrophotometer to check concentration (1.5 uL of each sample)

Nano drop

pIG101a 50.1 ng/ µL

pIG101b 33.2 ng/µL

pIG101c 43.2 ng/µL

Restriction digest of pIG101a b c

Materials for restriction digest:

- diH20
- 10X CutSmart buffer (NEB Lot # 10018445)
- NotI-HF restriction enzyme
- Template DNA from the miniprep from July 10 and July 11

Procedure for restriction digest:

Table1

	A	B	C	D	E	F	G
1	DNA Restriction Digest 1						
2	*Notes*	**Reactions**	3	**BSA?**	no		
3		**Excess**	4%	0%			
4		**Reactions**	**3.12**	**3**	***1 ref***		
5						< Warnings	
6		**diH2O**	**28.08**	27	**9**	µL	
7	CutSmart	**10× rxn buffer**	**4.68**	4.5	**1.5**	µL	
8		10× BSA	0	0	0	µL	
9	EcoRI-HF	**Enzyme 1**	**1.56**	1.5	0.5	µL	< 1.5 µL total
10	SpeI-HF	**Enzyme 2**	**0**	0	0	µL	
11		**Enzyme 3**	**0**	0	0	µL	
12		**DNA**	**12.48**	12	4	µL	Consider copy#
13							
14		**Total:**	**46.8**	**45**	**15**	µL	
15		***Total–DNA:***	**34.32**	**33**	**11**	µL	
16		***Total–Enz:***	**45.24**	**43.5**	**14.5**	µL	
17		***Total–DNA/Enz:***	**32.76**	**31.5**	**10.5**	µL	

a. Made a master mix according to the wetlab calculator - c di water, 25 uL of buffer, 4.16 uL of NotI and pipetted up and down to mix the buffer and water.
b. Added 12 uL of the mastermix to each tube
c. Added 2-4 uL of the DNA to each tube amount depends on the concentration)
   I. Don't need to digest plasmid # 447, 658, and 674
   II. 3 uL per tube except for 459, which got 4 µL, and 633, which got 2 µL
d. Flicked to mix, and place tubes in a 37C incubator for 15 min
   I. Start time: 10:32 pm
   II. End time: 10:53 pm

Making PN + other plates

1. Conditions: glucose, glucose + NaCl, sodium citrate, just NaCl
2. Concentration of glucose: 25 mM, 1 g/L sodium, 15 mM sodium citrate
3. Measured .112 g glucose and added to 5 mL, .025 g sodium, .111 g sodium citrate

August 8, 2019: Transformation of pIG101

Project: 2019 iGEM
Authors: Claire Young

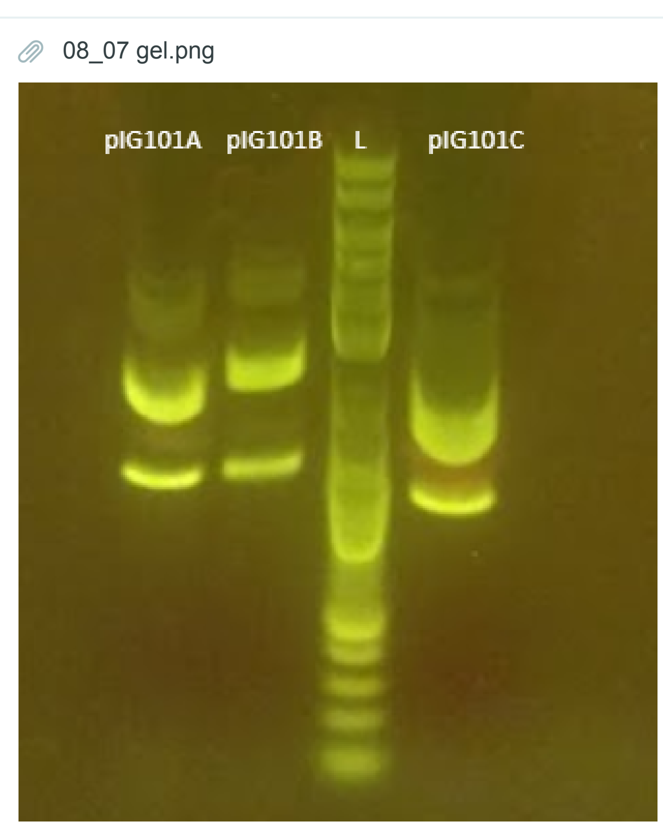
THURSDAY, 8/8/2019

Objectives: Today, we will retransform pIG101 onto LB+Amp plates containing arabinose and let those grow overnight to check for correct plasmid incorporation.

Notes from August 7, 2019:

- The restriction digest reactions were run using gel electrophoresis to determine if the plasmid was correct.
- Based on the band sizes, the plasmid was not correct and was likely unreacted AmpR plasmid

08_07 gel.png



- For the PN plate preparation, after all the reagents were added to water, the 5 mL of water was added to 20 mL PN media and poured into petri dishes

Procedure for tranformation: (Alicia)

a. Thaw comp cells on ice for 5 min
b. Pipetted 5 µL of each plasmid (pIG101a, pIG101b, pIG101c) into a tube containing the comp cells (on ice)
    I. flick to mix, DO NOT pippette or vortex
c. Left the tubes on ice for 30 min
    I. Start time: 5:42 pm
    II. End time: 6:12 pm
d. Heat shocked tubes for 45 s at 42 °C
e. Immediately placed on ice for 2 min
f. Added 150 µL of SOC to each tube, flick to mix
g. Placed in 37 °C incubator for 1 hr
    I. Start time: 6:17 pm
    II. End time: 7:17 pm

h. Placed LB Amp plates in 37 °C incubator to pre-warm
i. Used streaking method to plate ~200 μL of each tube onto the warm LB plates labeled with the plasmid number
    I. Divided the plate into thirds and plated one transformation on each third
    II. Plate half of the recovery on a regular LB amp plate and the rest on the +ara one. If you need my amp plates, they're in Fridge 0 with the blue stripe. Arabinose is in a bag in the Freezer 0 door labeled _Sara - arabinose_
j. Placed plates in 37 °C incubator
    I. start time: pm

Meanwhile preparation of LB30- Amp plates

a. Meanwhile, add 20% arabinose to a final 0.5% in one LB-amp plate, assuming 20 mL agar in a plate. Do this by mixing the arabinose in a final 400 μL SOC and spreading it evenly over the agar surface using a P1000 tip.
    I. 10 μl of arabinose and 390 μL
b. Dry this plate without a lid in incubator 1 during the ice incubation of the transformation, until 1.5 hr later when you plate (assuming it's dry by then).

August 12,2019 Putida can grow on PN-Glc, remaking linIG005 by digesting to form pIG101

Project: 2019 iGEM

Authors: Alicia Selvera

MONDAY, 8/12/2019

Putida grew on these plates for 2 days, we decided we are just going to add glucose to PN for the plant experiments

image.png

Remaking linIG005 by digesting the regions outside the CDS

we descovered that the primers bind nonspeicifcally to other terminators in the template. The plan is to run a digest for 1 hr using BsaAI, SalI-hf and XbaI. Gel purify. Then use that extracted plasmid ind an End on ligation to make pIG101. We are going to make both pcrIG005 and linIG005.

Table1

	A	B	C	D	E	F	G
1	DNA Restriction Digest 3						
2	*Notes*	**Reactions**	1	**BSA?**	no		
3		**Excess**	4%	0%			
4		**Reactions**	**1.04**	**1**	***1 ref***		
5						< Warnings	
6		**diH2O**	**11.44**	11	**11**	µL	
7		**10× rxn buffer**	**1.56**	1.5	**1.5**	µL	
8		10× BSA	0	0	0	µL	
9	BsaAI	**Enzyme 1**	**0.52**	0.5	0.5	µL	< 1.5 µL total
10	SalI-hf	**Enzyme 2**	**0.52**	0.5	0.5	µL	
11	XbaI	**Enzyme 3**	**0.52**	0.5	0.5	µL	
12		**DNA**	**1.04**	1	1	µL	Consider copy#
13							
14		**Total:**	**15.6**	**15**	15	µL	
15		***Total–DNA:***	**14.56**	**14**	**14**	µL	
16		***Total–Enz:***	**14.04**	**13.5**	**13.5**	µL	
17		***Total–DNA/Enz:***	**13**	**12.5**	**12.5**	µL	

August 13: taking araC from pBEST (Alicia)

Project: 2019 iGEM
Authors: Alicia Selvera

TUESDAY, 8/13/2019

Liquid Culture 1 mL of saturated 45789 into 10 mL LB30 amp, 1:10 dil, 28°C shaking 300 rpm 45° 6 h.

Miniprep

Procedure

- spin down in 14 ml conical tube
- 5 miniprep tubes
- 5 clear lysates go into one column
  - either vacuum manifold ir spin down and pour

Nanodrop resulted in ~600 ng/µl

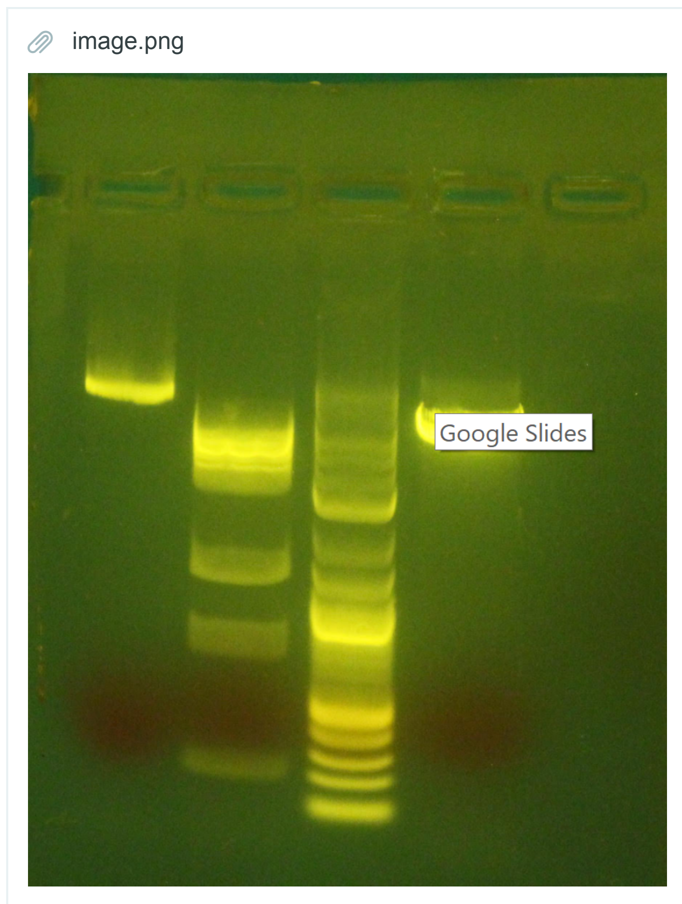
Restriction digest

Same as yesterday, but 3.5µl of dna and an xbaI diagnostic digest

Gel purification

Simultaneously run a diagnostic with XbaI

image.png



Nanodrop ~14ng/µL

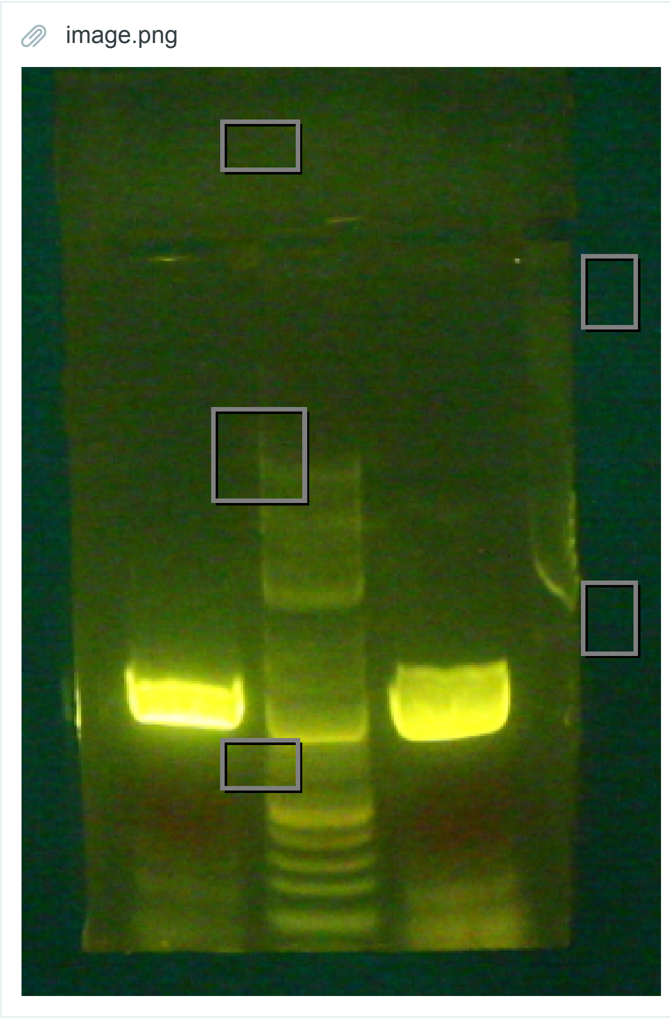
August 14, 2019 taking araC from pBEST part two (Alicia)

Project: 2019 iGEM
Authors: Alicia Selvera

WEDNESDAY, 8/14/2019

Gel purification of pcrIG005 and linIG005

image.png



Nanodrop

results are terrible. Graphs look jank.

pcrIG005 20.7 ng/µL

linIG005 15.2 ng/µL

Restriction digest part 3

since graphs looked terrible, like no noticable peak of DNA, I'm starting over

PCR

remade pcrIG005 and linIG005

Gel Purification

went terribly again

Summary

we have no idea why we keep getting beautiful gels and terrible purifications. We suspect it might be something wrong with the reagents we are using to perform the gel purification., but there has to be some major contamination. The plan for tomorrow is to re gel purify the restriction digest to get the pBEST fragment of interest (which we are doing since the template uses the same terminator in multiple spots some are primers are binding nonselectively). Since PCR is so sensitive the gel purification there should be fine. I will remake the PCR but in quadruples. I will then gel purify it one at a time until it works using new reagents. Instead of using the symo kit I will be using QXI, PE and EB. (2 washes, plus a dry spin before eluting)

August 15, 2019 Golden gate and transformation (Alicia and Stephanie)

Project: 2019 iGEM

Authors: Alicia Selvera

THURSDAY, 8/15/2019

hange of plans. I forgot I ordered a gblock weeks ago in case we couldnt get a PCR product. Well it finally arrived and look where we are. NO pCON araC. Hopefully that ends today. My new plan is to do a part assembly between pSPB440 and gblock (pIG005)

Golden Gate

Table1

	A	B	C	D	E	F
1	Golden Gate Assembly 1					
2	***Reactions***	1		***BSA?***	yes	
3	***# Unique Parts***	1			*1*	
4	***# Common Parts/Vector***	1			*1*	
5	***# Others (PCR pdts, oligo pts)***	0			*1*	
6	***Excess***	2%	0%	0%	-	
7						
8	**Reactions**	**1.02**	1	1	*1 ref*	
9						< Warnings
10	**diH2O**	**10.2**	10	10	**10**	μL
11	10× Ligase Buffer	**1.53**	1.5	1.5	**1.5**	μL
12	**10× BSA**	**1.53**	1.5	1.5	**1.5**	μL
13	**T4 DNA Ligase**	**0.51**	0.5	0.5	0.5	μL
14	Type IIs endonuclease	**0.51**	0.5	0.5	0.5	μL
15		***Total:***			***Each:***	
16	Unique Parts	**0.51**	0.5	0.5	0.5	μL
17	**Common Parts/Vector**	**0.51**	0.5	0.5	0.5	μL
18		**#N/A**	#N/A	#N/A		
19	**Others**	0	0	0	0.5	μL
20						
21	***Total:***	**15.3**	15	**15**	*15*	μL
22	***Total – DNA:***	**14.28**	14	**14**	**14**	μL
23	***Total – Uniques & Others:***	**14.79**	14.5	**14.5**	-	μL

Transformation - Alicia and Stephanie

Incubation: 4:40pm to 5:40pm

Plated on LB + Chl plates

Start time: ~6pm

End time: next day at ~9am

August 16, 2019: transformation results, golden gate of pIG101a and 102, and transformation (Alicia and Stephanie)

Project: 2019 iGEM

Authors: Stephanie Trejo

FRIDAY, 8/16/2019

Transformation Results

poor transformation colony results, very few colonies, golden gate reaction was a fail, only green colonies were visible

Golden Gate

pIG101a:BsaI EOL AmpR:: pSPB437, pSPB413, pcrIG005, pSPB480

Note: we are making this for when/if we need it later

pIG102

pIG102a:BsaI AmpR:: linIG102, pSPB413, pcrIG005, pSPB480

pIG102 postive control: BsaI AmpR:: linIG102, pSPB413, pSPB616, pSPB480

doubled the amount of linIG102 and pcrIG005 because they are too dilute

Table1

	A	B	C	D	E	F
1	Golden Gate Assembly 2					
2	***Reactions***	2		***BSA?***	yes	
3	***# Unique Parts***	1			*1*	
4	***# Common Parts/Vector***	3			*1*	
5	***# Others (PCR pdts, oligo pts)***	0			*1*	
6	***Excess***	2%	0%	0%	-	
7						
8	**Reactions**	**2.04**	2	1	*1 ref*	
9						< Warnings
10	**diH2O**	**18.36**	18	9	**9**	µL
11	10× Ligase Buffer	**3.06**	3	1.5	**1.5**	µL
12	**10× BSA**	**3.06**	3	1.5	**1.5**	µL
13	**T4 DNA Ligase**	**1.02**	1	0.5	0.5	µL
14	Type IIs endonuclease	**1.02**	1	0.5	0.5	µL
15		***Total:***			***Each:***	
16	Unique Parts	**1.02**	1	0.5	0.5	µL
17	**Common Parts/Vector**	**3.06**	3	1.5	0.5	µL
18		**#N/A**	#N/A	#N/A		
19	**Others**	0	0	0	0.5	µL
20						
21	***Total:***	**30.6**	30	**15**	*15*	µL
22	***Total – DNA:***	**26.52**	26	**13**	**13**	µL
23	***Total – Uniques & Others:***	**29.58**	29	**14.5**	-	µL

Transformation

into DH10B alt cells

August 17, 2019: inoculation of pIG101, pIG101 ara, and pIG102 (+ control), miniprep, restriction digest (Stephanie and Alicia)

Project: 2019 iGEM

Authors: Stephanie Trejo

SATURDAY, 8/17/2019

Inoculation

Made 3 LB + amp cultures each of pIG101, pIG101 ara, pIG102, and only one culture of the pIG102 positive control
Note: We picked one culture of pIG101, swirled in 4 mL LB+amp, then transferred 2 mL to another tube to make pIG101 ara cultures
Added 15 uL of 20% ara to pIG101 ara cultures

Start time: ~2pm
End time: ~9pm

Results: pIG101 ara did not appear green under blue light, will not miniprep

Miniprep

pIG102x, pIG102y, pIG102z (very small pellet), pIG101ax, pIG101ay, pIG101az, pIG102bz

NanoDrop Concentrations

Table1

	A	B
1	Name	Concentration (ng/µL)
2	101ax	153.8
3	101ay	168.5
4	101az	169
5	101bz	245.6
6	102x	212.1
7	102y	189.3
8	102z	8.4

Results: pIG102z was too dilute of a concentration, due to a very small pellet size

notI Restriction Digest

Table2

	A	B	C	D	E	F	G
1	DNA Restriction Digest 2						
2	*Notes*	**Reactions**	6	**BSA?**	no		
3		**Excess**	2%	0%			
4		**Reactions**	**6.12**	**6**	***1 ref***		
5						< Warnings	
6		**diH2O**	**73.44**	72	**12**	µL	
7		**10× rxn buffer**	**9.18**	9	**1.5**	µL	
8		10× BSA	0	0	0	µL	
9		**Enzyme 1**	**3.06**	3	0.5	µL	< 1.5 µL total
10		**Enzyme 2**	**0**	0	0	µL	
11		**Enzyme 3**	**0**	0	0	µL	
12		**DNA**	**6.12**	6	1	µL	Consider copy#
13							
14		**Total:**	**91.8**	**90**	15	µL	
15		***Total–DNA:***	**85.68**	**84**	**14**	µL	
16		***Total–Enz:***	**88.74**	**87**	**14.5**	µL	
17		***Total–DNA/Enz:***	**82.62**	**81**	**13.5**	µL	

Gel Electrophoresis

Table3

	A	B	C	D	E	F	G	H
1	Lane	1	2	3	4	5	6	7
2	Name	pIG101ax	pIG101ay	pIG101az	ladder	pIG102bz	pIG102x	pIG102y

Start time: 11:56 pm

End time: 12:15 am

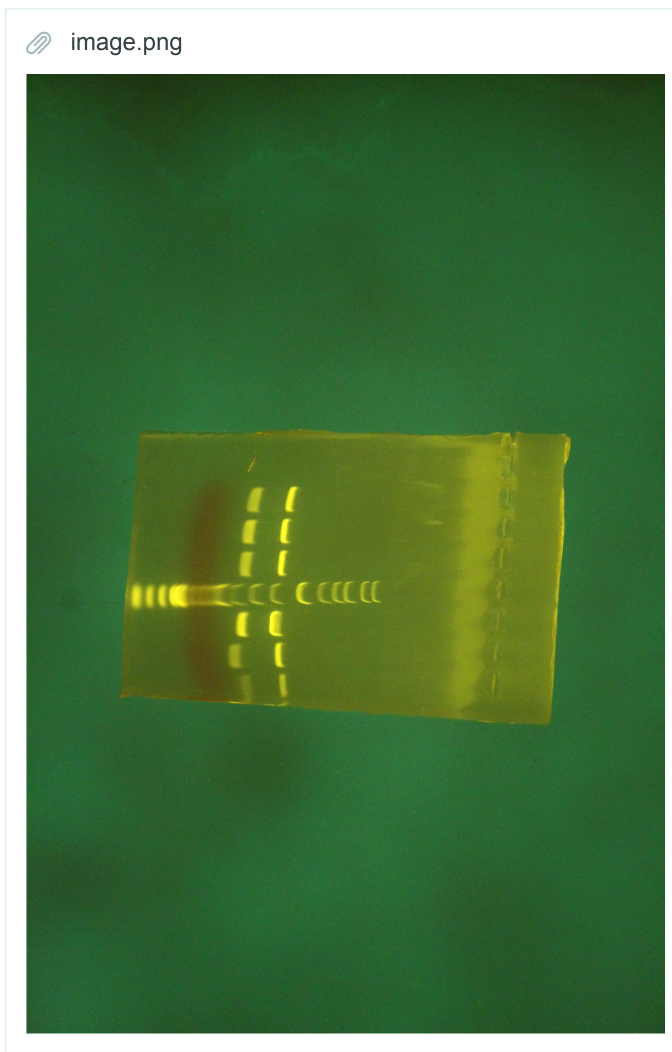
Note: pIG101 does not have any notI sites. So we are expecting one big band because we did not double check in Benchling. oops.

Expected bands for pIG102 notI digest: 4765, 970

Expected bands for pIG101 notI digest: 5639

Gel Results

image.png



Conclusion

EVERYTHING IS SAD. CLONING HATES US. The bands look terrible. We dont even know what they are. They are too small to be the cassette and too big to be part plasmids.

August 18, 2019 (Alicia)

Project: 2019 iGEM

Authors: Alicia Selvera

SUNDAY, 8/18/2019

Golden gate full of positive controls

- 1 = pIG101w, 2=pIG101x, 3 = pIG101y, 4= pIG101z
- after the disaster that was that gel, we decided to do a series of positive controls using shyams plasmids to see if there's any toxicity being resulted from the combination of araC and RK2
- We transformed them using electroporation in *E .coli* comp cells from the previous iGEM team

Table1

	A	B	C	D
1	437 (sfgfp dropout)	413 (AmpR)	linIG005b	480 (RK2)
2	437 (sfgfp dropout)	413 (AmpR)	linIG005b	418 (p15A)
3	437 (sfgfp dropout)	413 (AmpR)	616 (tonVT)	480 (RK2)
4	437 (sfgfp dropout)	413 (AmpR)	616 (tonVT)	418 (p15A)

August 19 (Alicia)

Project: 2019 iGEM

Authors: Alicia Selvera

MONDAY, 8/19/2019

Ran gblock lin005b on gel, it looked good

Did a pcr off that gblock to get more of both linIG005 and pcrIG005

DNA cleanup and concentrate the pcr products

- 5 vol of PB (125 μl), mix by inverting tube
- transfer to column
- vacuum manifold
- 2 x wash with PE (200μL)
- dry spin
- elute with EB 20μL

meanwhile, re-transform pIG101w,x,y,z and pIG012

Heat shock into DMB10 cells

Conclusion

bleh. pcr failed. I messed up the pcr protocol. next time, touch down pcr for pcrIG005 from 68- 63, use PCR stocks and use less template (0.01μL)(do a dilution) (1 μL into 99μL water, use 1μL of that) linIG005 do 67. We are retransforming the P. ara Marrionnette, becuase graduate students from the Bennett lab keep requesting it.

August 20: results from controls

Project: 2019 iGEM

Authors: Alicia Selvera

TUESDAY, 8/20/2019

Results from controls

The results from the control experiments were interesting to say the least. So only pIG101x and pIG101z produced green colonies. Incidentally, these two plasmids were the only ones that used the origin p15A instead of RK2. So we are going to use the p15A origin as the testing contructs for the thermometers moving forward, until we can figure out how to succesfully use RK2.

Redoing the PCR that I messed up on yesterday

I did the touch down procedure for pcrIG005 and did a normal protocol for linIG005. I did a PCR cleanup and then ran the product on a gel. The results were upsetting once again. We decided to abandon araC for the moment.

August 22nd: (Alicia) More assembly of testing cassettes

Project: 2019 iGEM
Authors: Alicia Selvera

THURSDAY, 8/22/2019

Recap

yesterday, Shyam performed a golden gate to make more verisions of a cassette we could use for testing. He used a KanR, sfGFP dropout, LacI, and P15A for one, and KanR, sfGFP dropout, LacI and also another origin (I forget the exact obe P10 something?, maybe pSC101, I need to double check) These had much much greater transformation efficiencies that we have been seeing with RK2. It's ridiculous how much better it is. We've been used to make 30 colonies for a plate, this had hundreds.

So around 12pm, we went in to pick 2 green colonies from each plate. We inncolated them with 2ml LB30 in 24 well culture plate thing. They were left shaking for aboout 6 hours. At 6pm, their was still not enough growth to do a minipreepp. I will miniprepp all of those cultures and some frozen pellets that were in the freezer in the morning.

August 23rd

Project: 2019 iGEM
Authors: Alicia Selvera

FRIDAY, 8/23/2019

Recap

I minipreped samples labeled 1a,1b,2a,2b,x,x,x and 12. I need to officially name these cassettes. Am currently thinked 1 = pIG104, 2=pIG105

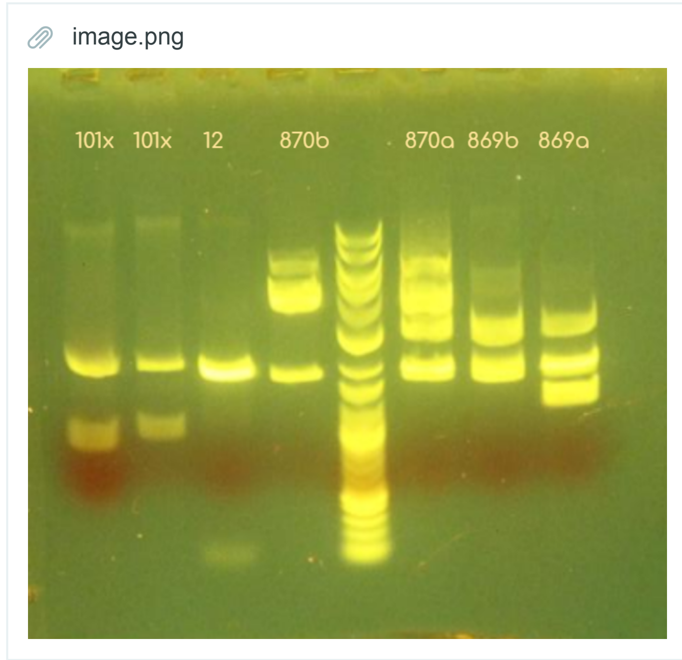
I eluted in 50uL of EB, following the standard Qiagen protocol.

The nanodrop results were good.

Table1

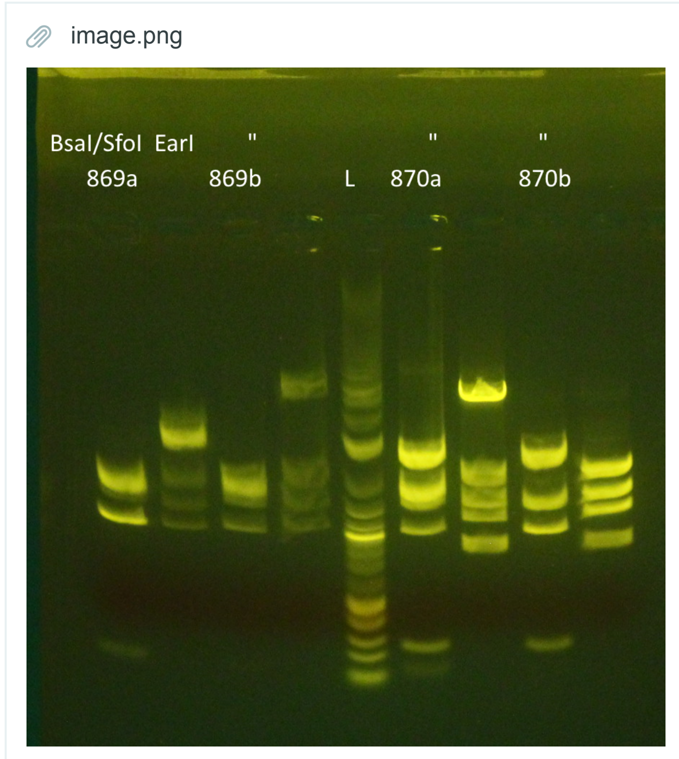
	A	B	C	D	E	F	G	H	I
1	Sample:	1a	1b	2a	2b	12	xa	xb	xc
2	ng/uL	116.3	114.8	455.1	366.3	113.8	9.4	33.1	33.2

image.png



So i'm going to say that, pIG101 failed. pIG012 passes. pSPB89b passes. pSPB689a fails. pSPB870b pass?, pSPB870a fail?

image.png



As expected, 869b and 870b were the only to pass

Golden gate

pSPB869b, ConLS, Pa1-LacO1, RBS BB0034,mCherry2, VoigtT, ConR2

pSPB869b, ConLS, PL-Lac1, RBS BB0034,sfGFP, VoigtT, ConR2

pSPB870b, ConLS, Pa1-LacO1,RBS B0034, mCherry2, VoigT, COnR2

pSPB870b, ConLS, PL-Lac1, RBS B0034, sfGFP, VoigT, COnR2

August 25

Project: 2019 iGEM
Authors: Alicia Selvera

SUNDAY, 8/25/2019

Transformed pSPB871-874, this was a 7 part golden gate with parts 1-6 plus the packbone from the 23rd. Transformation results were very good. Mostly white.

Todo list for tomorrow

- order primers
- spin down cultures and freeze at 8am
- miniprep plus restriction digest at 11am
-

August 26

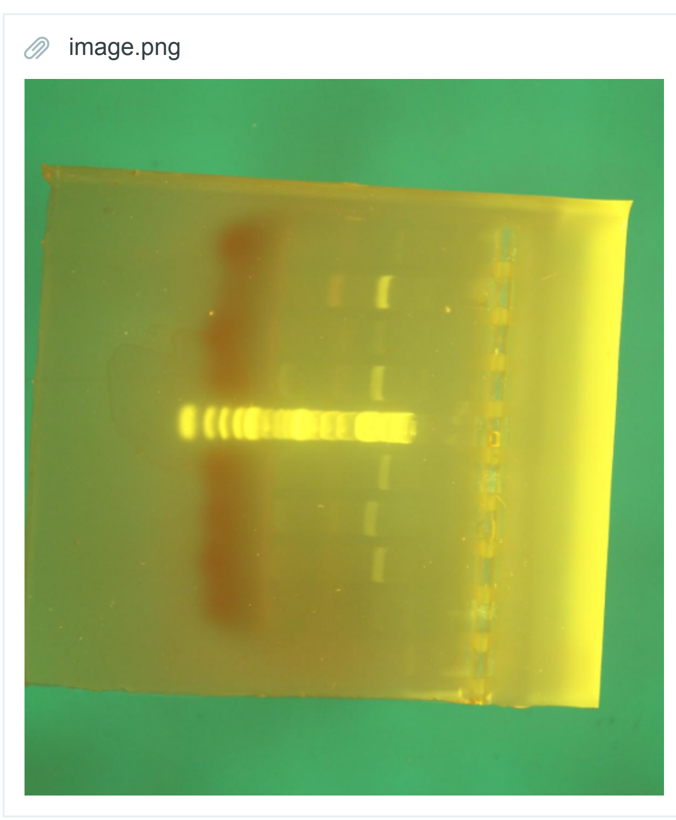
Project: 2019 iGEM
Authors: Alicia Selvera

MONDAY, 8/26/2019

Summary

I miniprepped the golden gates from yesterday, pSPB874-pSPB877, 874 is labeled 1, ..., 877 is labeled 4. I performed a restriction digest in NotI. I used too little DNA (around 30 ng/uL) and too much ladder (around 1 uL of non dilute). I put a good gel in the fridge. Its in a tubber ware in the back of the deli in shyams side.

image.png



August 27, 2019: Restriction digest of pSPB874-877

Project: 2019 iGEM
Authors: Claire Young

TUESDAY, 8/27/2019

Restriction digest of pSPB974-877

1. Made a mastermix for eight reactions out of 83.2 µL diH2O, 12.5 µL 10x cutsmart buffer, 4.16 µL NotI-HF
   a. aliquoted 12 µL into tubes labeled with the plasmid number and added 3 µL of miniprepped DNA to each tube
   b. 2 µL for 2B, 3B, 4B
   c. Tubes labeled 1A, 1B, 2A, 2B, 3A, 3B, 4A, 4B with 1 = pSPB874, 2 = pSPB875, 3 = pSPB876, 4 = pSPB877
2. Incubated for 20 min at 37 °C
   a. Start time: 9:21 am
   b. End time: 9:41 am
3. Miniprep products stored in -20 °C freezer

Gel electrophoresis of pSPB874-877

1. Added 3 µL 6X loading dye to each restriction digest product
2. Loaded gel in order of 1A, 1B, 2A, 2B, ladder, 3A, 3B, 4A, 4B
   a. Loaded 8 µL 1/15 dilution of the NEb 2-log ladder
3. Ran gel for 25 min at 120V
   a. Start time: 9:59 am
   b. End time: 10:24 am

Miniprep of pSPB971-874

Nanodrop concentrations

Table1

	A	B
1	Name	concentration (µg/mL)
2	871a	313
3	871b	605
4	872a	569
5	872b	504
6	873a	278
7	873b	318
8	874a	570
9	874b	253

August 28, 2019: Restriction digest of pSPB874-877 redo

Project: 2019 iGEM
Authors: Claire Young

WEDNESDAY, 8/28/2019

Due to the extremely high concentrations of the miniprepped plasmids yesterday, we redid the concentrations:

Redo of nanodrop concentrations

	A	B
1	Sample	Concentration (ng/μL)
2	pSPB871a	35.3
3	871b	61.9
4	872a	60.4
5	872b	54.1
6	873a	31.7
7	873b	32.5
8	874a	24.5
9	874b	27.6

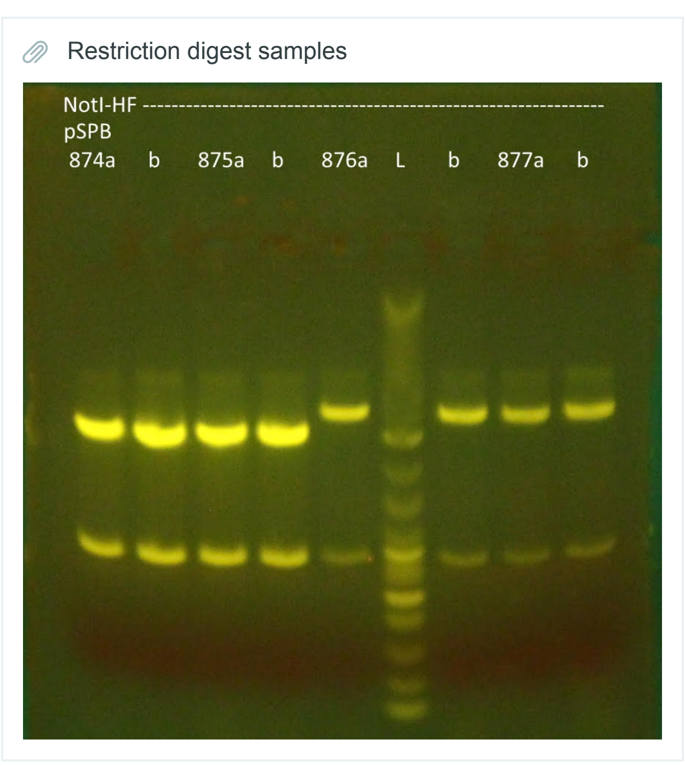
Ran a restriction digest on all miniprepped plasmids using the following conditions:

Table1

	A	B	C	D	E	F	G
1	DNA Restriction Digest 1						
2	*Notes*	**Reactions**	8	**BSA?**	no		
3		**Excess**	4%	0%			
4		**Reactions**	**8.32**	**8**	***1 ref***		
5						< Warnings	
6		**diH2O**	**86.528**	83.2	**10.4**	μL	
7	CutSmart	**10× rxn buffer**	**12.48**	12	**1.5**	μL	
8		10× BSA	0	0	0	μL	
9	EcoRI-HF	**Enzyme 1**	**0.832**	0.8	0.1	μL	< 1.5 μL total
10	SpeI-HF	**Enzyme 2**	**0**	0	0	μL	
11		**Enzyme 3**	**0**	0	0	μL	
12		**DNA**	**24.96**	24	3	μL	Consider copy#
13							
14		**Total:**	**124.8**	**120**	**15**	μL	
15		***Total–DNA:***	**99.84**	**96**	**12**	μL	
16		***Total–Enz:***	**123.968**	**119.2**	**14.9**	μL	
17		***Total–DNA/Enz:***	**99.008**	**95.2**	**11.9**	μL	

Ran the samples on a gel:

Restriction digest samples



All bands were at the expected size.

Plans for tomorrow: do a PCR reaction of both pSPB875 and 877 with primers AG 21 and 22

- Annealing temperature: 64 °C
- Extension time: 1:25 min
- Afterward, run on a gel and purify

August 29, 2019: PCR and gel purification of pSPB875 and 877

Project: 2019 iGEM
Authors: Claire Young

THURSDAY, 8/29/2019

PCR of pSPB875 and 877

1. Made a mastermix of 100 µL NEB High Fidelity 2x PCR master mix, 1 µL 100 µM primer AG21, 1 µL 100 µM primer AG22, 90 µL diH20
2. Split sample into two tubes (96 µL into each)
3. Added 4 µL of pIG875 to one tube and 4 µL pIG877 to the other
4. Split those into two more tubes, yielding a total of 4 50 µL reactions

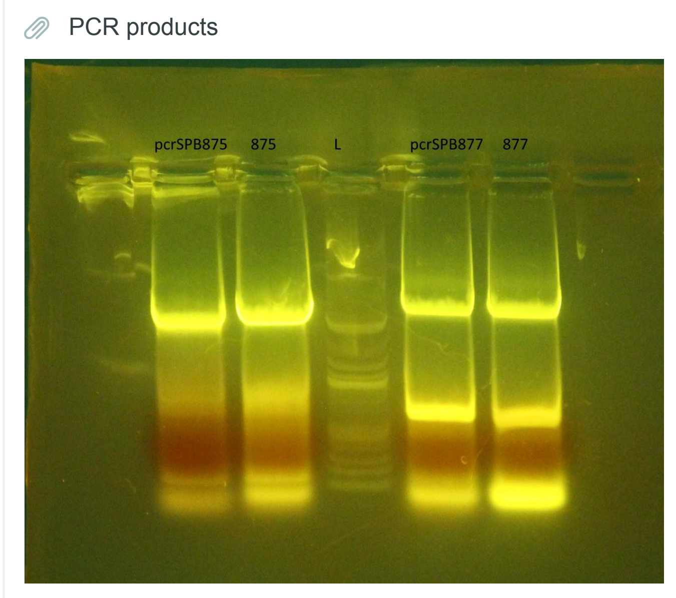
Cycling conditions:

I. Initial denaturation: 98 C for 1 min
II. Denaturation: 98 C for 10 sec
III. Annealing: 64 C for 30 sec
IV. Extension: 72 C for 1:25 min, 30X
V. Final extension: 72 C for 2 min

Gel Purification

Notes: Only purifying 877a and 875a, saving 877b and 875b for later if needed.

PCR products



weight of 875 a: 0.0964 g
weight of 875 b: 0.0826 g
weight of 877 a: 0.1186 g
weight of 877 b: 0.0955 g

Concentrations
875: 93.7 ng/uL, had a peak at 225 nm for some reason
877: 295.7 ng/uL

September 1, 2019: Golden gate and transformation of pcrSPB875 & 877 with the first round of RNA thermometers: RNAT1,3,4, 7

Project: 2019 iGEM
Authors: Alicia Selvera

SUNDAY, 9/1/2019

Objective: We are making the testing contructs for the intial set of thermometer

Golden Gate

We used BSA, BSAI hf, cutsmart, and T4 DNA ligase

pIG006, pIG008, pIG014, pIG015 (1µL)+ pcrSPB875 (.5µL)

pIG006, pIG008, pIG014, pIG015 (1µL)+ pcrSPB877 (.2µL)

It was ran on the long protocol.

Table1

	A	B	C	D	E	F
1	Golden Gate Assembly 1					
2	*Reactions*	4		*BSA?*	yes	
3	*# Unique Parts*	1			*1*	
4	*# Common Parts/Vector*	1			*1*	
5	*# Others (PCR pdts, oligo pts)*	0			*1*	
6	*Excess*	2%	0%	0%	-	
7						
8	**Reactions**	**4.08**	4	1	*1 ref*	
9						< Warnings
10	**diH2O**	**38.76**	38	9.5	**9.5**	µL
11	10× Ligase Buffer	**6.12**	6	1.5	**1.5**	µL
12	**10× BSA**	**6.12**	6	1.5	**1.5**	µL
13	**T4 DNA Ligase**	**2.04**	2	0.5	0.5	µL
14	Type IIs endonuclease	**2.04**	2	0.5	0.5	µL
15		*Total:*			*Each:*	
16	Unique Parts	**4.08**	4	1	1	µL
17	**Common Parts/Vector**	**2.04**	2	0.5	0.5	µL
18		**#N/A**	#N/A	#N/A		
19	Others	0	0	0	0.5	µL
20						
21	***Total:***	**61.2**	60	**15**	*15*	µL
22	***Total – DNA:***	**55.08**	54	**13.5**	**13.5**	µL
23	***Total – Uniques & Others:***	**57.12**	56	**14**	-	µL

Table3

	A	B	C	D	E	F
1	Golden Gate Assembly 1					
2	***Reactions***	4		***BSA?***	yes	
3	***# Unique Parts***	1			*1*	
4	***# Common Parts/Vector***	1			*1*	
5	***# Others (PCR pdts, oligo pts)***	0			*1*	
6	***Excess***	2%	0%	0%	-	
7						
8	**Reactions**	**4.08**	4	1	*1 ref*	
9						< Warnings
10	**diH2O**	**39.984**	39.2	9.8	**9.8**	µL
11	10× Ligase Buffer	**6.12**	6	1.5	**1.5**	µL
12	**10× BSA**	**6.12**	6	1.5	**1.5**	µL
13	**T4 DNA Ligase**	**2.04**	2	0.5	0.5	µL
14	Type IIs endonuclease	**2.04**	2	0.5	0.5	µL
15		***Total:***			***Each:***	
16	Unique Parts	**4.08**	4	1	1	µL
17	**Common Parts/Vector**	**0.816**	0.8	0.2	0.2	µL
18		**#N/A**	#N/A	#N/A		
19	**Others**	0	0	0	0.5	µL
20						
21	***Total:***	**61.2**	60	**15**	*15*	µL
22	***Total – DNA:***	**56.304**	55.2	**13.8**	**13.8**	µL
23	***Total – Uniques & Others:***	**57.12**	56	**14**	-	µL

Transformation using typical tss heat shock. Put in incubator at 8:20pm.

Tomorrow

Inoculate a colony in 10µL of LB30. Put that culture as a final 10% in a colony pcr using the oligos that made the thermometers as a test to see if the cassette was formed correctly. If the PCR is correct then you can at that colony in 2ml of LB30 and miniprep as normal. It's probably a good idea to start growing up the colonies as the PCR is running to save time.

9/3/2019

Project: 2019 iGEM

Authors: Alicia Selvera

TUESDAY, 9/3/2019

Recap: innoculated the thermometers, pulled the putida plate out of fridge (Lots of colonies, all green), performed a golden gate of spb 413,608,449,450 to try to make a testing cassette for the enzymes

9/4/2019

Project: 2019 iGEM

Authors: Alicia Selvera

WEDNESDAY, 9/4/2019

eTransform pIG123 from yesterday

9/6/2019: sequencing of pIG014, pcr test of thermometer cassettes, miniprep of pIG123

Project: 2019 iGEM

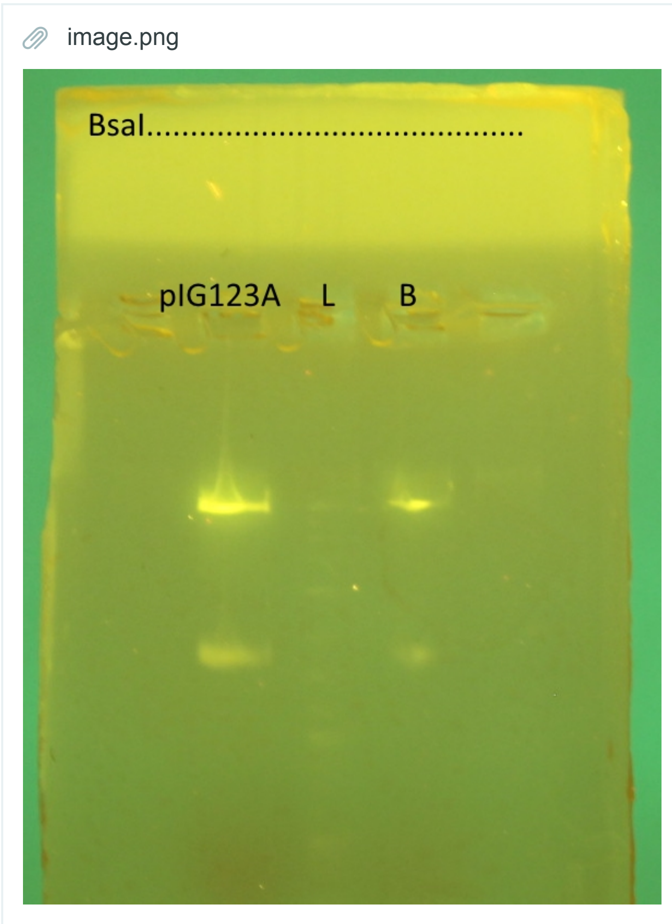
Authors: Alicia Selvera

FRIDAY, 9/6/2019

Recap: In a big picture. today we started finalizing some of the details to preparing our testing cassettes that way we can start testing the thermometers. pIG014 sequencing failed last tie so we are redoing it. I'm not sure exactly what happened but pIG015 DNE. I think we ordered two of the same primers and never got around to reodering. That needs to be done immediately. pIG123 will serve us in the enzyme testing.

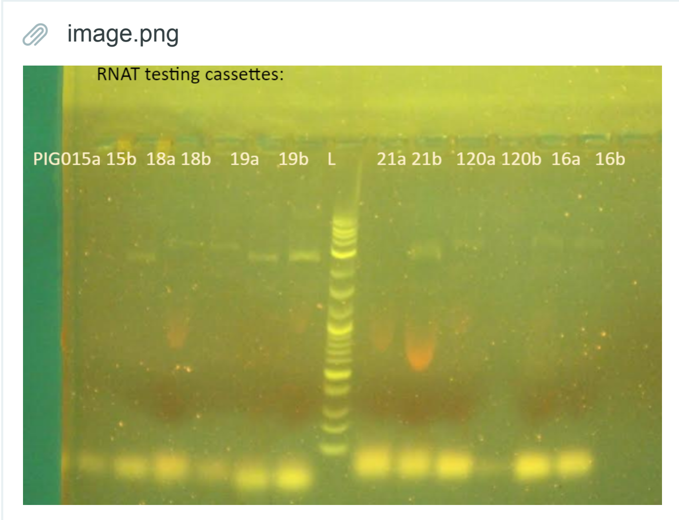
Test digest: The ladder was incrediably hard to see, but I wanna say the bands still look right.

image.png



A Testing PCR was done with AB25 and a forward oligo that binds to the thermometers in each of the testing cassettes as the primers

image.png



September 6, 2019: Miniprep of pIG123

Project: 2019 iGEM
Authors: Claire Young

FRIDAY, 9/6/2019

Miniprep of pIG123

1. Did a miniprep of the overnight cultures of pIG123
   a. Two total cultures, labeled 123A and 123B
   b. Note that the bacterial pellets after spinning down were significantly larger than normal and very green
2. Followed the standard miniprep protocol and eluted in 30 μL elution buffer
3. Concentrations:
   a. pIG123A: 313.4 ng/μL
   b. pIG123B: 254.6 ng/μL

Restriction digest of pIG123A,B

Table1

	A	B	C	D	E	F
1	*Notes*	**Reactions**	2	**BSA?**	no	
2		**Excess**	0%	0%		
3		**Reactions**	**2**	**2**	***1 ref***	
4						< Warnings
5		**diH2O**	**24**	24	**12**	μL
6	CutSmart	**10× rxn buffer**	**3**	3	**1.5**	μL
7		10× BSA	0	0	0	μL
8	EcoRI-HF	**Enzyme 1**	**1**	1	0.5	μL
9	SpeI-HF	**Enzyme 2**	**0**	0	0	μL
10		**Enzyme 3**	**0**	0	0	μL
11		**DNA**	**2**	2	1	μL
12						
13		**Total:**	**30**	**30**	**15**	μL
14		***Total–DNA:***	**28**	**28**	**14**	μL
15		***Total–Enz:***	**29**	**29**	**14.5**	μL
16		***Total–DNA/Enz:***	**27**	**27**	**13.5**	μL

Incubated at 37 °C

start time: 11:23 am

9/8/2019

Project: 2019 iGEM
Authors: Alicia Selvera

SUNDAY, 9/8/2019

notes for redoing PCR

10 µL rxn. AD25 + particular oligos. Tm 65. As little template as possible (10µL pippette. Set to 2µL. Suck up just visable amount. eject until bubble is formed. .5 µL of 10µM primers.

Table1

	A	B	C	D
1	Plasmid	Forward primer	reverse primer	Tm
2	115a	oIG011	AD25	65
3	115b	oIG011	AD25	65
4	116a	oIG011	AD25	65
5	116b	oIG011	AD25	
6	118a	oIG015	AD25	65
7	118b	oIG015	AD25	65
8	119a	oIG017	AD25	65
9	119b	oIG017	AD25	65
10	120a	oIG017	AD25	65
11	120b	oIG017	AD25	65
12	121a	oIG023	AD25	65
13	121b	oIG023	AD25	

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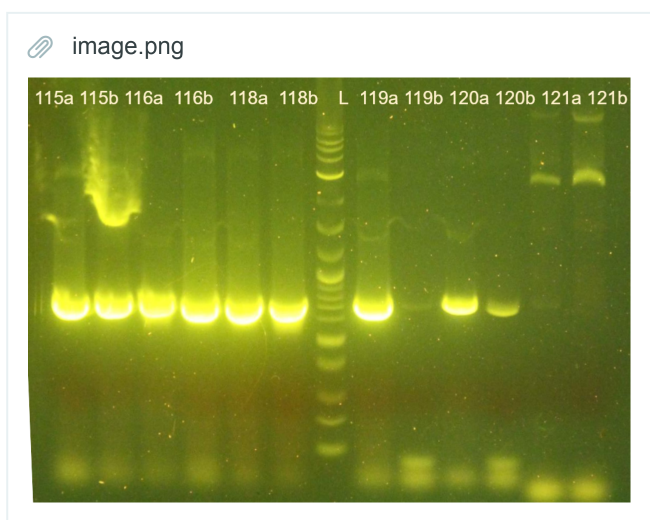


Transformation into NEB turbo of 115a, 116a, 118a, 119a, 120a on Kan Plates

9/9/2019

Project: 2019 iGEM

Authors: Alicia Selvera

MONDAY, 9/9/2019

Innoculation of pIG15, 16, 18,19, 20

There is a real possibilty i messed up the numbering when I transformed. I distinctly remember plating the single plate last, and I thought i went in plasmid order, but i also remember checking what the plates were numbered as. But the single plate was numbered as 18, so i innoculated following the numbering on the plates, but I have a suspicion that what is numbered 18 is actually 20, what is numbered 19 is actually 18, and what is numbered and what is numbered 20 is actually 19. When we go to do the digest, lets look really carefully at the size differences. 19 has a different origin that 18 and 20, so we should see a size difference there in a restriction digest.

9/10/2019 RNAT TESTING!!!

Project: 2019 iGEM

Authors: Alicia Selvera

TUESDAY, 9/10/2019

Objective: Trial run for testing RNA thermometers to get comfortable with the protocol.

Innoculation of pIG15,16,18,19,20 (a)

I inncoluted the a version of each construct. Thus , im dropping that a and added in a new a and b, since i picked 2 colonies from each plate section.

I innoculated in LB30. Turns out that was a mistake. But since this is just for testing the protocol. Add MgSo4 to media

Testing Thermometers

- Grow in 2ml LB liquid medium at 37 °C until OD600 = 0.1
  - From the 8 hour culture of 2ml LB, freeze 1 mL of the saturated culture
  - From overnight culture (8 hr growth), measure OD in a 1:30 dilution, dilute to 0.01, then grow to 0.1 (~3 hr)
    - Start: 7:06 pm
    - End: 9:00 pm
  - to measure OD, add 23.33μL of culture to a final 700μL (676.67μL)
  - LB shaking 250 rpm
  - Once it's at 0.1 add 2x IPTG, dilute 2x to get to ~.05 in LB
  - We don't have to use minimal media because of the mCherry2
    - We need to have absolute values, so use sulfur rhodamine to normalize (say it's equivalent to x amount of sulfur rhodamine)
    - Also do mCherry
- liquid media
  - Induce with no IPTG, 10 μM, 100 μM, 1 mM IPTG
  - measure the fluorescence every hour
    - saturates (df/dt=0) around OD = 6
    - 10 tubes for each temperature
    - Now we will use a plate reader over two days (one temp for each day)
      - Need to do 2 days because the maturation time for mCherry is ~30 min
    - inoculate into one container, vortex it well, aliquot into each of 30 tubes 2 mL [60 mL M9 minimal]

Going to test the a versions, do not need to make a b version

Make sure to shake the cultures vigourously before pipetting the culture, it precipitates very fast

Concentrations

	Sample	bad read OD600	diluted OD600	OD600 ×30 (to account for dilution)
1	15a	1.485	0.542	16.3
2	16a	0.667	0.620	18.6
3	18a	1.189	0.675	20.25
4	19a	0.490	0.538	16.14
5	20a	1.195	0.541	16.23
6				

Pipette 3 mL of the LB for each into the plate
Make sure to keep shaking the cultures while pipetting out

Table 2

	Sample	OD	Amount of LB to add to 0.1
1	15	0.117	189
2	16	0.143	391
3	18	0.127	276
4	19	0.164	507
5	20	0.116	179
6			

IPGT concentrations

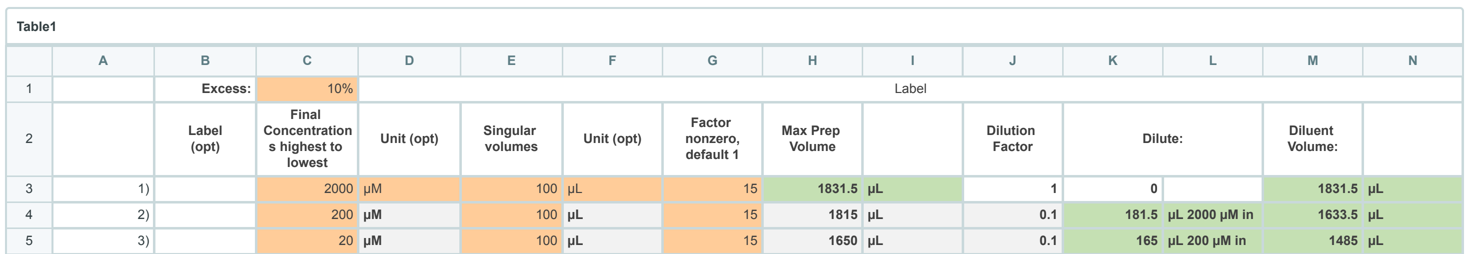


Table1

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		Excess:	10%	Label										
2		Label (opt)	Final Concentrations highest to lowest	Unit (opt)	Singular volumes	Unit (opt)	Factor nonzero, default 1	Max Prep Volume		Dilution Factor	Dilute:		Diluent Volume:	
3	1)		2000	μM	100	μL	15	1831.5	μL	1	0		1831.5	μL
4	2)		200	μM	100	μL	15	1815	μL	0.1	181.5	μL 2000 μM in	1633.5	μL
5	3)		20	μM	100	μL	15	1650	μL	0.1	165	μL 200 μM in	1485	μL

Started with 1M IPTG, so added 3. 66 μL to 1831.5 μL LB and diluted from there according to the chart

Plate is 100 μL of the IPTG solutions with 100 μL cultures diluted according to table 2

Cornin 3904 black clear flat bottom 96 well

Grown in plate reader at 30°C

Well Plate

	Row	0 µM	0 µM	0 µM	10 µM	10 µM	10 µM	100 µM	100 µM	100 µM	1000 µM	1000 µM	12
1	**115**												
2	116												
3	118												
4	119												
5	120												
6	Sulforhodamine 101												
7													
8													

Start: 9:30 pm

1 mL stocks of each culture: 650 µL glycerol with 650 µL saturated culture, stored in -80 °C freezer in box underneath the box labeled "common strains" (move the middle piece aside)

9/11/2019

Project: 2019 iGEM
Authors: Alicia Selvera

WEDNESDAY, 9/11/2019

Objective: Trial run for testing RNA thermometers to get comfortable with the protocol.

Innoculation of pIG15,16,18,19,20 (a)

I inncoluted the a version of each construct. Thus , im dropping that a and added in a new a and b, since i picked 2 colonies from each plate section.

I innoculated in LB30. Turns out that was a mistake. But since this is just for testing the protocol. Add MgSo4 to media

Testing Thermometers

- Grow in 2ml LB liquid medium at 37 °C until OD600 = 0.1
  - From the 8 hour culture of 2ml LB
  - From overnight culture (8 hr growth), measure OD in a 1:30 dilution, dilute to 0.01, then grow to 0.1 (~3 hr)
    - Start: 7:46 pm
    - End: 9:35 pm
  - Shake liquid culture in 24wellblock before diluting to ensure its homogenous
    then make dilution directly in cuvette. To measure OD, add 23.33μL of culture to a final 700μL (676.67μL)
  - LB shaking 250 rpm
  - Once it's at 0.1 add 2x IPTG, dilute 2x to get to ~.05 in LB
  - We don't have to use minimal media because of the mCherry2
    - We need to have absolute values, so use sulfur rhodamine to normalize (say it's equivalent to x amount of sulfur rhodamine)
- liquid media
  - Induce with no IPTG, 10 μM, 100 μM, 1 mM IPTG
  - measure the fluorescence every hour
    - saturates (df/dt=0) around OD = 6
    - 10 tubes for each temperature
    - Now we will use a plate reader over two days (one temp for each day)
      - Need to do 2 days because the maturation time for mCherry is ~30 min
    - inoculate into one container, vortex it well, aliquot into each of 30 tubes 2 mL [60 mL M9 minimal]

Going to test the a versions, do not need to make a b version
Make sure to shake the cultures vigourously before pipetting the culture, it precipitates very fast
Cornin 3904 black clear flat bottom 96 well

Concentrations

	pIGXXa Turbo	Diluted OD600	x10 dilution	amount of culture	amount of LB Kan
1	15a	0.406	4.06	7.3891625616	2992.610837…
2	16a	0.334	3.34	8.9820359281	2991.017964…
3	18a	0.346	3.46	8.6705202312	2991.329479…
4	19a	0.316	3.16	9.4936708861	2990.506329…
5	20a	0.348	3.48	8.6206896552	2991.379310…
6					

Pipette 3 mL of the LB for each into the plate
Make sure to keep shaking the cultures while pipetting out

Table 2

	pIGXXa Turbo	OD600	Amount of culture to add to 0.1
1	15		
2	16		
3	18		
4	19		
5	20		
6			

IPGT concentrations

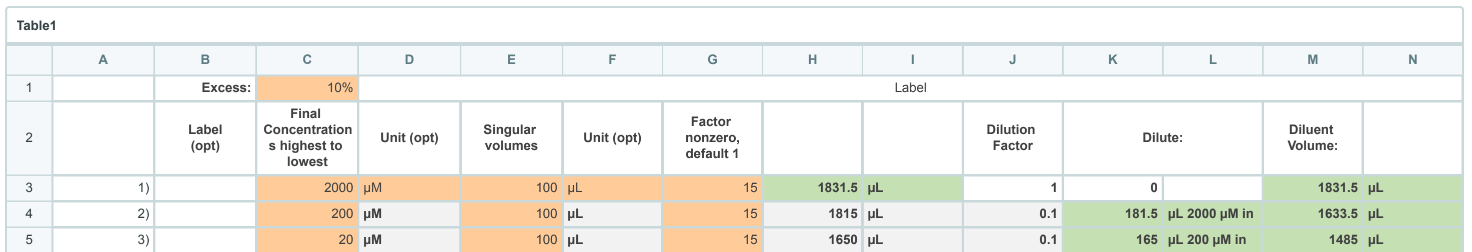


Table1

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		Excess:	10%	Label										
2		Label (opt)	Final Concentrations highest to lowest	Unit (opt)	Singular volumes	Unit (opt)	Factor nonzero, default 1			Dilution Factor	Dilute:		Diluent Volume:	
3	1)		2000	µM	100	µL	15	1831.5	µL	1	0		1831.5	µL
4	2)		200	µM	100	µL	15	1815	µL	0.1	181.5	µL 2000 µM in	1633.5	µL
5	3)		20	µM	100	µL	15	1650	µL	0.1	165	µL 200 µM in	1485	µL

Started with 1M IPTG, so added 3. 66 µL to 1831.5 µL LB and diluted from there according to the chart

Plate is 100 µL of the IPTG solutions with 100 µL cultures diluted according to table 2

Grown in plate reader at 25°C

Well Plate

	Row	0 µM	0 µM	0 µM	10 µM	10 µM	10 µM	100 µM	100 µM	100 µM	1000 µM	1000 µM	12
1	**115**												
2	116												
3	118												
4	119												
5	120												
6	Sulforhodamine 101												
7													
8													

Start: 9:30 pm

1 mL stocks of each culture: 650 µL glycerol with 650 µL saturated culture, stored in -80 °C freezer in box underneath the box labeled "common strains" (move the middle piece aside)

September 11, 2019: RNAT Testing day 2

Project: 2019 iGEM
Authors: Claire Young

WEDNESDAY, 9/11/2019

Inoculation of overnight cultures

- 200 µL of each overnight culture for pIG115, 116, 118, 119, and 120 were added to 1.8 mL LB+Kan for a total volume of 2 mL
- Left in the shaking 37 °C incubator for 9 hr
  - start time: 10:01 am

September 12, 2019:

Project: 2019 iGEM
Authors: Claire Young

THURSDAY, 9/12/2019

Objective: Anneal, phosphorylate, assemble, and transform two of the computationally designed thermometers to make sure they work.

Annealing:

1. Made a 10 µL reaction with 1 µL 10x NEBuffer 3.1, 8 µL water, and 0.5 µL of each 100 µM oligo (oIG046 and 47)
2. Cycling conditions (saved under Shyam's folder as the long annealing protocol):
    a. 95 °C for 1.5 min
    b. 95 °C for 12 s 146x
    c. 22 °C for 10 min

Phosphorylation

1. Made a 25 µL reaction with 0.5 µL annealing product, 2.5 µL 10x T4 ligase buffer, 0.5 µL T4 PNK, and 21.5 µL water
2. Incubated for 30 min at 37 °C
    a. Left tube directly on metal plate in incubator 1
    b. start time: 3:06 pm

Golden Gate

We used BSA, BSAI hf, cutsmart, and T4 DNA ligase
RNAT 101 + pcrSPB875

Table1

	A	B	C	D	E	F
1	Golden Gate Assembly 2					
2	***Reactions***	1		***BSA?***	yes	
3	***# Unique Parts***	1			*1*	
4	***# Common Parts/Vector***	1			*1*	
5	***# Others (PCR pdts, oligo pts)***	0			*1*	
6	***Excess***	2%	0%	0%	-	
7						
8	**Reactions**	**1.02**	1	1	*1 ref*	
9						< Warnings
10	**diH2O**	**10.2**	10	10	**10**	μL
11	10× Ligase Buffer	**1.53**	1.5	1.5	**1.5**	μL
12	**10× BSA**	**1.53**	1.5	1.5	**1.5**	μL
13	**T4 DNA Ligase**	**0.51**	0.5	0.5	0.5	μL
14	Type IIs endonuclease	**0.51**	0.5	0.5	0.5	μL
15		***Total:***			***Each:***	
16	Unique Parts	**0.51**	0.5	0.5	1	μL
17	**Common Parts/Vector**	**0.51**	0.5	0.5	0.5	μL
18		**#N/A**	#N/A	#N/A		
19	**Others**	0	0	0	0.5	μL
20						
21	***Total:***	**15.3**	15	**15**	*15*	μL
22	***Total – DNA:***	**14.28**	14	**14**	**14**	μL
23	***Total – Uniques & Others:***	**14.79**	14.5	**14.5**	-	μL

9/13/2019

Project: 2019 iGEM
Authors: Alicia Selvera

FRIDAY, 9/13/2019

Objective: We are aiming to get pIG017 sequenced by the end of today

Transformation happened at 1:45pm

Innolcuate pIG017 RNAT101 @ 11pm

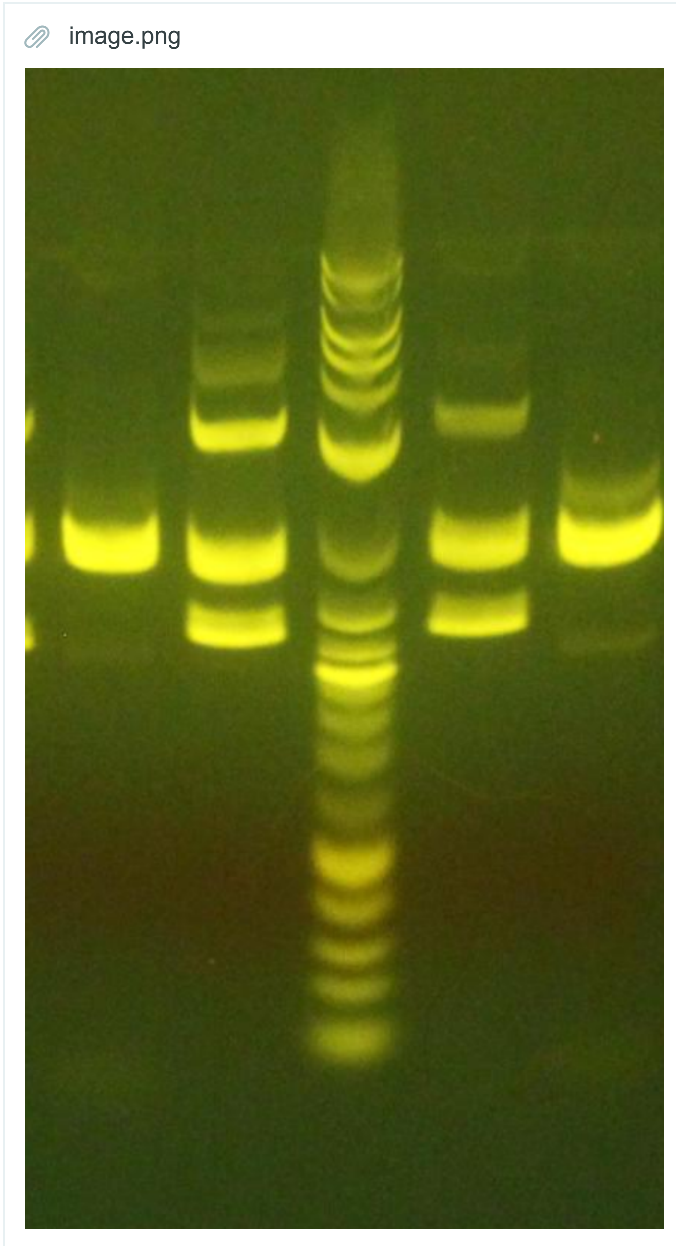
PCR off of liquid culture using primers: AC58 / 59

Performed a PCR coloumn cleanup

Sent off for sequencing

Did a BSAI digest: looks good (its in the 1st and 4th well) (ignore 2nd and 3rd. I piggibacked offf of shyams gel)

image.png



Notes: DpnI digest unreacted pcr product (pcrSPB875/877) before assembling them into thermometer testing cassettes. Tomorrow putida + arabidopsis experiments are to be done

9/14/2019: Putida/arabidopsis control experiments began

Project: 2019 iGEM
Authors: Alicia Selvera

SATURDAY, 9/14/2019

Inncoluation of P putida in LB Kan 1 has 3mL the other has 4ml

Inncoluation of arabiopsis with p putida

1. collect the bacterial cells by centrifugation at 2,600 *x g* for 20 min at room temperature.
2. resuspend pellet in 45mL of sterile water
3. flood inncoluate the seeds with 5 mL of culture

Notes: the first plate i put the water on the seeds. This caused the seeds to just float away. (this was a very bad idea) . On the second plate, i put the water right under the seeds and the water spread out to the seeds, bt didnt caused the seeds to float away.

September 19, 2019:Victors thermometers have arrives

Project: 2019 iGEM

Authors: Alicia Selvera

THURSDAY, 9/19/2019

Objective: resuspend oligos, anneal, phosphorylate

Materials for oligo anneals:

- Thermometer oligos 102-123
- Nuclease-free water
- neb buffer 3.1
- Thermocycler

Procedure for oligo anneals: (Alicia)

1. Resuspended oligos to 100µL
2. For a 10 µL reaction, added .5 µL of each oligo to a PCR tube and 8 µL nuclease-free water and .5 of each oligo
3. Placed in thermocycler under anneal long

Materials for phosphorylation

- Oligo anneals 102-123
- nuclease-free water
- T4 ligase buffer
- T4 PNK

Procedure for phosphorylation

1. Mixed 1040.52 µL water, 119.6 µL 10X T4 ligase buffer, 11.96 µL T4 PNK into a master mix
2. Pipetted 49 µL into each tube
3. Added 1 µL of each anneal product into each tube
4. Incubated at 37 °C for 30 min

Golden Gate

5.61 uL of 430

11.22 uL of M

aliquot 14.5

might need to redo 23

Table1

	A	B	C	D	E	F
1	Golden Gate Assembly 1					
2	***Reactions***	22		***BSA?***	no	
3	***# Unique Parts***	1			*1*	
4	***# Common Parts/Vector***	1			*1*	
5	***# Others (PCR pdts, oligo pts)***	1			*1*	
6	***Excess***	2%	0%	0%	-	
7						
8	**Reactions**	**22.44**	22	1	*1 ref*	
9						< Warnings
10	**diH2O**	**252.45**	247.5	11.25	**11.25**	µL
11	10× Ligase Buffer	**33.66**	33	1.5	**1.5**	µL
12	10× BSA	0	0	0	0	µL
13	**T4 DNA Ligase**	**11.22**	11	0.5	0.5	µL
14	Type IIs endonuclease	**11.22**	11	0.5	0.5	µL
15		***Total:***			***Each:***	
16	Unique Parts	**11.22**	11	0.5	0.5	µL
17	**Common Parts/Vector**	**5.61**	5.5	0.25	0.25	µL
18		**#N/A**	#N/A	#N/A		
19	**Others**	**11.22**	11	0.5	0.5	µL
20						
21	***Total:***	**336.6**	330	**15**	*15*	µL
22	***Total – DNA:***	**308.55**	302.5	**13.75**	**13.75**	µL
23	***Total – Uniques & Others:***	**314.16**	308	**14**	-	µL

September 20, 2019

Project: 2019 iGEM

Authors: Alicia Selvera

FRIDAY, 9/20/2019

Made plates, transformed and innoclulated thermometers. Need to redue transformation 105, plus assembly 123.

September 22,2019: One big miniprep

Project: 2019 iGEM

Authors: Alicia Selvera

SUNDAY, 9/22/2019

Miniprepped all the thermometers. Was going good, then Alicia forgot to hit centrifuge botton twice before eluting. And through out coloumn before checking for flowthrough. She then preceeded to dumpster dive and recover all the coloumns and spin them down. Before doing such a thing she added an extra 50 µL EB to some of the coloumns. The spectra still looked nice though, so we decided to roll with it.

September 23, 2019: Sequencing of RNAT102-123, except 105

Project: 2019 iGEM
Authors: Claire Young

MONDAY, 9/23/2019

NotI restriction digest of RNAT102-123

Set up the reaction with a master mix according to the following table, and added 1 µL of each RNAT to 9 µL master mix to each tube

Note: The NotI was accidentally diluted 22/174 X in water, or ~12% (stored in iGEM antibiotic box)

Incubated for 30 min at 37 °C

Table1

	A	B	C	D	E	F	G
1	DNA Restriction Digest 1						
2	*Notes*	**Reactions**	21	**BSA?**	no		
3		**Excess**	5%	0%			
4		**Reactions**	**22.05**	**21**	***1 ref***		
5						< Warnings	
6		**diH2O**	**154.35**	147	**7**	µL	
7	CutSmart	**10× rxn buffer**	**22.05**	21	**1**	µL	
8		10× BSA	0	0	0	µL	
9	EcoRI-HF	**Enzyme 1**	**22.05**	21	1	µL	< 1 µL total
10	SpeI-HF	**Enzyme 2**	**0**	0	0	µL	
11		**Enzyme 3**	**0**	0	0	µL	
12		**DNA**	**22.05**	21	1	µL	Consider copy#
13							
14		**Total:**	**220.5**	**210**	**10**	µL	
15		***Total–DNA:***	**198.45**	**189**	**9**	µL	
16		***Total–Enz:***	**198.45**	**189**	**9**	µL	
17		***Total–DNA/Enz:***	**176.4**	**168**	**8**	µL	

Materials for sequencing

- AB17 sequencing primer
- Golden gate reactions for RNAT102-123, except for 105 which didn't form colonies
- Water

Procedure for sequencing

1. Making master mixes

September 25, 2019: Preparation of kit thermometers parts and RNAT105

Project: 2019 iGEM

Authors: Alicia Selvera

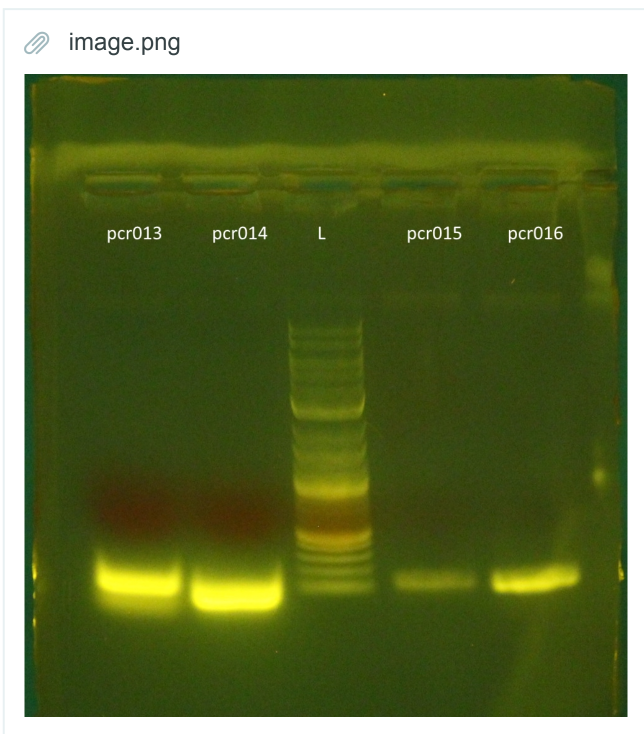
WEDNESDAY, 9/25/2019

PCR of kit thermometers

pcrIG013-pcrIG016

Loaded on a gel and ran at 120 V, start time: 6:37 pm

image.png



Annealing for RNAT105:

1. Made a 10 µL reaction with 1 µL 10x NEBuffer 3.1, 8 µL water, and 0.5 µL of each 100 µM oligo (oIG56 and 57)
2. Cycling conditions (saved under Shyam's folder as the long annealing protocol):
    a. 95 °C for 1.5 min
    b. 95 °C for 12 s 146x
    c. 22 °C for 10 min
    d. Start time: 5:42 pm

Phosphorylation

1. Made a 25 µL reaction with 0.5 µL annealing product, 2.5 µL 10x T4 ligase buffer, 0.5 µL T4 PNK, and 21.5 µL water
2. Incubated for 30 min at 37 °C
    a. Left tube directly on metal plate in incubator 1
    b. start time: 6:50 pm
    c. end time: 7:20 pm

Gel Purification

1. Added the volume of agarose dissolving buffer listed in the "3x gel weight" from the gel purification table to each tube
2. Heated gels at 42 °C until melted
   a. Start time: 7:34
   b. End time: 7:56
3. Followed the procedure listed on the Zymoclean Gel DNA Recovery Kit protocol
   a. Included a dry spin for 2 min
   b. Eluted in 8 μL DNA elution buffer

Gel purification values

	Sample	tube weight	tube+gel weight	gel weight (mg)	3x gel weight
1	pcr013	979	1200	221	663
2	pcr014	997	1192	195	585
3	pcr015	986	1250	264	792
4	pcr016	996	1184	188	564

Concentrations
pcr013: 17.2 ng/μL
pcr014: 58.5 ng/μL
pcr015: 6.9 ng/μL
pcr016: 11.0 ng/μL

Golden GAte

pSPB413, 449, 608, 918 combined in a 4 part golden gate with BsaI to to make araC cassette vector
pcr013, pcr014, pcr015, pcr016, RNAT105 were all combined with pSPB430. pcr013 and pcr016 do not have Esp31 cut sites and should not have been included, so don't transform them.

Table1

	A	B	C	D	E	F
1	***Reactions***	5		***BSA?***	yes	
2	***# Unique Parts***		0		*1*	
3	***# Common Parts/Vector***		1		*1*	
4	***# Others (PCR pdts, oligo pts)***		1		*1*	
5	***Excess***	3%	0%	0%	-	
6						
7	**Reactions**	**5.15**	5	1	*1 ref*	
8						< Warnings
9	**diH2O**	**52.7875**	51.25	10.25	**10.25**	µL
10	10× Ligase Buffer	**7.725**	7.5	1.5	**1.5**	µL
11	**10× BSA**	**7.725**	7.5	1.5	**1.5**	µL
12	**T4 DNA Ligase**	**2.575**	2.5	0.5	0.5	µL
13	Type IIs endonuclease	**2.575**	2.5	0.5	0.5	µL
14			***Total:***		***Each:***	
15	Unique Parts	**0**	0	0	0.5	µL
16	**Common Parts/Vector**	**1.2875**	1.25	0.25	0.25	µL
17		**#N/A**	#N/A	#N/A		
18	**Others**	**2.575**	2.5	0.5	0.5	µL
19						
20	***Total:***	**77.25**	75	**15**	*15*	µL
21	***Total – DNA:***	**73.3875**	71.25	**14.25**	**14.25**	µL
22	***Total – Uniques & Others:***	**74.675**	72.5	**14.5**	-	µL

Sept 27, 2019: inoculation and miniprep

Project: 2019 iGEM

Authors: Stephanie Trejo

FRIDAY, 9/27/2019

Inoculation of RNATs 114 and 115

End: 8:20 pm

Miniprep

RNATs 008, 114, 115

pIG names: 015, 030, and 031 respectively

Table1

	A	B
1	Name	Concentration (ng/uL)
2	pIG015	35.9
3	pIG030	239.7
4	pIG030a	168.2
5	pIG031	205.2
6	pIG031a	177.8

Note: pIG015 concentration is probably low because I resuspended it first, and left it sitting for a bit while I spun down the other ones. I probably should have vortexed it again, but I didn't think about it at the time. My bad.

Samples stored in pIG box, in the fourth row.

September 28,2019

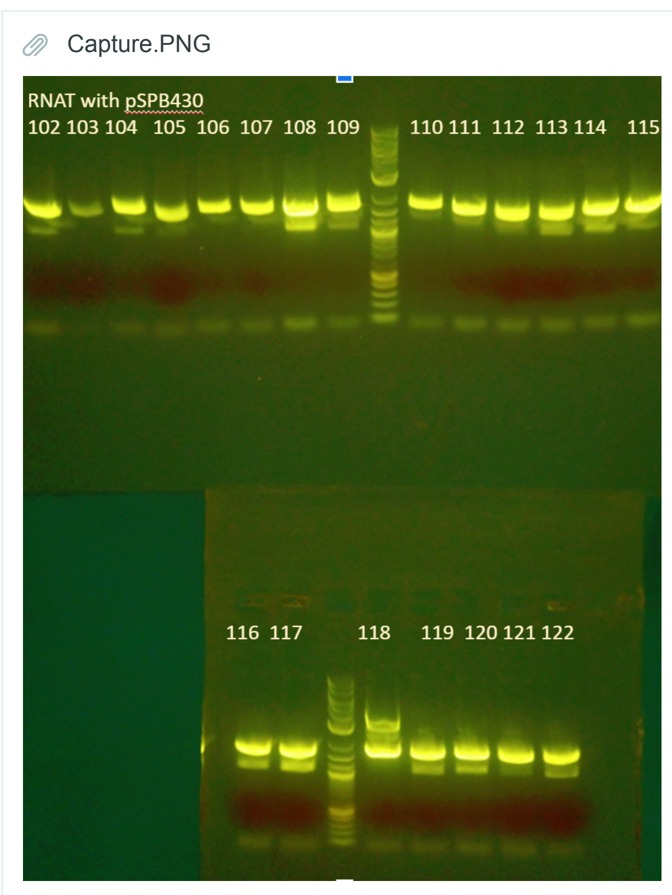
Project: 2019 iGEM

Authors: Alicia Selvera

SATURDAY, 9/28/2019

Picture of Restictrion digest from the 23rd

Capture.PNG



PCR of pSPB875

1. Made a mastermix of 100 µL NEB High Fidelity 2x PCR master mix, 1 µL 100 µM primer AG21, 1 µL 100 µM primer AG22, 90 µL diH20
2. Split sample into two tubes (96 µL into each)
3. Added 4 µL of pIG875 to each tube
4. Split those into two more tubes, yielding a total of 4 50 µL reactions

Cycling conditions:

I. Initial denaturation: 98 C for 1 min
II. Denaturation: 98 C for 10 sec
III. Annealing: 64 C for 30 sec
IV. Extension: 72 C for 1:25 min, 30X
V. Final extension: 72 C for 2 min

Gel purification

I ran 4 lanes of 50uL rxns. The band appear about 4.4kb. I cut out those bands and ran it in two seperate gel extractions. Each weighed about .2 g

Summary: Cut bands into tubes. Add 3 volume ADB. Melt for 10 min. Pass through column. Wash column twice with 200 µL wash buffer. Spin dry 2 min.

Septemper 29, 2019: Cleanup of pcrSPB875

Project: 2019 iGEM

Authors: Claire Young

SUNDAY, 9/29/2019

DNA Cleanup of pSPB875

1. Mixed 125 µL buffer PB (quiagen protocol) with ~25 µL of pcrSPB875
2. Centrifuged for 1 min at 17,900 xg
3. Added 750 µL buffer PE and centrifuged for 1 min
4. Dry spun for 1.5 min
5. Transfered column to 1.5 mL tube and added 50 µL buffer EB, let sit for 3 min
6. Centrifuged for 1 min

DpnI digest

(6 µL of cutsmart and 1 µL of DpnI was added straight to the pcr product)

Golden gate

0.5 µL of <200ng/µL, 1 µL of 100-200, 0.5 µL of >100 of each plasmid. 2µL of vector no BSA, BSAI-hf

Notes

- To save extra transformation, take miniprep culture, make sure its homogenized. Take 1µL onto two gridded plates
  - take a ruler, draw a grid on round plates
- retransform control tomorrows (pSPB875 and pSPB877)

September 30: Transformation of thermometer cassettes and controls

Project: 2019 iGEM

Authors: Alicia Selvera

MONDAY, 9/30/2019

October 1, 2019:

Project: 2019 iGEM

Authors: Claire Young

TUESDAY, 10/1/2019

Inoculation of colonies for RNA thermometer testing

Picked colonies of all the thermometers, pSPB841, 875, and 877 and incoculated them in 3 mL LB+Kan

Incubated at 37 °C

Start time: 9:35 am

For the 12 hr cultures at different temperatures, inoculated 15 µL saturated culture into 1.5 mL LB+Kan+1mM magnesium

Start time: 12:30 am

Gel order

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	RNAT 006	7	8	9	102	104	106	L	107	116	117	118	119	120	121
2	122	123	108	109	110	111	112	L	113	114	115				

October 2 (Alicia) Thermometer cleanup and otsBA

Project: 2019 iGEM
Authors: Alicia Selvera

WEDNESDAY, 10/2/2019

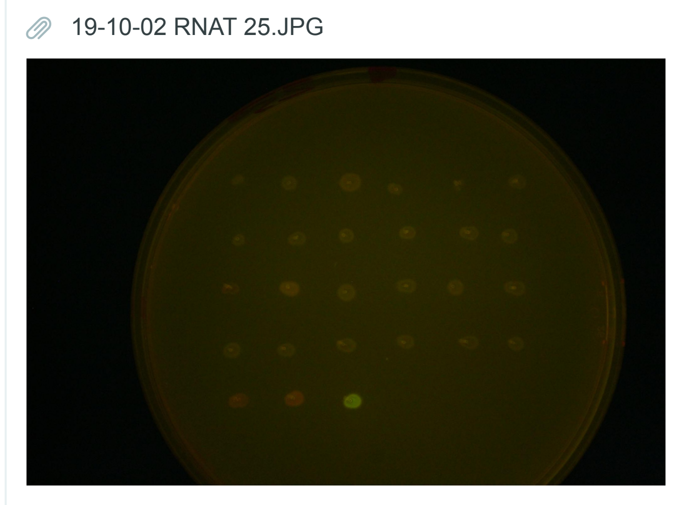
Notes from October 1st.

IPTG plates were made with 0.59 mM of IPTG.
There is a real possibility of cross contamination between wells in the 24 well block
Plates were spoting with 1 uL of culture and put into 25, 30. and 37 degree incubators

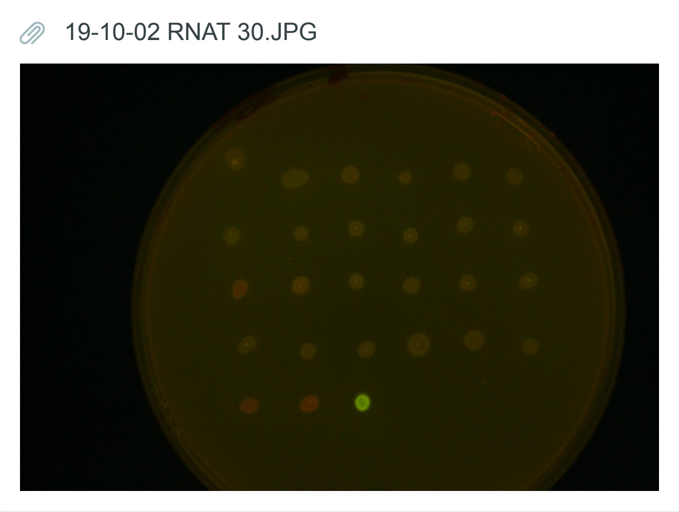
Summary of today

Took macroscope images of spot

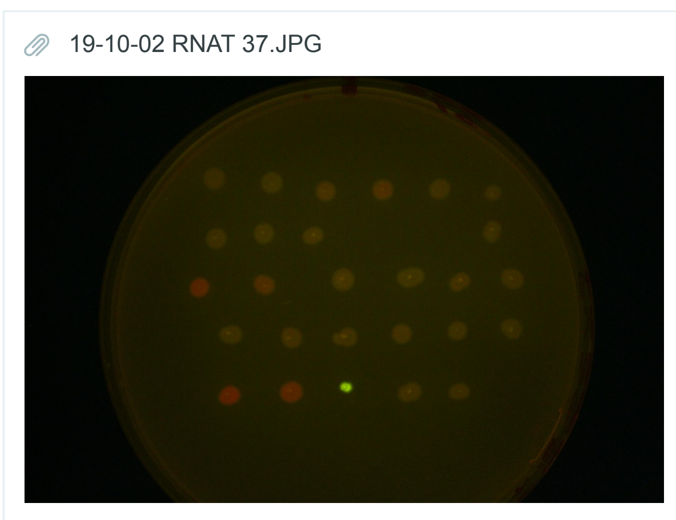
19-10-02 RNAT 25.JPG



19-10-02 RNAT 30.JPG



19-10-02 RNAT 37.JPG



Redid colony pcr on RNAT009 and RNAT021 using AD65 and a TM of 53 and Taq Polymerase

It failed. There was so bands.

Obtained E. coli genome from Illene. Amplified otsBA from it used oIG42 and 43. Ran a gel. It looked good. Then I Gel purified it and assembled with pSPB440. This was then transformed using NEB turbo on Chl plates and stuck in incubator at 10:15pm.

I Innocculted all the thermometers in duplicate on one 96 well block and stuck in incubator at 10:15 pm.

October 3, 2019:

Project: 2019 iGEM

Authors: Claire Young

THURSDAY, 10/3/2019

Inoculation of pIG010

At 7:50 am, plate showed no colony growth so returned to 37 °C incubator

Plate at 2 pm still showed no growth, so redid the golden gate

Note about the thermometers

There was significant splashing of the cultures in the 96-well plate onto the seal and possibly other wells, so it's possible the samples are contaminated.

Colony PCR of thermometers

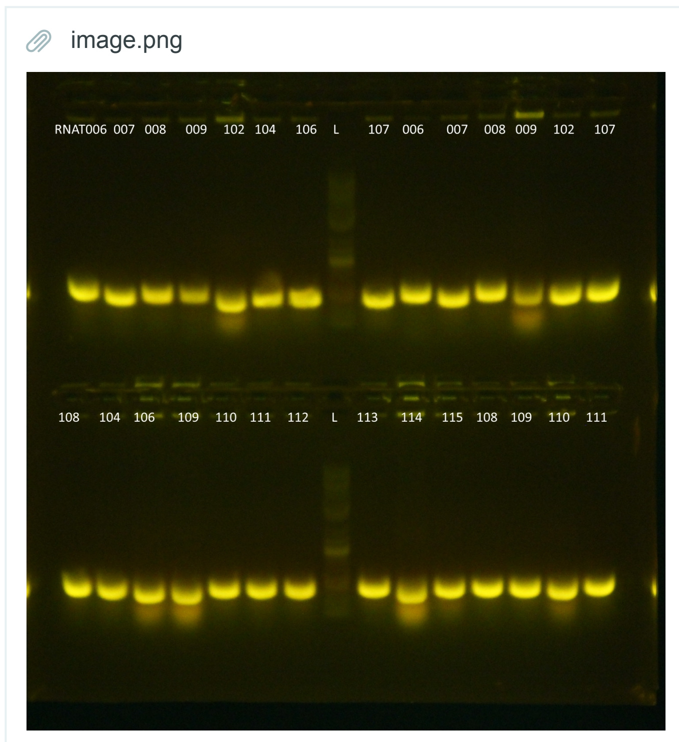
15 µL Taq reactions using 1 µL template, the forward primer for each thermometer, and the reverse primer as AD65

Annealing temp 53 °C, extension time 30 sec

Table1

	A	B	C	D	E	F	G	Ladder	I	J	K	L	M	N	O
1	RNAT006	7	8	9	102	104	106		107	6	7	8	9	102	107
2	108	104	106	109	110	111	112		113	114	115	108	109	110	111
3	112	113	114	115	116	117	118		119	120	121	122	123	116	117
4	118	119	120	121	122	123	pIG003								

image.png



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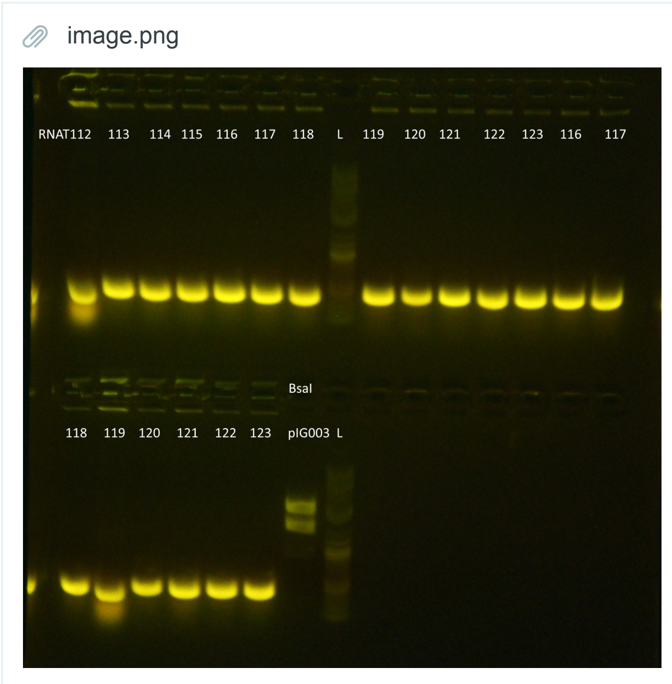

Golden gate to make pIG010

Assembled pcrIG010 with pSPB440 with Esp3I using the long Esp31 protocol

Electroporation transformation of putida

- .4uL of pSPB874 in p putida electrocomp cells
- placed cells in middle of cuvvette sheets
- tap
- wipe
- align notch
- 1200V for 1mm cuvette
- 2hrs at 30°C

Inoculation for temperature testing

Inoculated 3 μL of each thermometer and control into 300 μL LB+Kan+IPTG+Mg

Incubated at 25, 30, 37 °C

Start time: 11:15 pm

October 4, 2019:

Project: 2019 iGEM
Authors: Claire Young

FRIDAY, 10/4/2019

Plate read final time point of thermometers

Restriction digest of pIG010 a, b

Using 2 µL pIG010 a1, 1 µL pIG010 a2, and 8 µL pIG010 b1+b2

BsaI digest

Incubated at 37 °C

Start time: 5:20 pm

Prepping Putida for the plant experiments tomorrow

Innculating Arabidopsis with Putida

- Grow *P. putida* culture at 30 °C for 24 hr in shaking incubator
  - In 3 mL LB-kan
- spin down bacterial culture in 2600 g for 15min
- Resuspended in 2 mL sterile distilled water
- dilute to an OD of .2 (~10^8 CFU/mL)
- using a known volume, using a spreader plate 400µL onto PN plates
- then plate seeds (in ABL)

(drop inoculate 10µL of culture onto each seed)

October 6th

Project: 2019 iGEM
Authors: Alicia Selvera

SUNDAY, 10/6/2019

Yesterday Alicia and Stefanie seeded plates with p putida (transformed w/ pSPB874) to test flood inncolulating versus drop innoculating. They were plated horizontally in the Bartel Lab.

We miniprepped the thermometers. We also performed a 7 part golden gate to make the enzyme cassettes. They were transformed into both turbo and DH10B-alt. DH10B produced many more colonies. Mostly white, some green. I came in at 4 and innoculated them into a 24 well block in 2 mL of culture. I will miniprep tonight.

October 8th

Project: 2019 iGEM

Authors: Alicia Selvera

TUESDAY, 10/8/2019

Electroporation transformation of putida

- .4uL of pIG104,5,6 in p putida electrocomp cells
- placed cells in middle of cuvvette sheets
- tap
- wipe
- align notch
- 1200V for 1mm cuvette
- 2hrs at 30°C
- plate on amp, also plate wildtype on ampt

October 9th

Project: 2019 iGEM

Authors: Alicia Selvera

WEDNESDAY, 10/9/2019

Innoculated 5mL of p putida into 7 different cultures

+/- refers to the additon of 1mM of IPTG

OD of putida cultures at different time points to track growth

	pIG104+	pIG104-	pIG105+	pIG105-	pIG106+	pIG106-	wild type	H
1								
2								
3								
4								

October 11, 2019 Preparing thermometers for time course testing

Project: 2019 iGEM
Authors: Alicia Selvera

FRIDAY, 10/11/2019

Transformation of RNAT 006, 007, 102, 106, 107, 118, 121 and controls into NEB turbo using 30sec 1 min * 2 heat shock

Experiement to figure out how much Carb to add

1. Alliquot 2mL of LB30 into 10 wells in 24well block
2. From a 5mg/mL stock of Carb, add 20µL to first well, 18µL to second well, and so on to 2µL in 10 well
3. pick a colony from Putida pSPB874 and add to each well
4. put in 30 C incubator

October 12, 2019

Project: 2019 iGEM

Authors: Alicia Selvera

SATURDAY, 10/12/2019

Results from Putida/Carb experiments

The wild type putida grew in every concentration of Carb. Just to test whether our carb is bad or putida is resistant, I started a culture of wild type putida in four concentrations of Amp.

RNAT testing

Bacterial Strain Preparation

Grow in 2ml LB liquid medium at 37 °C until saturation (about 8 hours)

Start: 12:10pm

End: 8:00

From the 8 hour culture of 2ml LB, freeze 1 mL of the saturated culture

From saturated culture, measure OD in a 1:30 dilution, dilute to 0.01, then grow to 0.1 in LB shaking 250 rpm (~1.5 hr)

1:30 dilution: Add 23.33μL of culture to 676.67μL LB (final 700μL)

Table1

	A	B	C	D	E
1	pIGXXa Strain	Diluted OD600	x30 dilution	amount of culture	amount of LB Kan
2		0.215	6.45	4.6511627907	2995.348837…
3			#VALUE!	#VALUE!	#VALUE!
4			#VALUE!	#VALUE!	#VALUE!
5			#VALUE!	#VALUE!	#VALUE!
6			#VALUE!	#VALUE!	#VALUE!
7			#VALUE!	#VALUE!	#VALUE!

Start:8:17 pm

End:

Plate Preparation

Add 100μL of 2x IPTG to each well 1 mM IPTG (consult wetlab dilution series calculator)

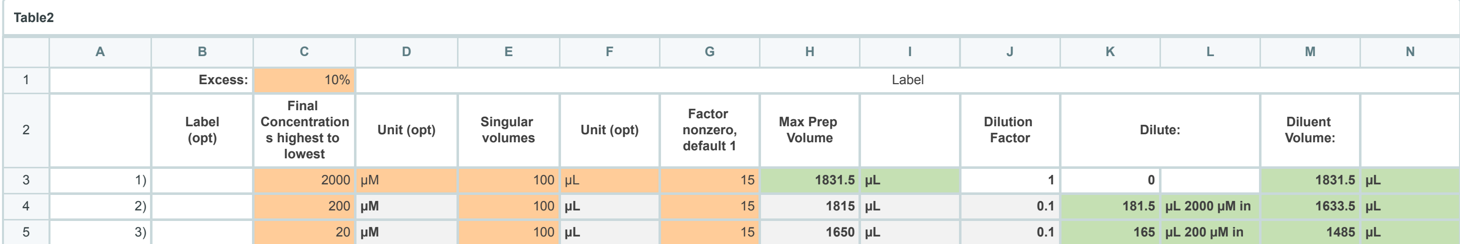

Table2

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		Excess:	10%	Label										
2		Label (opt)	Final Concentrations highest to lowest	Unit (opt)	Singular volumes	Unit (opt)	Factor nonzero, default 1	Max Prep Volume		Dilution Factor	Dilute:		Diluent Volume:	
3	1)		2000	μM	100	μL	15	1831.5	μL	1	0		1831.5	μL
4	2)		200	μM	100	μL	15	1815	μL	0.1	181.5	μL 2000 μM in	1633.5	μL
5	3)		20	μM	100	μL	15	1650	μL	0.1	165	μL 200 μM in	1485	μL

add sulfur rhodamine to normalize

10mg/L and then 2x dilute from there

Add 100μL of culture to each well. Do all samples in triplicate.

Grown in plate reader at 30°C

Table3

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	**Row**	**0 µM**	**0 µM**	**0 µM**	**10 µM**	**10 µM**	**10 µM**	**100 µM**	**100 µM**	**100 µM**	**1000 µM**	**1000 µM**	**12**
2													
3													
4													
5													
6													
7	Sulforhodamine 101												
8													
9													

Start:

1 mL stocks of each culture: 650 µL glycerol with 650 µL saturated culture, stored in -80 °C freezer in box underneath the box labeled "common strains" (move the middle piece aside)

October 14th

Project: 2019 iGEM
Authors: Alicia Selvera

MONDAY, 10/14/2019

Electroporation transformation of putida

- .4uL of pIG104,5,6 in p putida electrocomp cells
- placed cells in middle of cuvvette sheets
- tap
- wipe
- align notch
- 1200V for 1mm cuvette
- .75mL SOC
- 2hrs at 30°C shaking
    - a put the epi tubes in a bigger conical vial so that it can be shaking at 30 C
- plate on amp, also plate wildtype on normal plate
- transformation started at 1:30

October 16,2019

Project: 2019 iGEM

Authors: Alicia Selvera

WEDNESDAY, 10/16/2019

Putida + arabidopsis

Procedure

- I grew putida pIG104,5,6 and wild type in 2 mL of LB over night
- left on bench, shaking, room temp for a few hours
- spun down at 2500 g for 10 minutes
- resuspended in 1.5 mL sterile water
- measure OD and dilute to an OD of .2 (~10^8 CFU/mL) in 1.5mL stile water
- split each contruct, including wild type
- add 10mM Arabinose to half of each construct
  - 750µL water * 10mM arabinose = v *1000mMiPTG
  - 7.5µL IPTG
- plated on 16 plates

  - (2x) 104 induced
  - (2x) 104 not induced
  - (2x) 105 induced
  - (2x) 105 not induced
  - (2x) 106 induced
  - (2x) 106 not induced
  - Col-0 with normal putida
  - Col-0 no bacteria
  - 104+105+106 induced
  - 104+105+106 not induced

Time course for the thermometers with ramping temperatures

Project: 2019 iGEM

Authors: Alicia Selvera

FRIDAY, 10/18/2019

102, 106, 107, 118, 121 and the kit thermometers, plus 875 and 877 as controls. we have induced and non induced conditions. It was ran as a time course and the temperature was ramped up. So we started at 25C then went to 30 and then 37.

The plate is laid out as followed

A1-6 is 102 induced
A7-12 in 106 induced
B1-6 is 107 induced
B7-12 is 118 induced
C1-6 is 121 induced
C7-12 is kit 8 induced
D1-6 is kit 9 induced
D7-9 is 875
D10-12 is 877
E1-3 is 102 uninduced
E4-6in 106 uninduced
E7-9 is 107 uninduced
E10-12 is 118 uninduced
F1-3 is 121 uninduced
F4-6in 8 uninduced
F7-9 is 9 uninduced
the last row is sulfur rodamine

Sulfur rodamine was used in the following concentrations
1.25 mg/L <
0.625 mg/L <
0.3125 mg/L <
0.15625 mg/L <
0.078125 mg/L <