Experiments

Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Bryan and Phillip	• Membrane test (Dialysis tubing on top of an agar plate) • Prototype for transdermal patches		• Dialysis tubing mimics the transdermal patches as a semi-permeable membrane • Goal is to have a semi-permeable membrane that lets the protein through but holds the bacteria back from being delivered to the human body	• 3/25 collect the membrane tests
Andrew	• Liquid culture of UV promoter for miniprep			• Miniprep for sequencing

Experiments

Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Bryan	• Collected the membrane test	• One leaked bacteria (not sterile enough) • One didn’t leak	• We've concluded that the experiment isn't sterile enough • Two tubes had contradicting results	• Redo with liquid membrane test
Bryan	• Liquid membrane test • Dialysis tube inserted into a 50 ml centrifuge tube		• Liquid membrane test could have a better result than the plate test because it has a greater surface area in contact with the LB • If any bacteria leaks, it will grow in the LB	• 3/26 pickup the liquid membrane test
Andrew	• Mini-prepped UV Promoter for sequencing • Liquid culture of UV promoter		• Finished UV promoter PP for sequencing	• Running another protocol for UV mini-prep sequencing

Experiments

Thursday, April 16, 2015
12:30 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Prototype Testing	Bryan	• Collected the 50 ml centrifuge tube		• The experiment wasn't sterile enough • The tube had bacteria both inside and outside	• 4/7 Redo Liquid plate test
Temp+ GFP Testing	Andrew	• Placing Temp + GFP plates into 42 degrees to see if the promoter works as described on the iGem website	• No GFP expression after 1h, leaving it overnight	• Designed an experiment to test the Temp + GFP construct • Experiment- Grow re-streaks of Temp+ GFP at Room Temp, 37 Degrees and 42 Degrees	• Ran experiment as described in conclusion • Going to check on 3/27

Experiments

Thursday, April 23, 2015
5:01 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Check if Temp+ GFP construct works	Andrew	• Checked re-streaks of Temp + GFP that has been grown overnight at RT, 37 Degrees and 42 Degrees	• RT did not grow • Both 37 and 42 degrees grew and had GFP expression	• Expression at 32 Degrees is problematic • Redesigning experiment	• Grow Re-streaks of Temp + GFP at 32 and 37 degrees to see which one allows bacteria to grow well while not expressing GFP
Checking Dialysis tube experiment	Andrew	• Checked Dialysis tube experiment (50 ml tubes) • Checked Dialysis tube (agar plates)	• 50 ml tube setup seems to be contaminated • No colonies on plates	• 50 ml tube seems to be contaminated since the LB in it does not have any resistance	• Redo 50ml setup and leave the plates our during spring break to check if the plates will grow if given enough time

Weekly plan

Friday, April 17, 2015
8:03 PM

Group	Goal
Monica + Joseph	• Finish temperature sensitive promoter (front insert) + GFP gen

Outside of class schedule

Friday, April 17, 2015
8:03 PM

Group	Date	Time (e.g. period, after school, etc.)	Experiment
Monica + Joseph	4/20	A3	• Streak + liquid culture temp sens promoter + GFP gen in different temperatures

Weekly summary

Friday, April 17, 2015
8:03 PM

What we accomplished:	
What we need to do:	

Experiments

Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Evonne	• Agar plate test • Liquid culture (RFP + UV) for new dialysis tube testing		• Preparing for the 4th prototype	• 4/14 collect the liquid culture
Bryan	• LB testing • Liquid culture (RFP + UV) for new dialysis tube testing		• Preparing for the 5th prototype	• 4/14 collect the liquid culture
Andrew	• Digested Temp at ES • Did gel check of digestion		• Passing on Temp + GFP testing to Joseph + Monica	• Gel purification of Temp, ligation + transformation

Experiments

Friday, April 17, 2015
3:21 PM

Group	Experiments	Results	Conclusions/Notes	Next steps

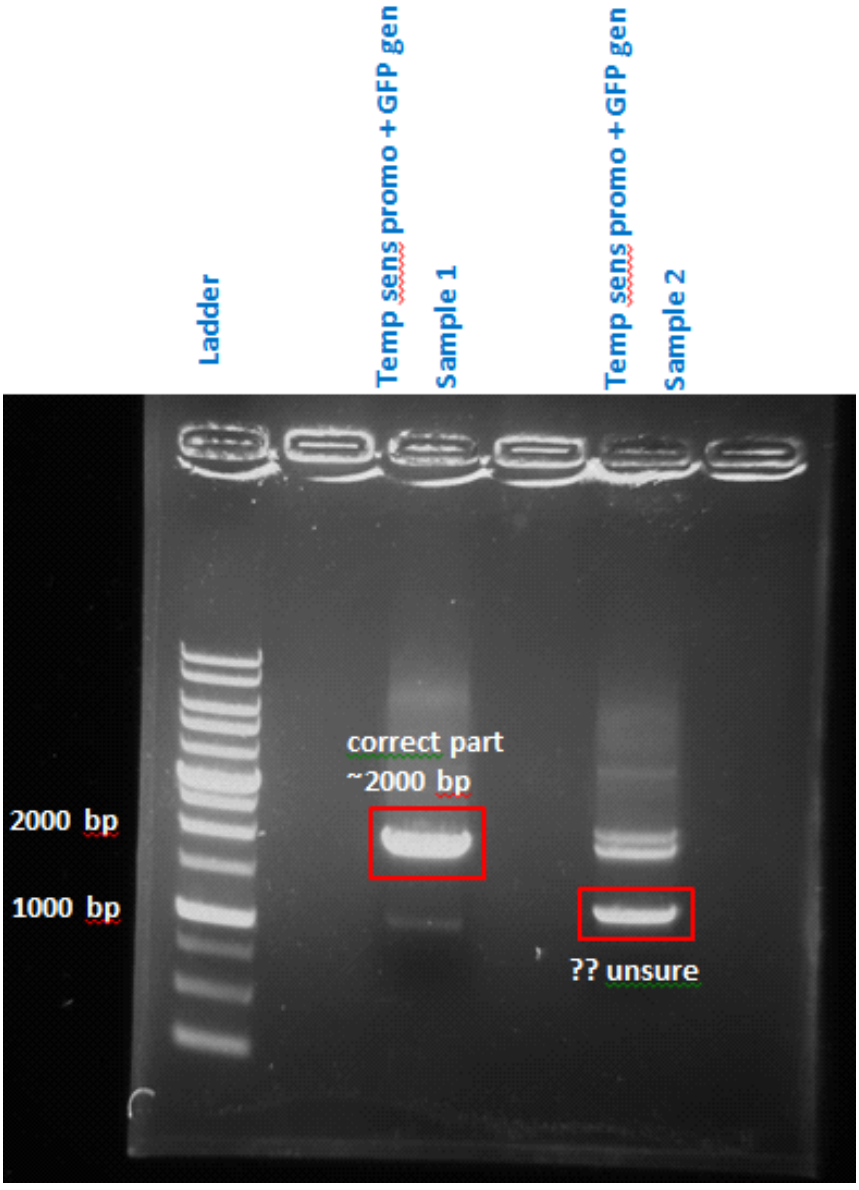
Joseph + Monica	• Gel check for temp sens promoter + GFP gen
	  • Picked 2 colonies from plate that glowed
	  • 2 colonies, 2 lanes
	• Expected size: ~2000 bp
	  • GFP gen: ~900 bp
	  • Promoter: ~900 bp
		• Band at ~2000 bp in both lanes
		• However, Lane 2 had a band at ~1000 bp
		• Gel pic **(Figure 1)**
			• Lane 1 showed successful ligation of temp sens promoter + GFP gen
			• Don't know what was the extra band in Lane 2 so only kept sample in Lane 1
				• Experiment with what temperatures bacteria would produce GFP at 4, 25, 30, 37, and 42 degrees C

Evonne and Bryan	• Collect liquid cultures			• 4/15 Set-up membrane tests
Andrew	• Ligation and transformation ofTemp+ RBS			• 3 in 1 of the Temp + RBS

Photos

Friday, April 17, 2015
3:23 PM

Figure 1. Temp sens promoter + GFP gen construct gel check



Experiments

Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps

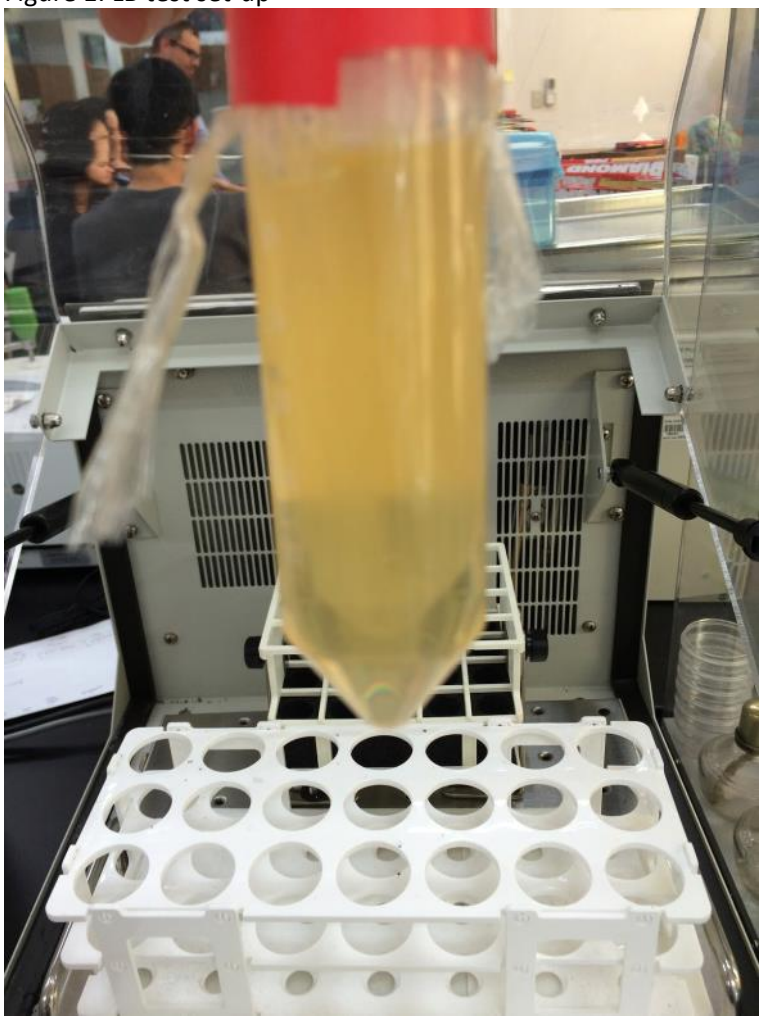
Joseph + Monica	• Planning for Temp Sens + GFP construct testing:
	  • Use OD600 to balance pop.
	  • Use 395nm as excitation, 509nm as emission for GFP
	  • Test groups: 4, Room, 30,37, 42 C
	  • Plate and tube for each
			• OD600 is to make sure that concentration of bacteria would be same in each cuvette sample as bacteria in the liquid culture would grow at different rates in different temperatures
			• Use 395 nm and 509 nm to measure GFP production
			• 30 degrees C = skin temperature
			  • Could be useful for drug delivery
				• 4/20: streak + liquid culture bacteria; place in different temperatures
				• 4/21: spectrovis

Evonne	• Membrane test (Dialysis tubing on top of an agar plate) • Prototype for transdermal patches • In contrast to the first prototype, the bacteria used has a UV promoter and RFP as a reporter • Two trials		• Dialysis tubing mimics the transdermal patches as a semi-permeable membrane • Goal is to have a semi-permeable membrane that lets the protein through but holds the bacteria back from being delivered to the human body • The 4th prototype must be more sterile	
Bryan	• Membrane test (Dialysis tubing within a 50ml centrifuge tube) • Prototype for transdermal patches • In contrast to the 3rd prototype, the bacteria used has a UV promoter and RFP as a reporter • Two trials		• The 5th prototype must be more sterile • Set-up --> see Figure 1	
Andrew	• Temp + RBS 3 in 1			• Run gel for PCR

Photos

Thursday, April 16, 2015
12:30 PM

Figure 1: LB test set-up



Within the dialysis tube: Liquid culture of UV + RFP
Outside the dialysis tube: LB

Experiments

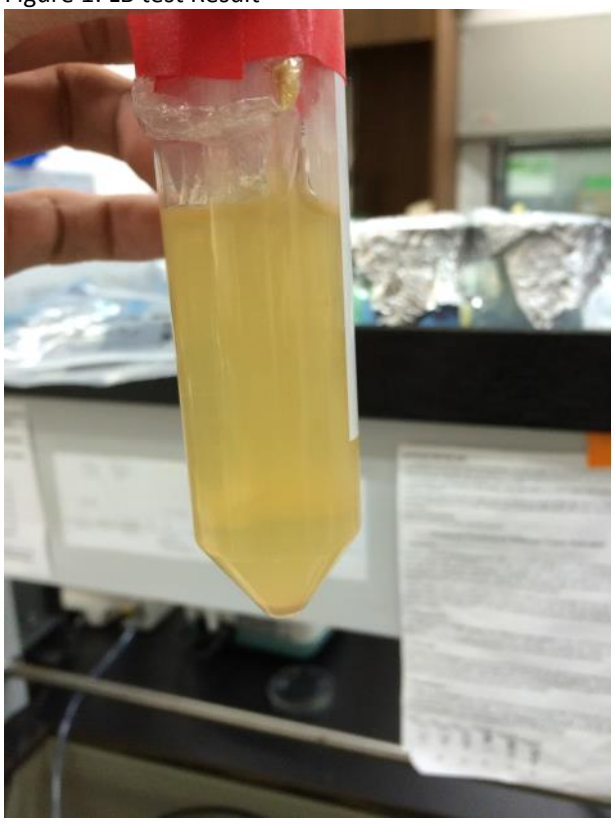
Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Evonne	• Collected the membrane test	• Both membrane tests had bacteria growing on the agar plate	• The testing was sterile but still had bacteria growing • Should revise the protocol and review mistakes that can occur during the experiment	4/23 Seek for further directions regarding membrane tests
Bryan	• Collected the membrane test	• Both membrane tests had bacteria growing outside the dialysis tube	• The testing was sterile but still had bacteria growing • Should revise the protocol and review mistakes that can occur during the experiment	4/23 Seek for further directions regarding membrane tests
Andrew	• Ran gel for Temp + RBS PCR • Liquid culture of temp	• Band is incorrect	• Need to re-digest RBS	• Mini-prep temp and digestion of both temp and RBS

Photos

Tuesday, April 21, 2015
3:36 PM

Figure 1: LB test Result



Bacteria grew both inside and outside the dialysis tube

Figure 2: Plate test Result

Bacteria grew outside the tube

Experiments

Thursday, April 23, 2015
5:40 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Andrew	• Mini prep of Temp • Digestion of RBS at EX • Digestion of Temp at ES • Ran Gel for digestion			• Gel digestion + Ligation + Transformation of Temp + RBS

Guidelines

Friday, April 17, 2015
2:21 PM

For each separate experiment, keep adding rows.

Figures should be fully labelled on program of your choice (has to be able to add solid text, arrows, shapes, and rotate those), saved as a jpg file, and uploaded to daily folders. Fully labelled = arrows identifying different parts, boxes pointing things out, text labelling plasmid sizes on gels, etc.

Group: who are involved in the experiments
Experiments: what did you do? include specific details like restriction sites, inserts, etc. note changes to protocols and should be specific to the point others can follow along what you did
Results: what were the results? what did you observe? what were some significant things to point out? if you say successful/unsuccessful explain what makes it so
Conclusions/notes: what do your results mean? propose a reason why the results turned out the way it did
Next steps: what are you going to do next and why?

2015年7月24日
下午 04:41

Summary

Thursday, April 23, 2015
4:18 PM

Temperature sensitive promoter
This week we focused on testing for the activation temperature of the temperature sensitive promoter. We grew plates at temperatures from 4 degrees C to 42 degrees C. We found that the promoter is activated at 37 degrees C. We could further this experiment by looking at the amount of production of GFP.

Mutagenesis
This week we finished the first point mutation of ACT. We completed PCR and miniprep for this and are ready to send it to sequencing to check if the point mutation was successful.

New UV promoter
This week we got the UV promoter back from sequencing and found that the promoter provided by the iGEM headquarter was a constitutive promoter rather than one activated only under UV light. Therefore, that explains for why the bacteria with UV promoter + RFP construct always produced RFP whether it was under the light or not. The iGEM headquarter sent us another package with the new UV promoter; however, it included the constitutive promoter too. Therefore, we are planning to send it back to sequencing to ensure that we got the correct promoter.

Temperature sensitive promoter + RBS
Last week we confirmed that the temperature sensitive promoter works. So this week we are building the promoter + RBS construct.

Experiments

Friday, April 17, 2015
3:21 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Testing temperature sensitive promoter	Joseph + Monica	Growing temperature test cultures - liquid and plates • 4*C, Room temp, 30*C, 37*C, 42*C			To check fluorescence for promoter expression
Mutagenesis	Leon	1. Transform ACT Muta 1 Trial 2 long protocol 5 min ice 30 second 42c 5 min ice 1000uL LB 60 min 37c 250 rpm plate all 2. LB Agar plate test prepared 100mL samples 6 groups: 1. 15 g/L 2. 17 g/L 3. 20 g/L 4. 22 g/L 5. 25 g/L 6. 30 g/L 3. grew liquid cultures for temp sens promoter 5mL LB + 5uL amp	1. Transform ACT Muta 1 Trial 2 - FAIL plates were inverted to incubate right side up no single colonies on plate 2. LB Agar plate test autoclaved and poured into large plates viscosity is obvious 3. temp sens promoter cultures all grew	1. salvage adding 500uL lb to lid and plate shake it around to pick up some bacteria re-plate to attempt to salvage single colonies 2. unsure yet, going to have to actually streak or transform some stuff will update in future 3. cultures still grow and resistance is correct	1. salvage tomorrow 2. testing in future updates in later days 3. miniprep for sequencing and keeping

Pictures

Thursday, April 23, 2015
8:22 AM

Experiments

Tuesday, April 21, 2015
3:34 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Mutagenesis	Phillip	Started reattempt at PCR for mutagenesis of PstI sites. This is our 3rd attempt for the first mutagenesis of the PstI site. The first attempt has turned out to be unsuccessful. I do not have any good ideas of why that is the case. We have made certain revisions to the PCR procedure and hopefully this will work. I also transformed ACT-A and ACT-B samples so that we may have more plasmid later on to purify	Will update tomorrow Plates: PCR: Need gel check tomorrow	Only time can tell.	Digestion and then transform at the end of the day. Hopefully, I will also be able to finish plasmid purification to have more ACT plasmids.
UV promoter	Paul and Dylan(Dylan is absent)	Today Paul helped get the new UV promoter and the constitutive promoter from the mail office. Afterwards Paul took the new promoters and had them streaked onto Ampicillin LB agar plates using inoculated loops (UV sterilized)	Paul has successfully streaked the promoters onto the plates, and Paul has also successfully retrieved the packages from the mail office.	Successful streaking of the plates, however the content of the mail package from the iGEM headquarters is slightly questionable because the iGEM headquarters sent wrong promoters along with the correct promoters. Therefore we have decided that we would have the promoters sequenced to make sure that they are of the correct kind. Just a small note the promoter that we are trying to test to see if it works in our potential construct is the UV promoter and is not the constitutive promoter that the IGEM headquarters keeps on sending to us.	Paul will come in tomorrow to come retrieve the plates that he streaked out today and he will put the plates into the refrigerator that is located under the lab bench. Afterwards the promoters will be shipped off for sequencing by a third-party contractor.
Testing temperature sensitive promoter	Daphne and Jon	Helped to streak temperature sensitive promoter. We labeled each plate with different temperatures and set three incubators according to those temperatures: 32 C, 34 C, 35C.	Successfully streaked the promoter onto the plates and placed into the incubators.	Results will be out tomorrow	Joseph will take over from now and check the results of whether if it glows or not tomorrow.
Mutagenesis	Leon	1. Checked on ACT Muta 1 Trial 2 transformation 2. setup hotplate temperature gradient incubation 3. autoclave + pour LB Agar plates 4. salvaged act Muta 1 trial 2 plates	1. ACT Muta 1 Trial 2 initially failed, waiting on salvage 2. seems to work, might use this setup for temperature gradient, unsure 3. plates are good, ready in fridge	1. not sure if m1t2 should be restreak salvaged 2. picture of set up in **(Figure 3)** 3. test on ACT stuff 4/22 , 4/23 4. this is a very sketch method added 500ul of lb to lid shook it a little poured into a new plate repeat	1. ACT Muta 1 Trial 2 is done 2. further tests 3. streak stuff to test plates 4. liquid culture + miniprep + sequencing?
Testing temperature sensitive promoter	Joseph Monica	1. Checked cultures for temp sense + GFP from yesterday 2. Measured population of liquid cultures with spectrovis	1. Cells did not grow at 4 degrees and RT 2. Cells grew at 30, 37 and 42 degrees **(Figure 1)** 3. Cells glowed at 37, 42 degrees **(Figure 2)** 4. Still doing spectroanalysis	1. Promoter activated somewhere between 30-37 degrees 2. Note: temperature was hard to maintain in black incubator (30 degrees)	1. New plates - from 34, 35 and 32 degrees to pinpoint temperature that promoters are activated at 2. Repeat procedure - may use data for modeling
Temp + RBS construct	Andrew	• Did gel digestion + ligation of Temp+RBS • Transformed the Temp RBS • Heat deactivated and saved ligation mix just in case			• Checking plates next day and 3 in1

Pictures

Wednesday, April 22, 2015
4:56 PM

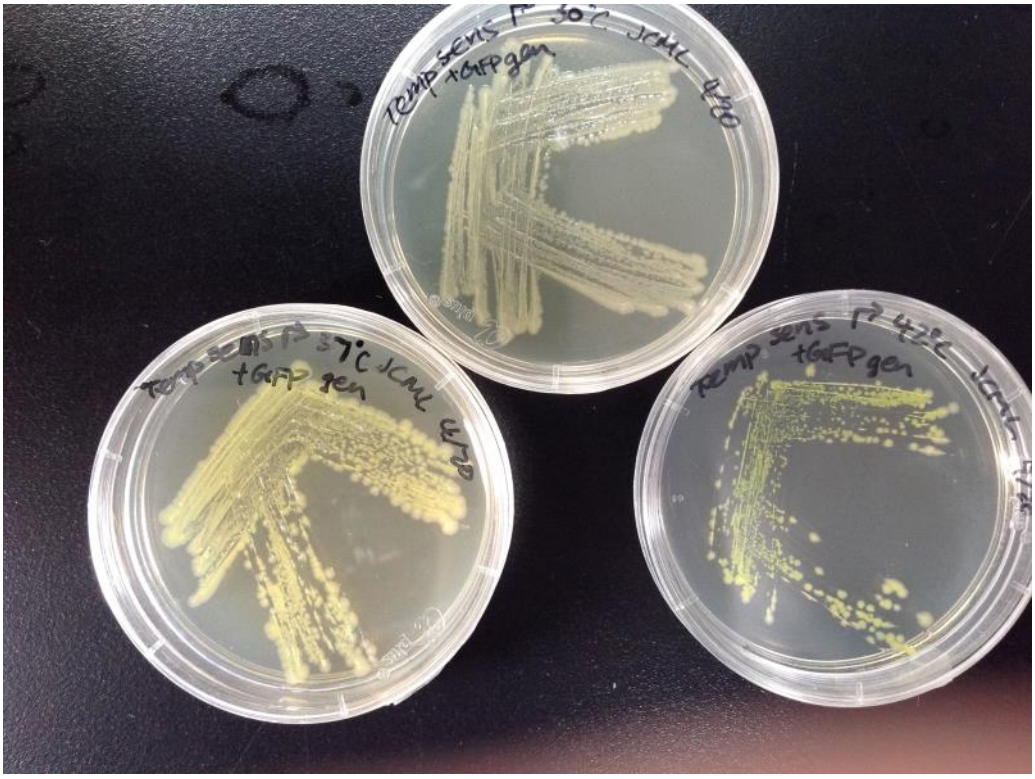


Figure 1. Bacteria carrying GFP generator driven by a temperature-sensitive promoter grow at 30, 36, and 42°C.

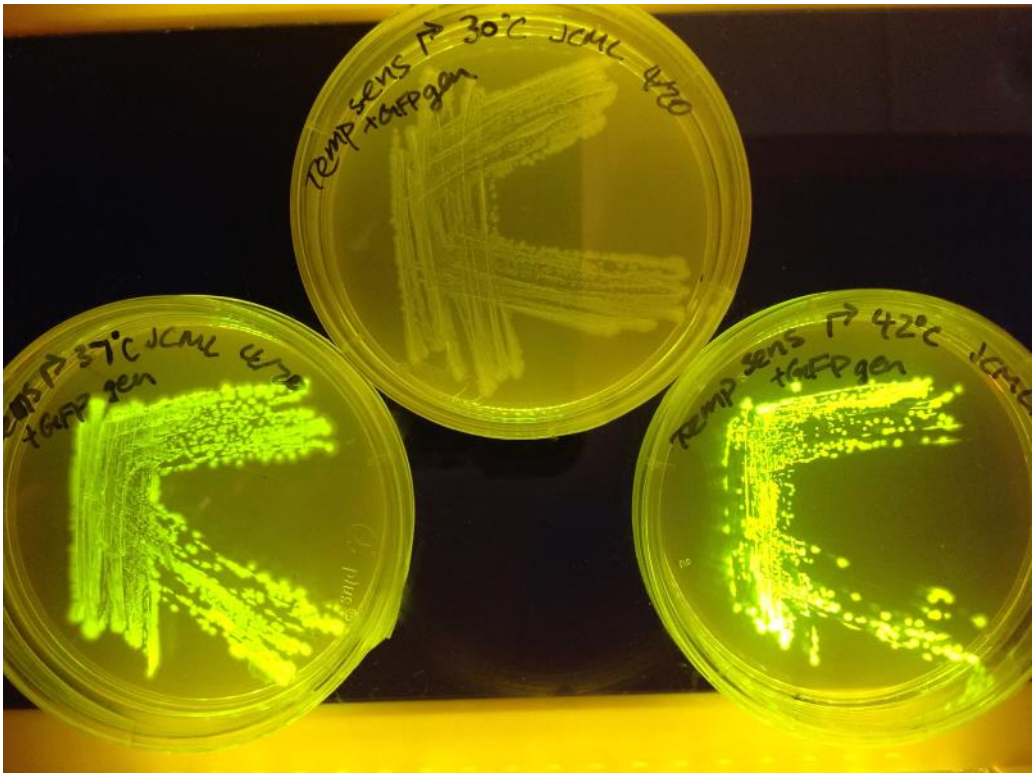


Figure 2. GFP generator is expressed at temperatures above 36°C.

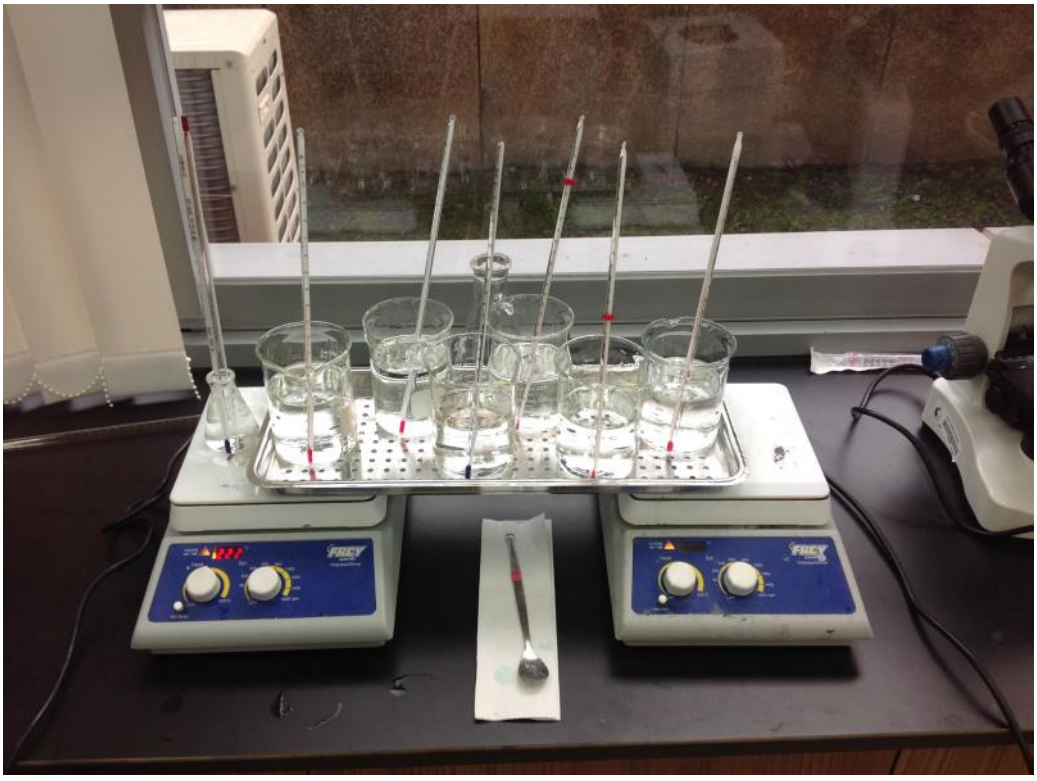


Figure 3. hotplate setup for multi temperature water bath incubation

Dialysis Tubing Tests

Tuesday, April 21, 2015
2:28 PM

Evonne and Bryan

1st membrane test (Plate):

March 24th, 2015

- Membrane test (Dialysis tubing on top of an agar plate)

March 25th, 2015 (PD Day)

- Collected the membrane test
    - One leaked bacteria (not sterile enough)
    - One didn't leak

2nd membrane test (LB)

March 25th, 2015 (PD Day)

- Made the liquid culture membrane test
    - Dialysis tube inserted inside a 50 ml centrifuge tube

March 26th, 2015 (PD Day)

- Pickup liquid culture membrane test

3rd membrane test (Plate)

April 7th, 2015

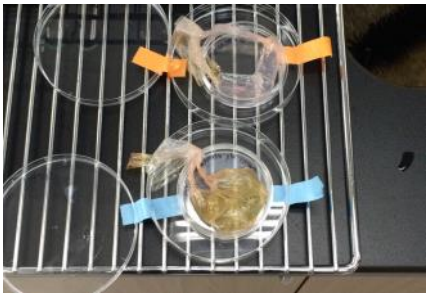
- Liquid Culture

April 9th, 2015

- Set-up membrane test for liquid culture

April 10th, 2015

- Both membrane tests dried out, one leaked



4th membrane test (Plate and LB)

April 13th, 2015

- Liquid culture (RFP + UV) for new dialysis tube testing
- Evonne - plate
- Me - liquid testing
    - More preferable because greater surface area

LB testing setup:
20ml Liquid culture in dialysis tube
15ml LB outside the dialysis tube

- If any bacteria leaks it's contaminated

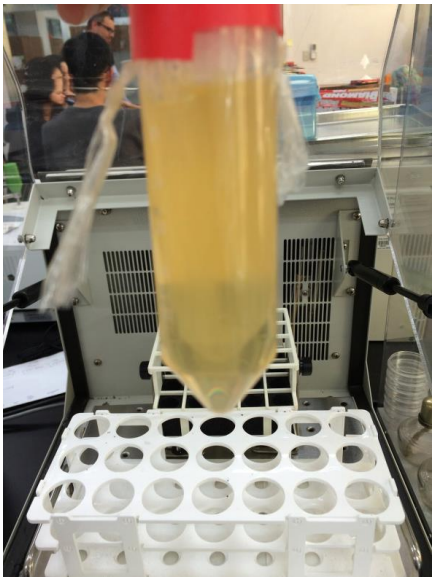
April 14th, 2015

- Pickup liquid cultures

April 15th, 2015

- Set-up membrane test

LB test:



April 16th, 2015

LB test:

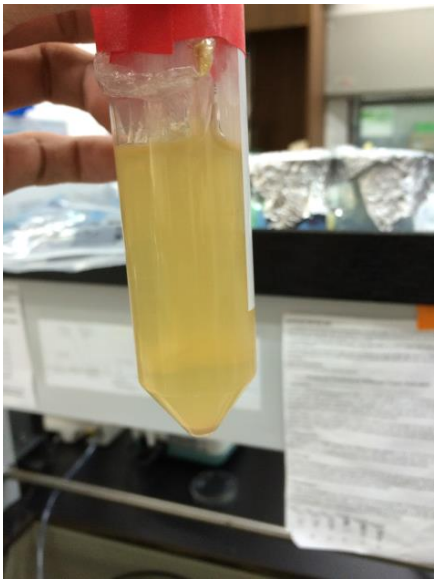


Plate:

Dr. Chiang said experiment failed.
Just because the plates showed red color does not mean that proteins passed through dialysis tube and bacteria didn't
Need to think of new method/membrane

Experiments

Tuesday, April 21, 2015
4:02 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
	Phillip				
Testing temperature sensitive promoter	Joseph, Monica	Plates failed - temperatures were altered some time Doing reruns at 30, 32, 35 and 37			
Testing UV promoter	Daphne	Finished 4 tubes of liquid culture (with amp)on the 2 plates of new UV promoter that was streaked by Paul on 4/21 I gave the tubes to Joseph who will put them into 37 degrees incubator for me.	Oops I used out all the small gloves	Update tomorrow	Come back tomorrow to proceed!!!!!!!!
Temp + RBS construct	Andrew	• Plates got contaminated- Redo • Mini-preped Temp promoters to restock PP			• Retransform ligation mix of Temp + RBS
ACT and ACT m1t3	Leon	• plates were wet and perhaps contaminated (figure 3,4) • M1t3 looks fine (figure 5,6)	no single colonies, bacteria smeared	if plates are wet then single colonies won't form	culture + restreak

Pictures

Tuesday, April 21, 2015
4:03 PM

Figure 1: This is a gel check. Without ladder because I loaded the PCR Mastermix instead. Oops

Figure 2: Plates for UV promoter

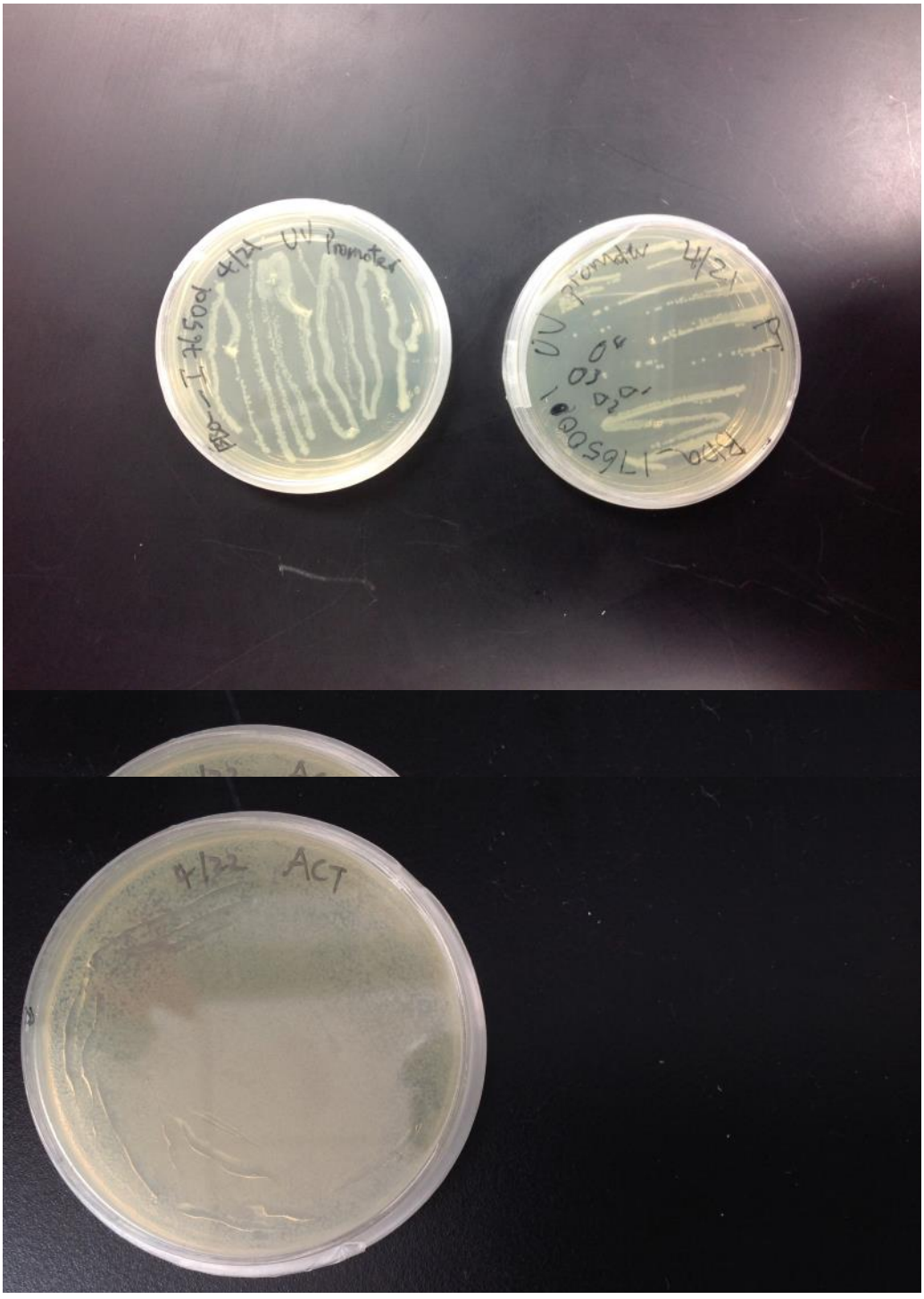


Figure 3 ACT transformation plate 1 smeared

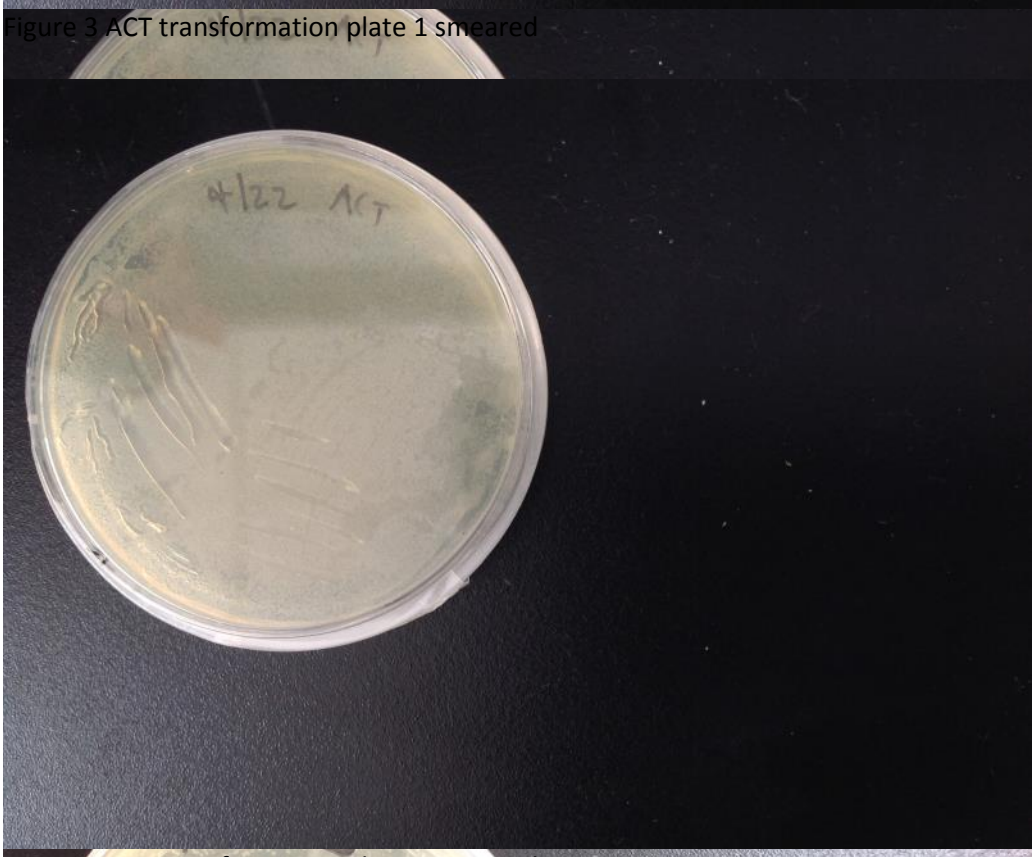


Figure 4 ACT transformation plate 2 smeared

Figure 5 ACT M1T3 B transformation: looks good

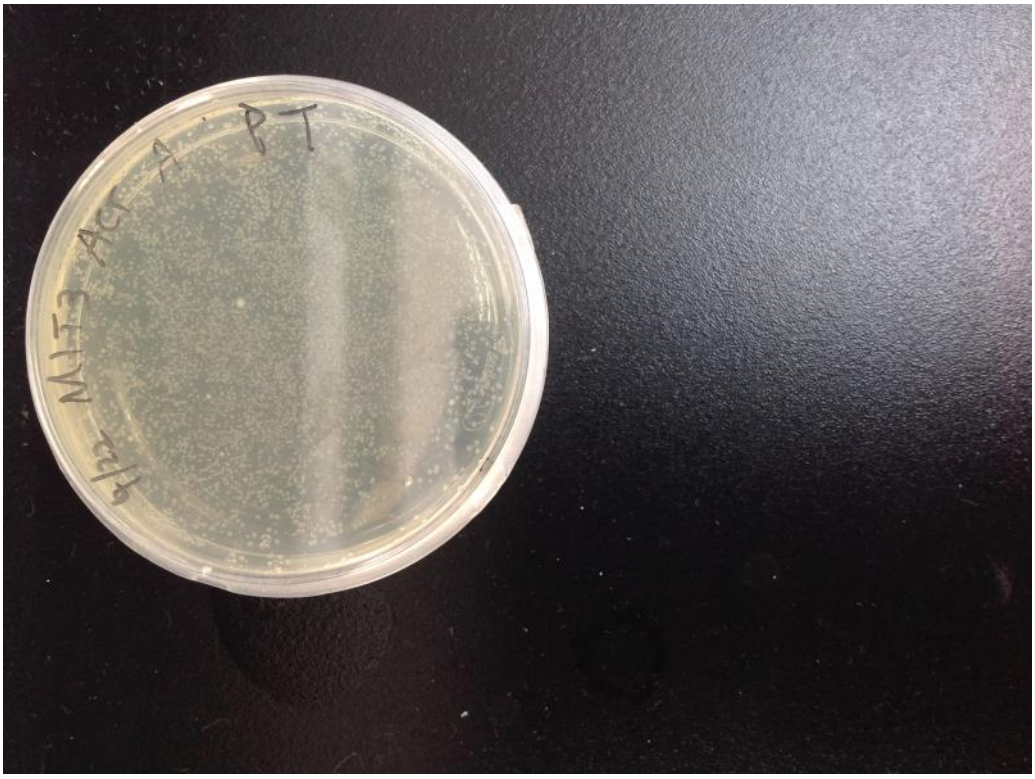


Figure 6 ACT M1T3 A transformation: looks good

Experiments

Wednesday, April 22, 2015
6:11 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Testing temperature sensitive promoter	Joseph + Monica	1. Checked results for temp sens promoter + GFP gen at 30, 32, 35, 37 degrees C **(Figure 1 and 2)** 2. Streaked new plates for temp sens promoter + GFP gen at 35 and 36 degrees C	1. Colonies grew in all plates; however, only glowed at 37 degrees C	• We think that the activation temperature is somewhere in between 35 and 37 degrees C	• Check results for plates at 35 and 36 degrees C
Mini-prep of Temp Sens Promoter	Andrew	Mini Prep Temp Sens Promoter for sequencing			
Testing of human body temperature for the temperature sensitive promoter	Paul and Dylan with Dylan being absent	Took Mr. Tsao's laser temperature scanner and used it to take measurements of the community.	I collected the results and compiled them onto an excel spreadsheet on my Google doc folder.	No additional notes apart from collecting the body temperatures of various people in the school community.	Next step is to analyze the data that has been put into the Google Doc spreadsheet
Testing the new UV promoter	Daphne and Jon	Finished miniprep of Philip's 3 liquid cultures. Jon did 3 tubes (with WI and water)and Daphne did 3 tubes of miniprep using regular protocol.	Daphne finished miniprep, Jon's tubes added elution instead of water so need to be redone tomorrow.		Daphne's tubes move on to digestion and Jon redo miniprep • Digestion for testing • Miniprep for sequencing
Temp + RBS	Andrew	• Transformed ligation mix from 4/21			• Checking plates next day
Temp promoter sequencing	Andrew	• Mini-prepped Temp promoter with sequencing protocol			• Sending PP for sequencing

ACT M1T3	Leon	• streaked 7 plates for A
		  • varying concentration agar
		  ○ 15, 17, 20, 22, 25, 30 g/L
		• streaked 7 plates for B
		  • varying concentration for agar
		  ○ 15, 17, 20, 22, 25, 30 g/L
		• streaked 4 extra plates
		• grew cultures for m1t3 A and m1t3 B
			• all grew didn't keep all due to spatial constraints (figure 3,4,5,6,7,8,9,10)	• 20 g/L, 22 g/L and 25 g/L are most easily streaked	• restreak/lb
					• lb cultures for act dna
					• sequence to make sure it's correct

ACT	Leon	• streaked 12 big plates + 2 small plates
		  • big plates varying agar conc.
		  ○ 15, 17, 20, 22, 25, 30 g/L
			• all plates grew very well
			  • kept most of the plates for a restreak
				• bacteria are happy and growing on amp plates
				• agar concentration does not inhibit growth
					• restreak single colonies

Pictures

Thursday, April 23, 2015
8:18 AM

Figure 1. Temperature sensitive promoter + GFP @ 30, 32, 35, and 37 degrees C

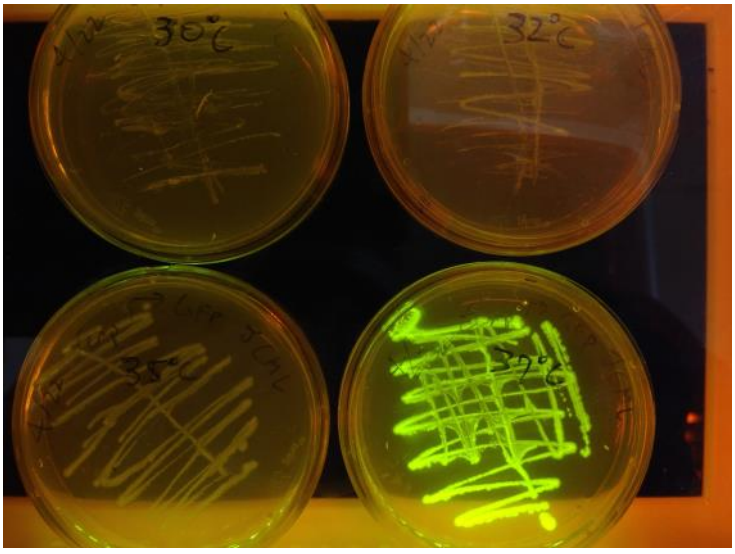
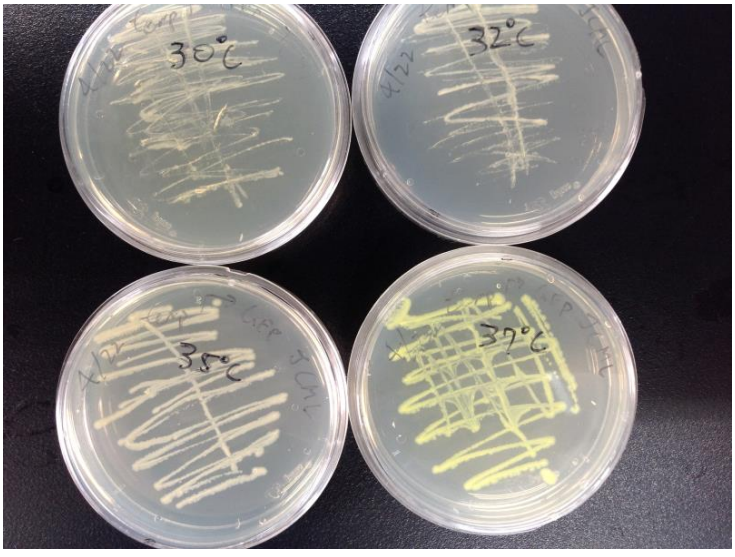


Figure 2. Temperature sensitive promoter + GFP @ 30, 32, 35, and 37 degrees C



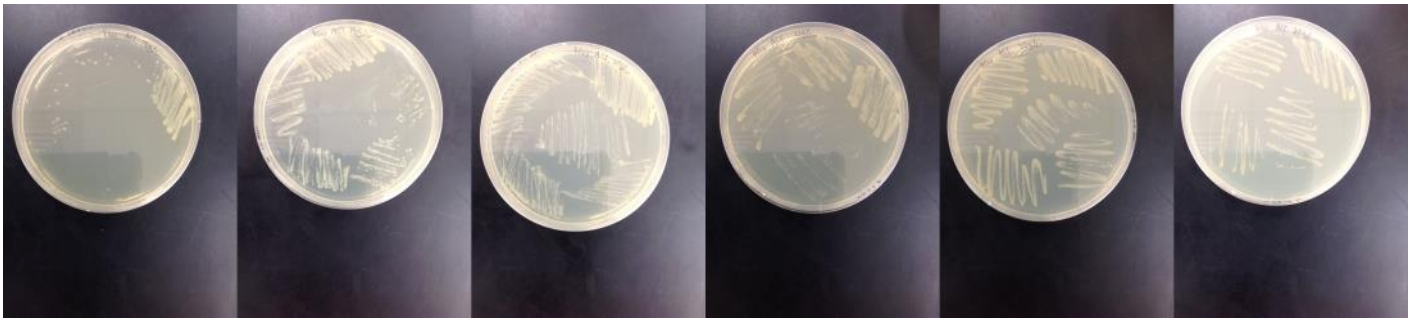


Figure 3 ACT plate 1 restreaks from left to right: 15g/L 17g/L 20g/L 22g/L 25g/L 30g/L

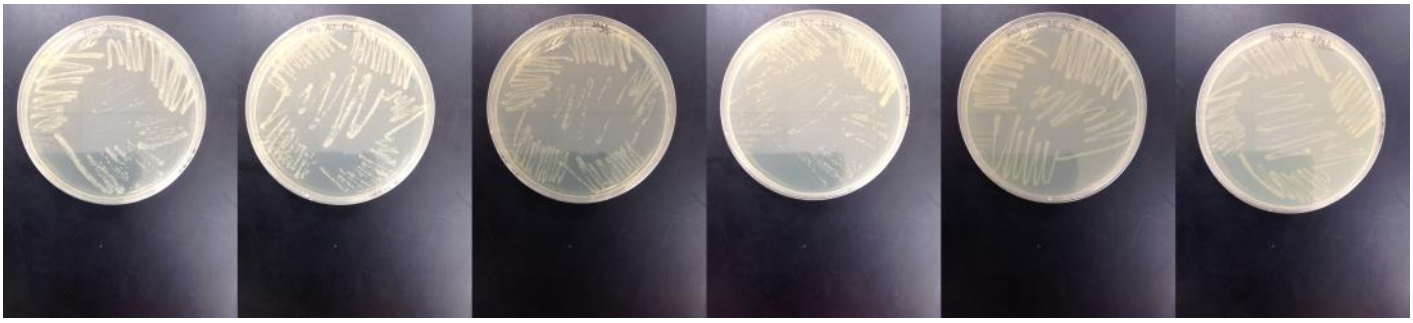


Figure 4 ACT plate 2 restreaks from left to right: 15g/L 17g/L 20g/L 22g/L 25g/L 30g/L

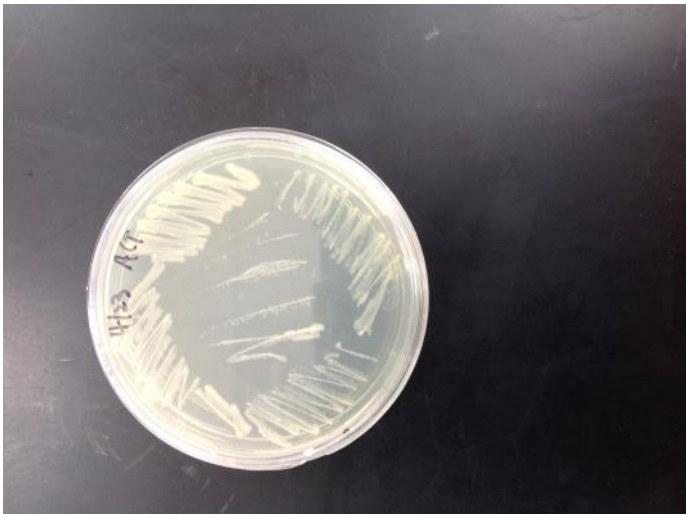


Figure 5 ACT plate 1 restreak

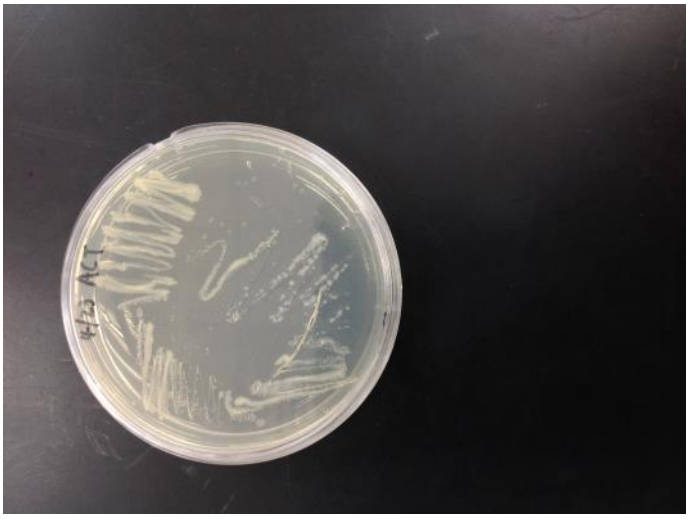


Figure 6 ACT plate 2 restreak

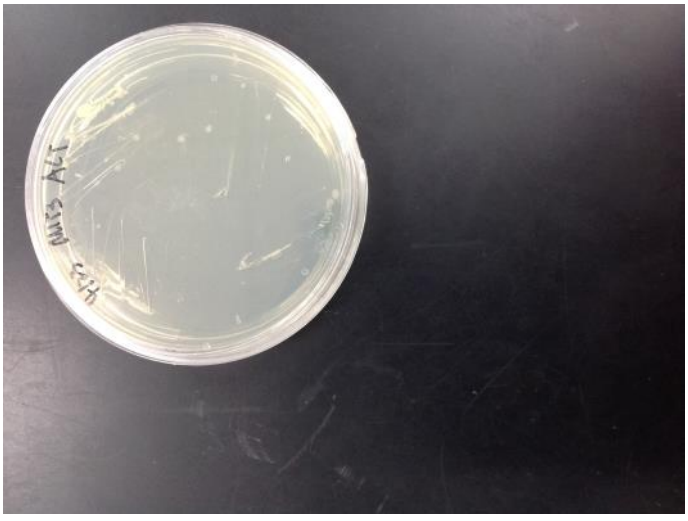


Figure 7 ACT plate 3 restreak

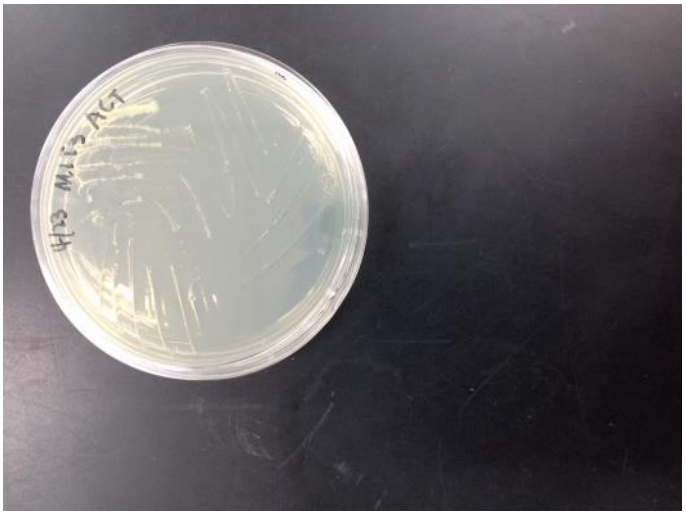


Figure 8 ACT plate 4 restreak

Figure 9 ACT M1T3 B restreak

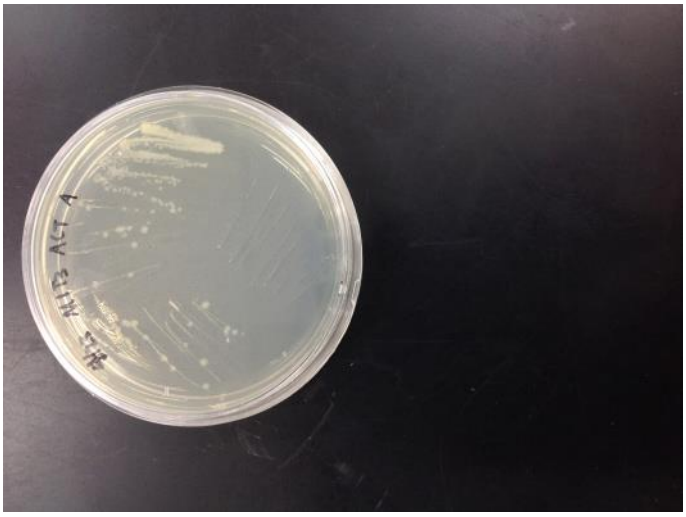


Figure 10 ACT M1T3 A restreak

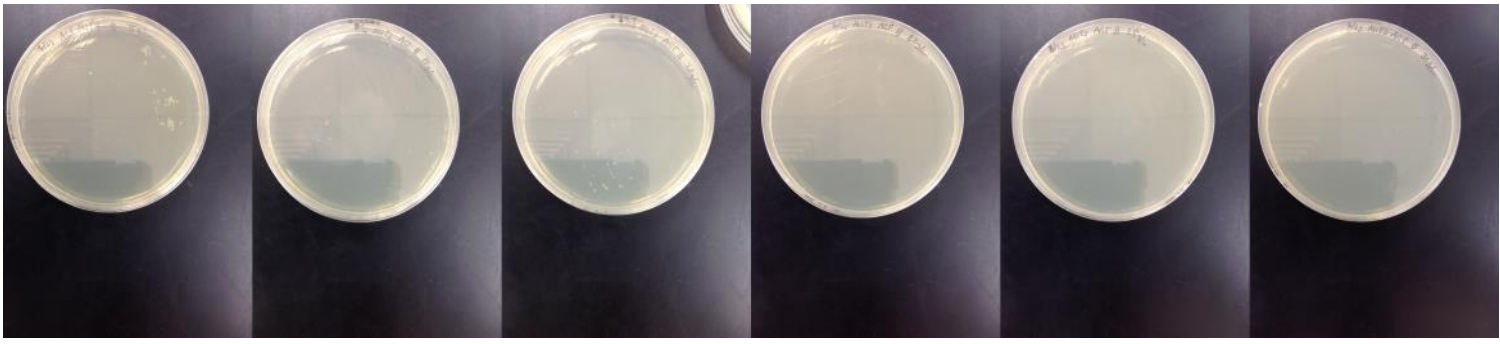


Figure 11 ACT M1T3 B restreaks varying concentrations

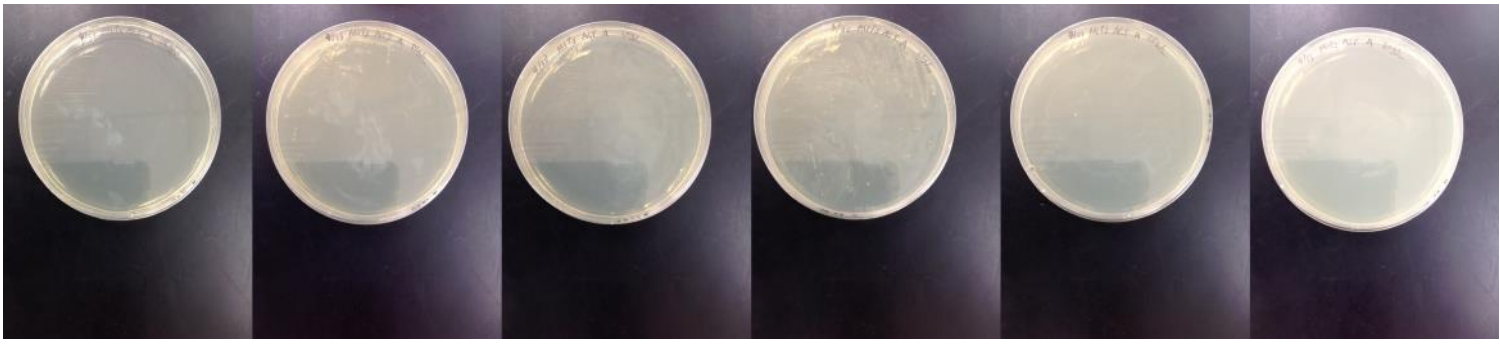


Figure 12 ACT M1T3 A restreaks varying concentrations

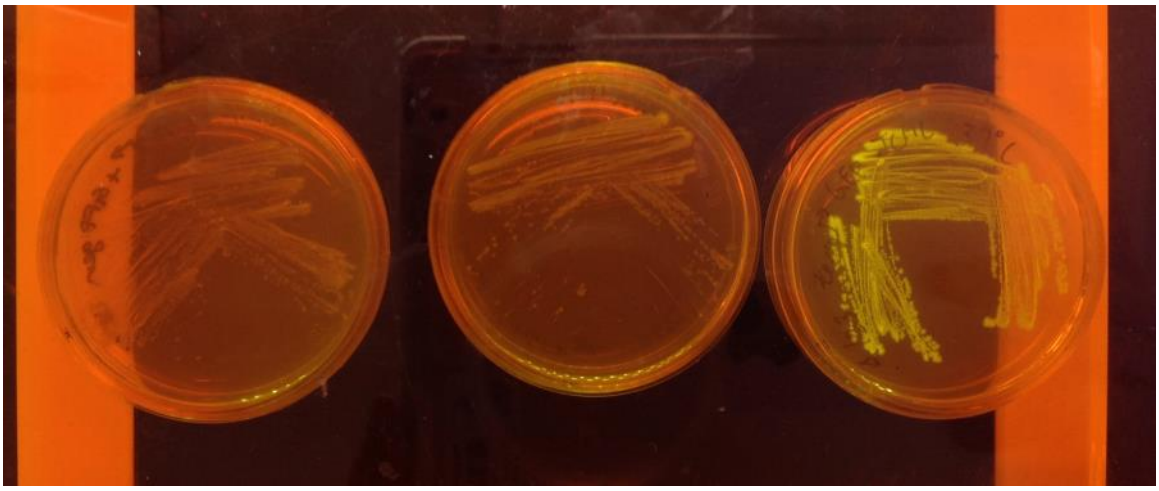
Experiments

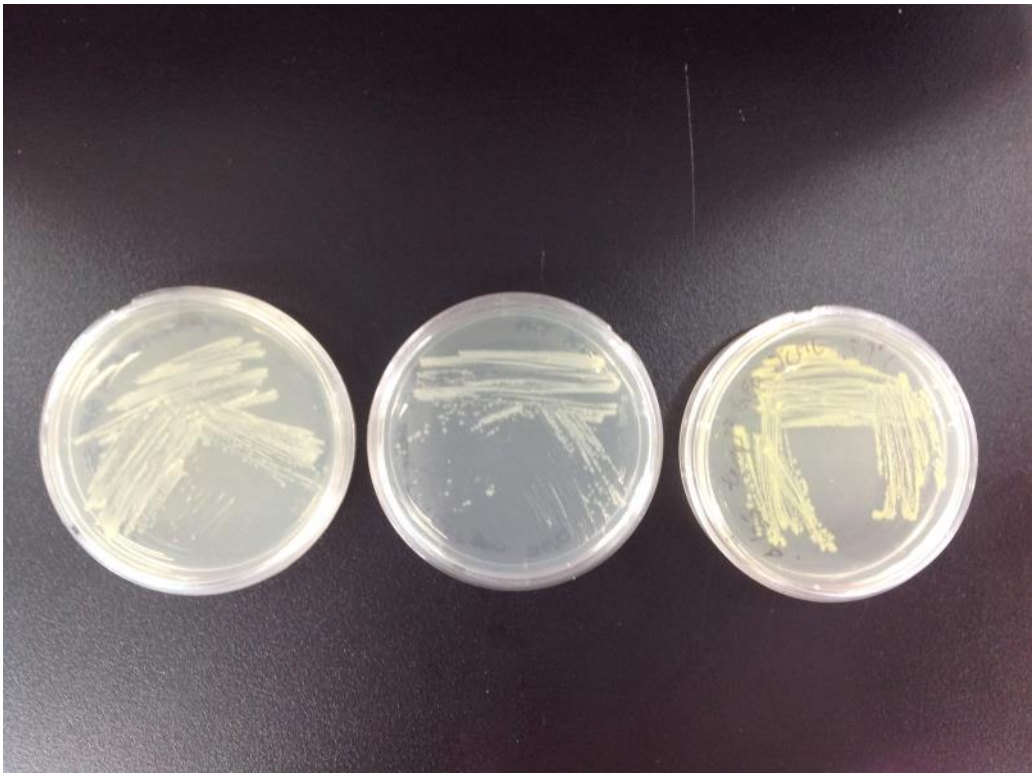
Wednesday, April 22, 2015
6:11 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Testing temperature sensitive promoter	Joseph + Monica	1. Checked temp sens GFP expression	1. See pics 1. Strong at 37, weak at 36 (?), barely at 35	• A gradient?	Either split it down further or try to get liquid culture spectro data
Mini-prep of Temp Sens Promoter	Andrew				
Testing of human body temperature for the temperature sensitive promoter	Paul and Dylan with Dylan being absent				
Testing the new UV promoter	Daphne and Jon				
Temp + RBS	Andrew	•			•
Temp promoter sequencing	Andrew	•			•
Miniprep ACT M1T3 for sequencing	Leon	• Minipreped completed	• 337.0 ng/ul • 471.4 ng/ul	Need sequencing results	Send it out on Monday/Tuesday for sequencing
ACT m1t3 plates and ACT plates	Leon	storage + photograph	storage is nice, bacteria still alive	ready for restreaks and cultures	restreak + culture then sequence

Pictures

Thursday, April 23, 2015
8:18 AM





Experiments

Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Bryan and Phillip	• 4 Liquid Culture tubes		• Preparing for the 3rd prototype	• 4/9 Collect the liquid culture and set-up the membrane tests
Andrew	• Started Temp + RBS experiment • Liquid cultured Temp, Already have RBS digested part(EX) • Re-streaked Temp + GFP at 32 and 37 degrees			• Mini-prep of Temp • Digestion of Temp • Check plates

Experiments

Thursday, April 23, 2015
5:15 PM

Group	Experiments	Results	Conclusions/Notes	Next steps

Andrew	• Mini-prep of Temp
	• Digestion of Temp (ES)
	• Ran gel for Temp
	• Checked plates that were grew on 4/7
	• Re-streaked Temp +GFP, incubated at 37 degrees
		• Plates both grew and both expressed	• New design of way to test Temp+ GFP
			  • Grow at 37 degrees since it grows better
			  • Place plates into different temperatures, RT, 32, 37, 42 degrees
			  • See which ones still glow when RT stops glowing
				• Place Temp+ GFP restreaks into RT, 32, 37, 42 degrees to run experiment next day
				• Ligation and Transformation of Temp + GFP

Experiments

Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Bryan and Phillip	• Redo plate membrane test • Prototype for transdermal patches • Two trials • Placing a dialysis tubing on top of an plate filled with LB instead of Agar plate		• Dialysis tubing mimics the transdermal patches as a semi-permeable membrane • Goal is to have a semi-permeable membrane that lets the protein through but holds the bacteria back from being delivered to the human body • This time as the 3rd prototype and has to be more sterile • Hopefully combine the benefits of both a plate test and a LB test	• 4/10 The membrane tests
Andrew	• Ligation of Temp and RBS • Transformation of Temp and RBS • Left restreaks into RT, 32, 37 and 42 Degrees, check if RT stops expressing every 4 hours	• RT did not stop expressing	• Keep checking RT plates	• Checking Transformation on 4/10 • Checking RT plate on 4/10

Experiments

Thursday, April 16, 2015
12:30 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Bryan	• Collected the membrane test	• Both membrane tests dried out • One leaked	• Never put LB open in incubator, it will dry out	• 4/13 Redo with plate test with agar rand liquid LB membrane test
Andrew	• Transformation of Temp RBS • Did liquid culture for Temp • Temp + GFP experiment	• Transformation of Temp RBS was plated on wrong resistance • RT still glows	• Redo the Temp RBS experiment • Keep Checking	• Mini-prep of Temp

Photos

Tuesday, April 21, 2015
3:15 PM

- Both membrane tests dried out, one leaked
- Figure 1: LB leakage

- Figure 2: Result for the two experiments

Top:

- LB leaked
- Exterior LB dried out in the incubator

Bottom:

- LB didn't leak
- Exterior LB dried out in the incubator

Experiment

Thursday, April 23, 2015
5:31 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Andrew	• Temp + RBS • Temp + GFP testing	• Did mini-prep for Temp • RT still glows	• Have to throw Temp + GFP plates since no one will be in over the weekends	• Digestion of Temp and hopefully ligation + transformation

Summary

Monday, May 04, 2015
8:03 AM

Temperature promoter + RBS
This week, we successfully constructed the temperature promoter + RBS plasmid and ran PCR for it. The results showed that the DNA were the right size.

UV promoter
We've been having trouble with the UV promoter. After testing the new promoters provided by the iGEM headquarter, we found that this promoter also had problems. When we ran gel for the UV promoter, we consistently found bands at 3000 bp instead of at 2000 bp as expected. We also discovered problems when we grew the bacteria on plates. We grew bacteria with the UV promoter on two plates, and one of the plates grew normally white the other plate turned red. We are not sure what is wrong with the promoter, so we have sent it to sequencing.

Temperature sensitive promoter
This week we have been able to conclude that the temperature sensitive promoter is activated at 37 degrees C. This means that when we design our delivery method, we need to consider adding external heat to activate the bacteria as the skin temperature is lower than 37 degree C.

M1T3
We have completed the mutation. However, after sending it to sequencing, we found that the mutation of ACT was unsuccessful. Thus, we are now seeking for an alternative way of accomplishing this point mutation.

M2T4

Prototype
This week we tested our prototype with 0.2 um blue membrane, syringe filter, white filter membrane (size..?), and bandage membrane (positive control). For each design, we did two trials. Even though for each design, one trial was contaminated, the other turned out to filter out bacteria successfully.

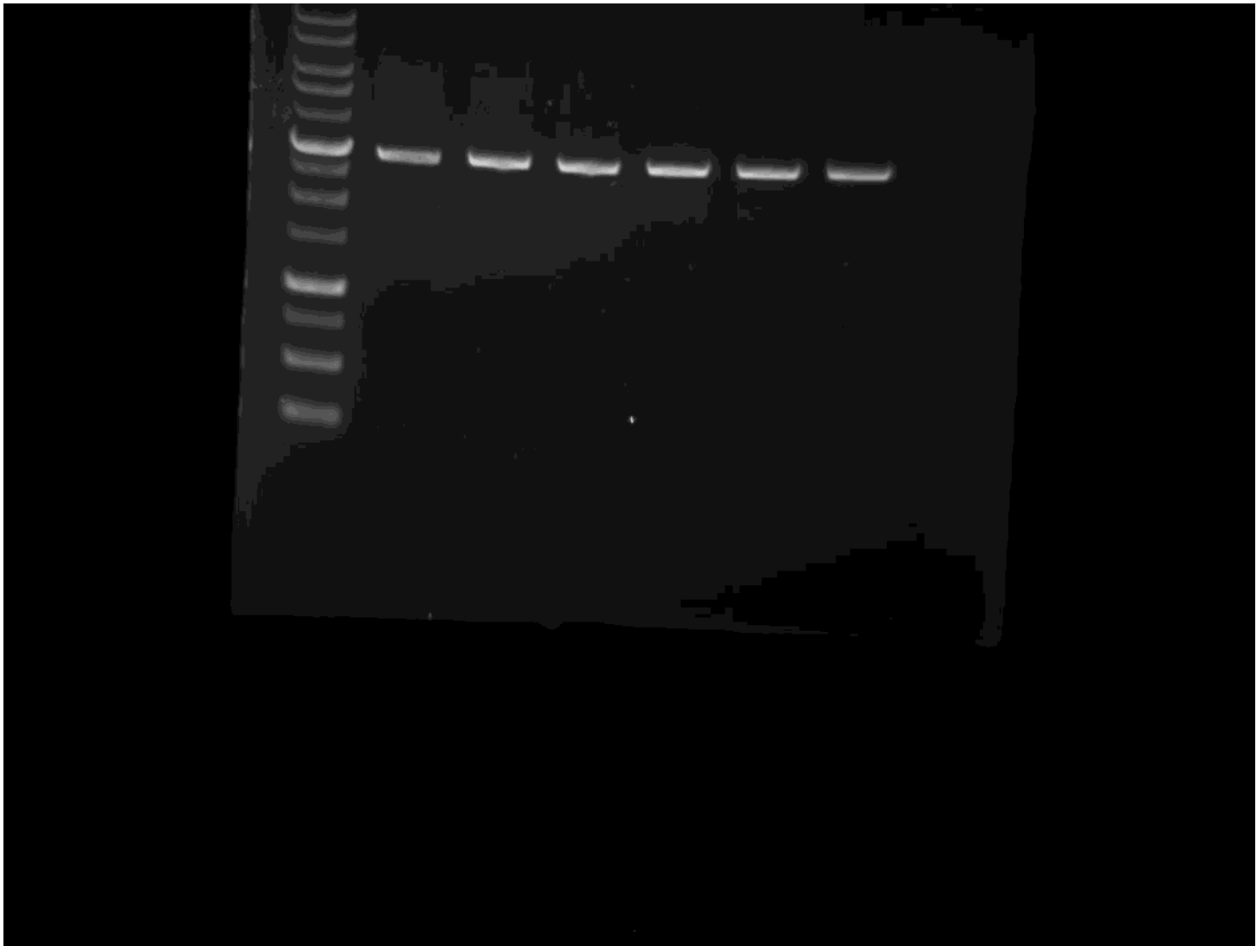
Experiments

Wednesday, April 22, 2015
6:11 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
UV Promoter	Jon	Liquid Culture for UV Promoter	Successful Liquid Culture and Placing it into the incubator	Wait for the result tomorrow	Mini Prep
Temperature Sensitive Promoter	Jon	Liquid Culture for Temperature Sensitive Promoter	Successful Liquid Culture and Placing it into the incubator	Wait for the result tomorrow	Mini Prep
Temperature Sensitive RBS Promoter	Jon	Liquid Culture for Temperature Sensitive RBS Promoter	Successful Liquid Culture and Placing it into the incubator	Wait for the result tomorrow	Mini Prep
ACT M1T3 A	Jon	Liquid Culture for ACT M1T3 A	Successful Liquid Culture and Placing it into the incubator	Wait for the result tomorrow	Mini Prep
ACT M1T3 B	Jon	Liquid Culture for ACT M1T3 B	Successful Liquid Culture and Placing it into the incubator	Wait for the result tomorrow	Mini Prep
UV Promoter (Back insert with GFP)	Daphne	Digestion for UV promoter, cut with S and P	Digestion and gel check completed but it came out the wrong size on the gel. The bands were at near 3,000 bps instead of the expected 2,100 bps of UV promoter.	Redo digestion again tomorrow and cut at E and P.	Digestion
Temp RBS construct	Andrew	Gel check Of Temp RBS PCR	Size is right		Liquid culture and miniprep
Temp RBS construct	Andrew	PCR 2 more colonies Restreaked all 3 colonies used for PCR			Do gel check and check restreaks
Temperature promoter	Paul and Dylan (Dylan was absent so it was Evonne who served as the recording partner)	Collecting body temperature data recordings from members of the TAS student and faculty body using Mr. Tsao's IR laser temperature sensor.	Successfully recorded the sufficient amount of data sets from the TAS public. We collected 20 data points from the female members of the TAS community while we collected 14 data points from the male members of the TAS public	Successful collection of the data points, now we will process the data points on the shared Google Doc spreadsheet with the rest of the team	Data processing and possible experimental work

Pictures

Thursday, April 23, 2015
8:18 AM



Experiments

Tuesday, April 28, 2015
2:39 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
ACT M2T3	**Leon**	**PCR ACT M2T3 Standard protocol**	**Notsure yet**	**None yet**	**Dpn1 + transform**
ACT M1T3	**Leon**	**Miniprep ACT M1T3 A1 A2 B1 B2**	**Good concentrations**	**Should sequence them next time**	**Sequence**
NEW UV Promoter testing	**Daphne+ Phillip**	**Digestion (at E and P) and gel check**	**The bands were still at 3 kb.** **Please refer to figure 1 under the pictures tab.**	**Restreak to send for sequencing.** **The results make it very confusing because we are not expecting the**	**miniprep**
		1.		**New**	

Pictures

Tuesday, April 28, 2015
3:12 PM

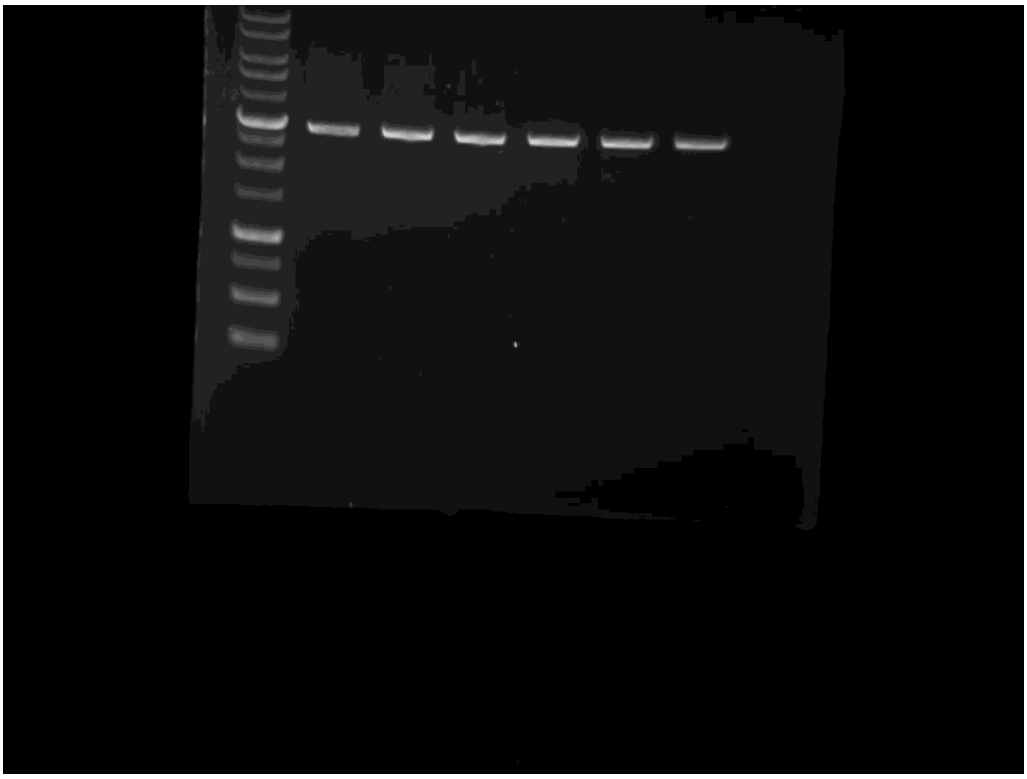


Figure #1: The gel check for the UV promoter cut at EP is shown here to be around a length of 3k.

Experiments

Tuesday, April 28, 2015
2:39 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
ACT M2T3	**Leon**	**Dpn1 + transform (figure 2)**	**Not sure yet**	**None yet**	
ACT M1T3	**Leon+ Phillip**	**Restreaked on to plates** **Grew cultures for plate a and plate b (figure 3,4)**	**Gonna check tomorrow**	**They can be found in the Mutagenesis box.** **They were sent for sent for sequencing but we didn't have enough concentration.** **We now learn that sequencing can take liquid culture and bacteria**	
ACT M2T4	Leon	Setup PCR for ACT M2T4 New PCR program test 5 cycles of reduced heat 30 cycles of normal heat Potential problems reduced heat by too much?	None yet	We'll see how we will edit the PCR program	Should definitely run gel and dpn1 + transform
NEW UV Promoter testing	**Daphne**	**Streak from** **MIT3 ACT B** **MIT3 ACT A** **Bba_1765001A** **BBA_1765001B** **Temp + RBS**			**Liquid culture**
Temperature promoter data processing and lab maintenance	**Paul**	**I refilled the pipette boxes that have been running low on pipettes, and I also looked at the data tables for the data collected during our field testing of people's body temperatures.**	**Boxes have been refilled and are going under the decontamination processes of autoclaving. I am also working on the data processing**	**Lab is now in a more desirable condition since the pipette boxes have now been refilled and I have started the data processing procedure for the body temperature community data points**	**Continue the data processing process and also see if the lab needs any maintenance or experimental work that needs to get worked on**
UV Promoter Testing	**Jonathan**	**Miniprep 5mL each.** **1 for sequencing and one for further experimentation**	**Concentration:** **For sequencing** **For further experimentation:**	**F9 Leon's Box you can find it :D** **Right next to it is**	
Andrew	Temperature Promoter+ RBS	Ran gel check for the PCR results for Temp Prom+RBS. Additionally, did miniprep for the Temp Prom+ RBS.			
Joseph, Monica	Temperature promoter + GFP Temperature promoter alone	Starting miniprep by liquid cultures			Follow up with miniprep

Pictures

Tuesday, April 28, 2015
3:12 PM

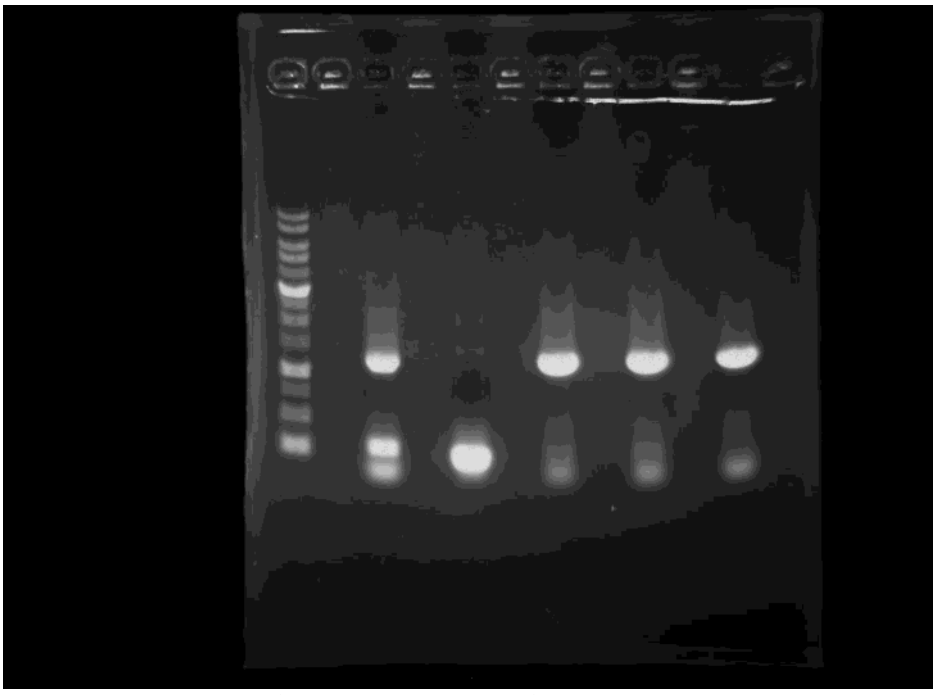


Fig. 1 this shows the gel check for Temperature+RBS result from the PCR.
This is the order of the gel starting from the left to the right. 1kb ladder, Positive control (pLac+GFP Gen), negative control (RBS vector), And everything else is Temperature Promoter + RBS.
Please refer to experiments to see conclusion

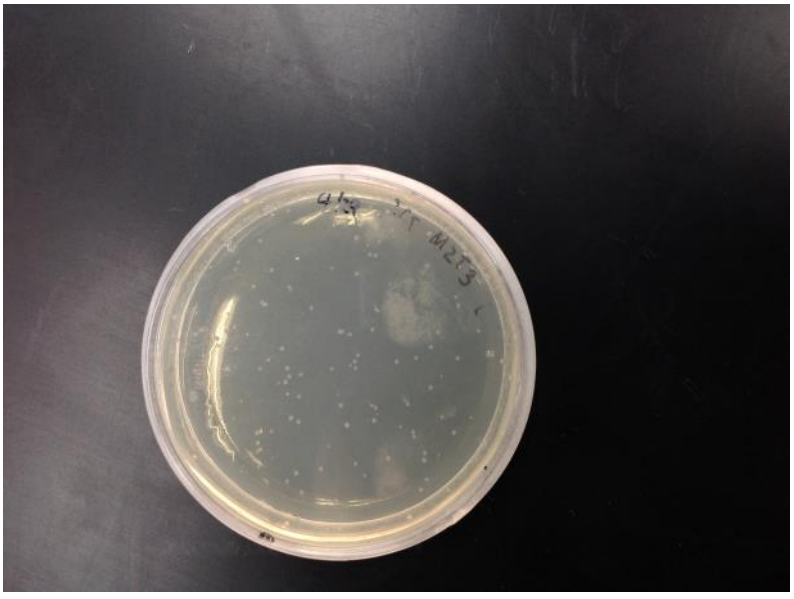


Figure 2 ACT M2T3 transformation needs to be sequenced for results

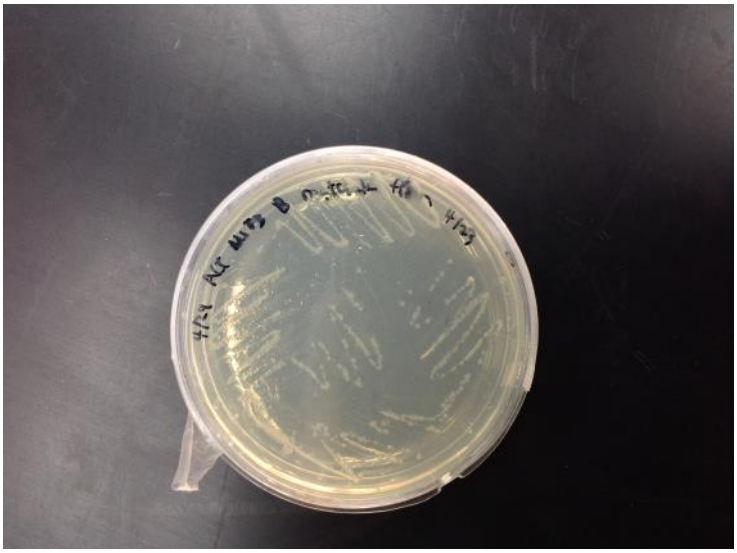


Figure 2 ACT M1T3 B restreak

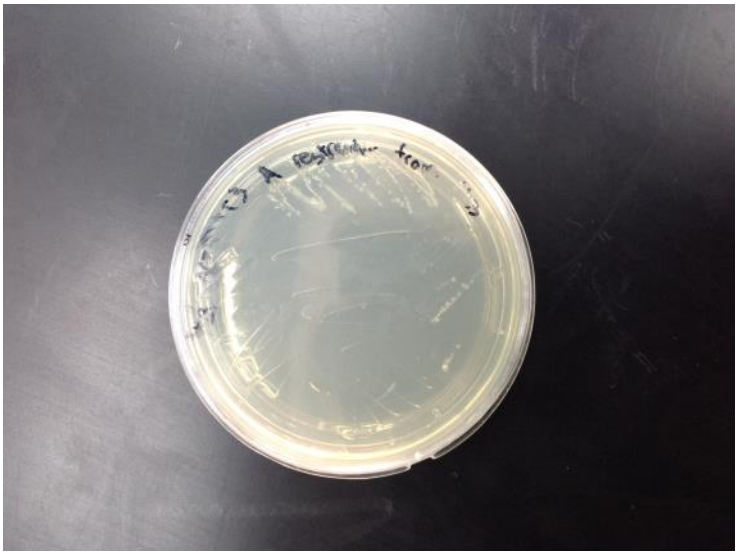


Figure 3 ACT M1T3 A restreak

Sequencing Protocol and Notes

Wednesday, April 29, 2015
2:49 PM

Any questions, ask Phillip

To get sequencing results

Experiments

Wednesday, April 29, 2015
2:46 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
M2T4	**Leon+ Phillip**	**Took the PCR product, digested then transformed**	**Both grew, negative control too**	**Can't use this stuff troubleshoot sent out for other companies to do for us**	
M1T3	**Leon**	**Miniprep + sequencing step**	**2 tubes at 400,500 ng/ul 4 tubes at 100~ ng/ul**	**Not sure if they are mutated**	**Wait for sequencing results**
I765001 UV	**Leon**	**3 in 1**	**One plate was red one was white labeled individually**	**Not sure why one is red and one is white (Figure 1,2)**	Wait for sequencing
ACT M1T3, ACT M1T2, ACT	Leon	Restreaks	Fun	They shall grow Figure 5,6,7,8, 9 Figure 10,11 Figure 12	Save em'
Miniprep	Jo and Monica	Miniprep from last time	More fun		Awaiting further orders from central *What are the concentrations? -central* Pretty low - will try re-miniprepping
UV promoter	Monica	Miniprep 2 tubes of 5 mL UV promoter + RPF (white) and 1 tube of 5 mL UV promoter + RPF (red)	White = 100.1 ng/ul Red = 495.1 ng/ul		Send to sequencing

Pictures

Wednesday, April 29, 2015
3:24 PM

Figure 1 I765001 white

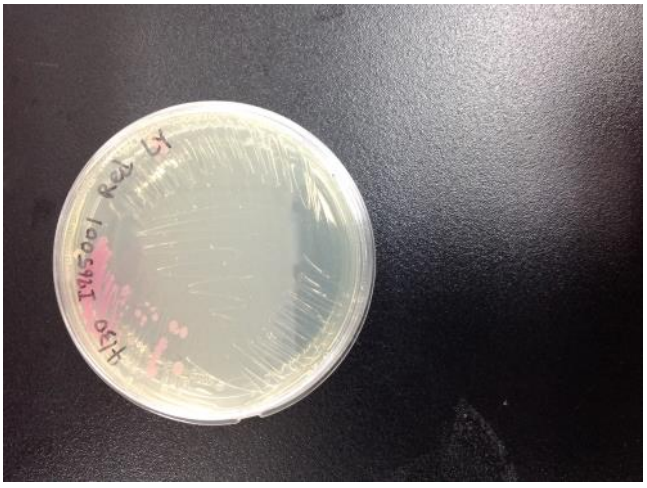


Figure 2 I765001 Red

Figure 3 ACT M2T4

Figure 4 ACT M2T4 Negative (failed)

Figure 5,6,7,8 left to right: ACT M2T3 A, B, C, D

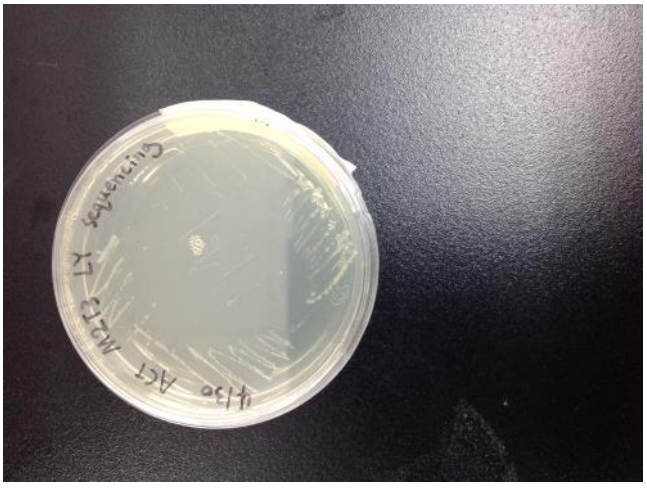


Figure 9 ACT M2T3 sequencing restreak

Figure 10 ACT M1T2 restreak from transformation salvage plate 1

Figure 11 ACT M1T2 restreak from transformation salvage plate 2

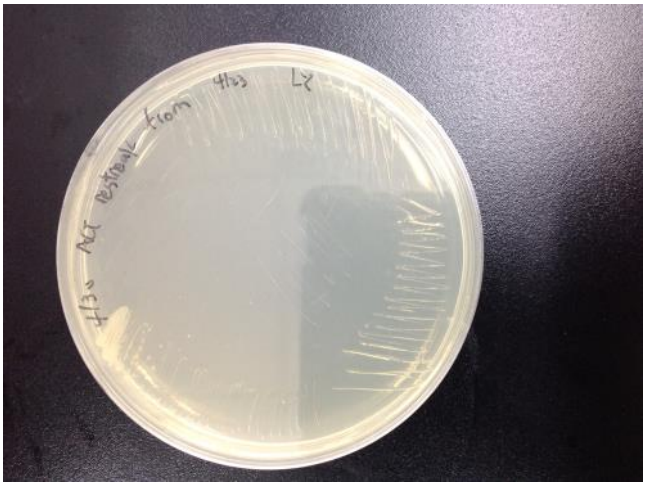


Figure 12 ACT restreak from restreak (needs one more restreak)

Experiments

Friday, May 01, 2015
7:23 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Mutagenesis	**Leon + Phillip**	**Called people**	**8000nt to mutate ACT**	**We're gonna do it**	**Let's do it**
Organization	**Leon**	**Cleaned out fridge plates**	**Very clean**	**Dr. Chiang approved**	**Throw out all the plates that are being neutralized as I speak/type**

Experiment

Thursday, May 07, 2015
3:15 PM

Prototype	**Evonne and Byran**	**1. New blue membrane 2. Syringe filter 3. White filter membrane 4. Bandage membrane (positive control)**	**1. Successful! Colonies grew on the top LB+agar plate and corresponding side of the membrane, but none passed through the membrane so the bottom LB+agar plate had no grown colonies. However, the other plate had two red RFP colonies, indicating contamination and sterilization techniques. There were no GFP colonies though. 2. Syringe filter had two plates: 1 failed while 1 succeeded. The positive control was full of colonies while the failed plate had several colonies. The other plate had no grown colonies 3. Both worked and had no colonies. 4. Both showed positive control and had colonies growing on the bottom LB+agar plates**

Pictures

Thursday, May 07, 2015
3:16 PM

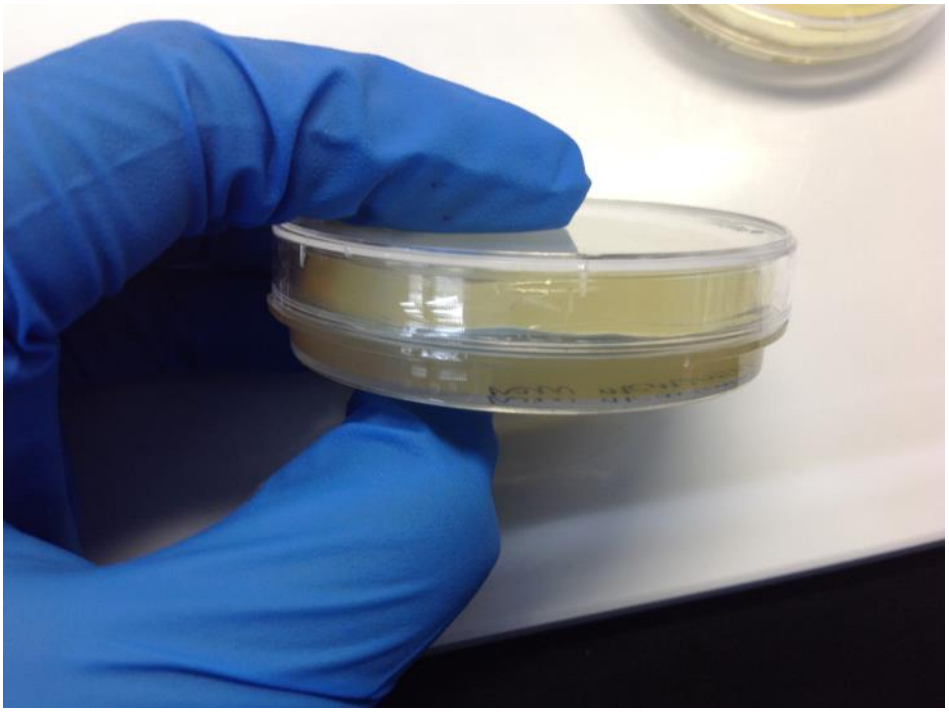


Figure 1: Set up for Prototype

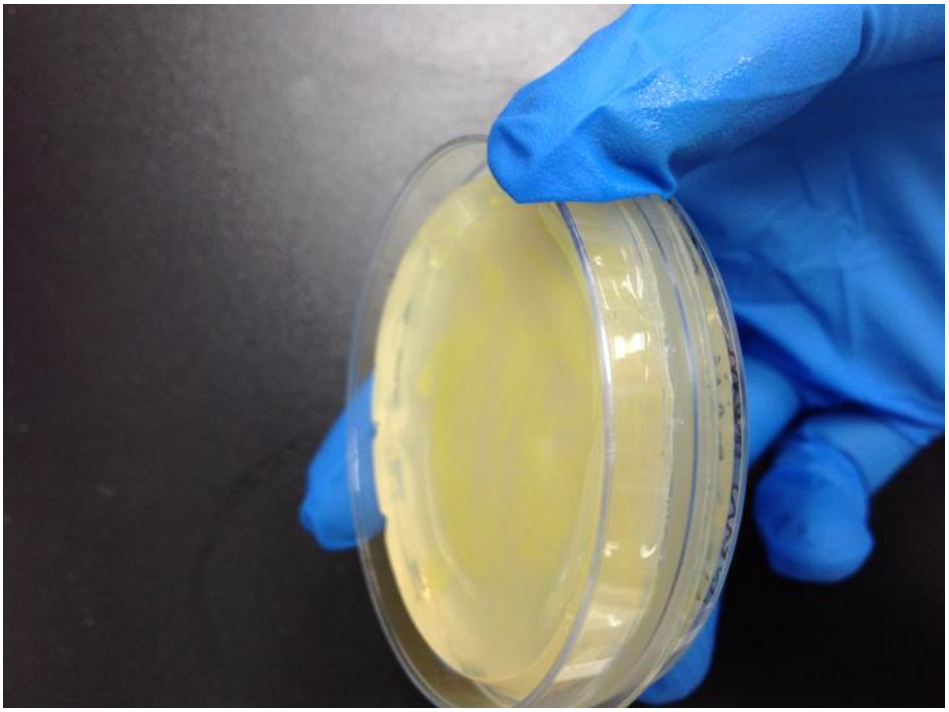


Figure #2: Set up for prototype (with membrane between 2 LB agar plates)

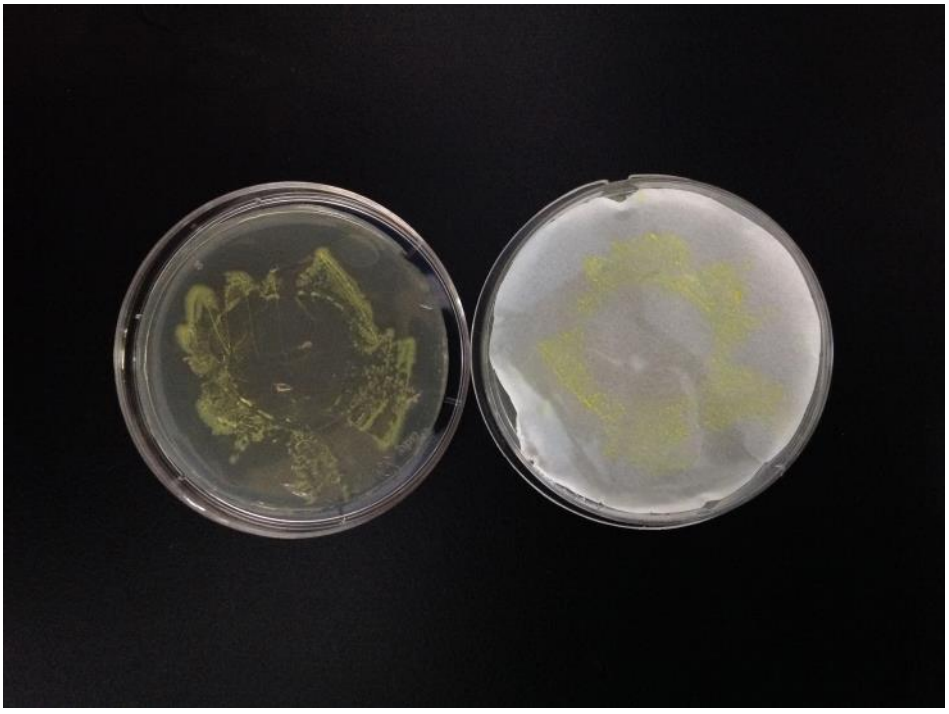


Figure #3: Set up for Prototype with white filter membrane (before sandwiching top LB agar plate over membrane)

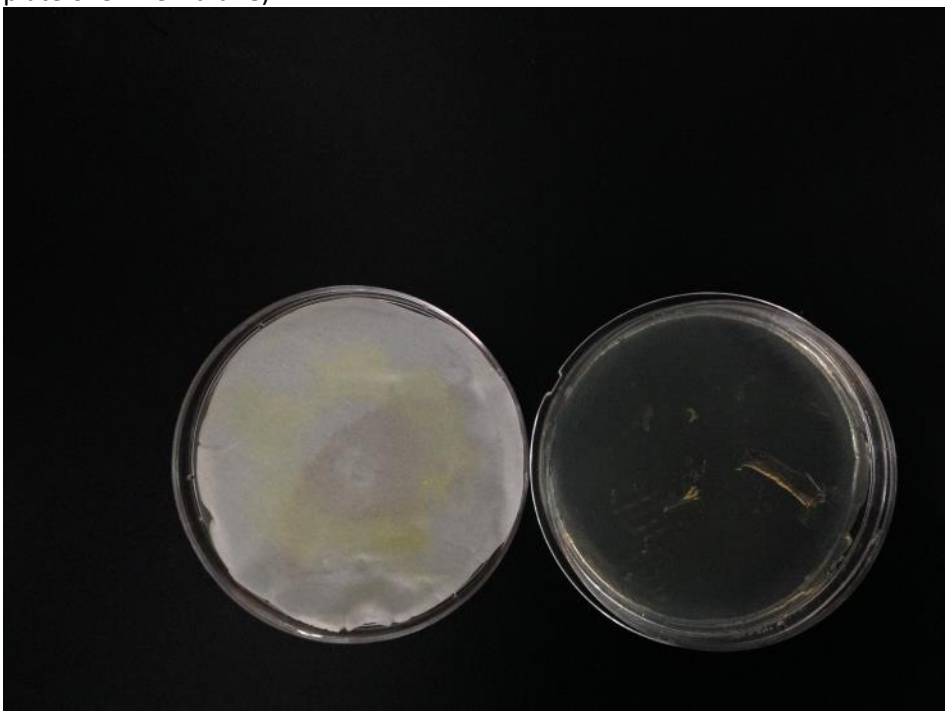
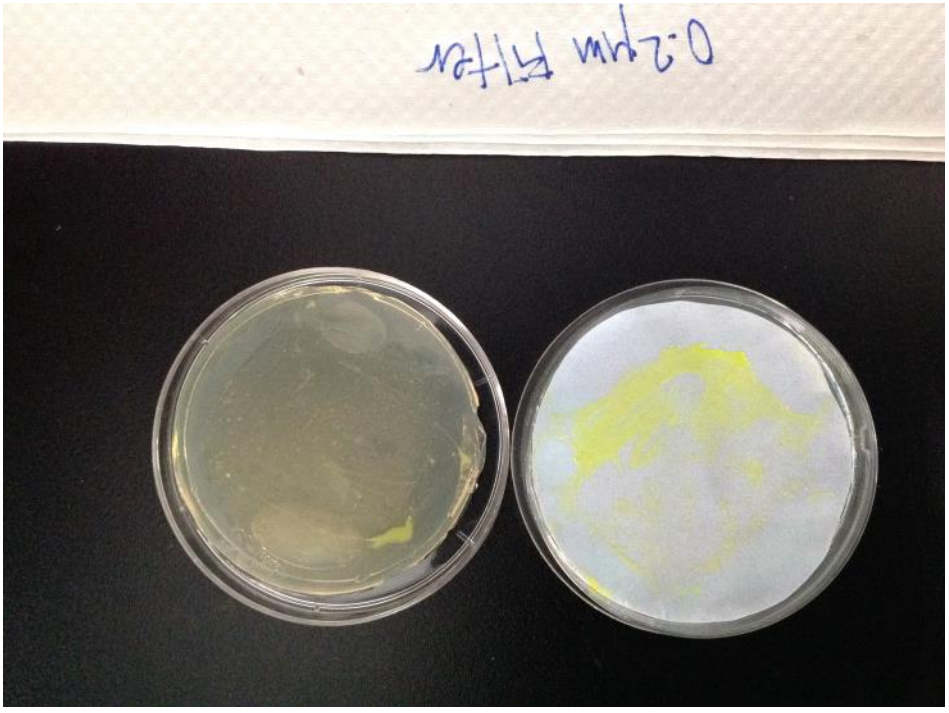


Figure #4: Set up for Prototype with white filter membrane (before sandwiching top LB agar plate over membrane)- 2nd plate



? Figure #5: Set up for new 0.2 micrometer blue membrane (1st plate)

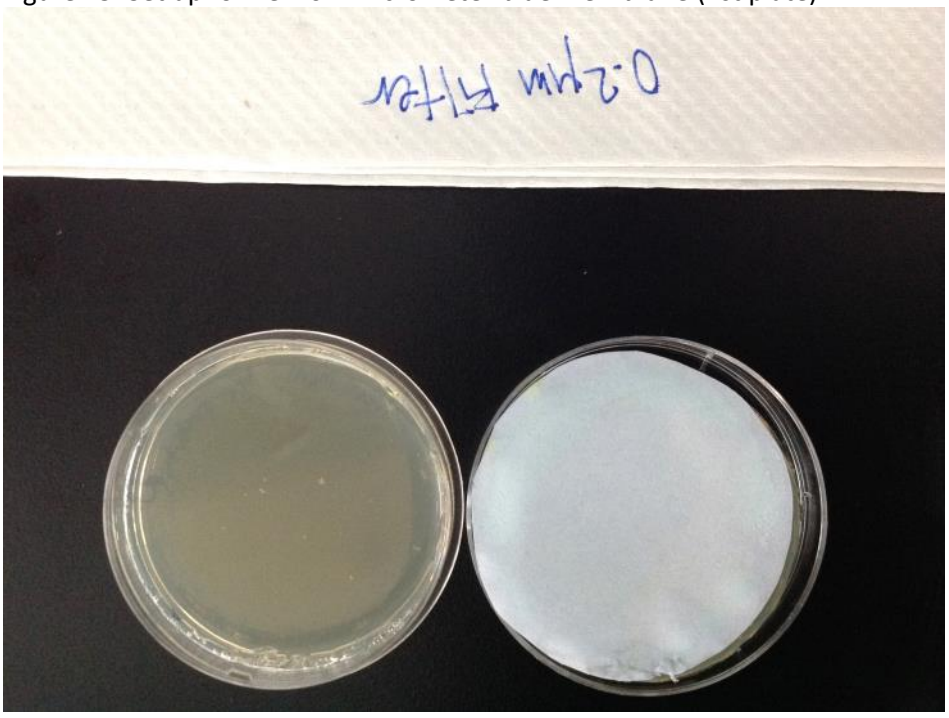


Figure #6: Set up for new 0.2 micrometer blue membrane (showing bottom LB agar plate)

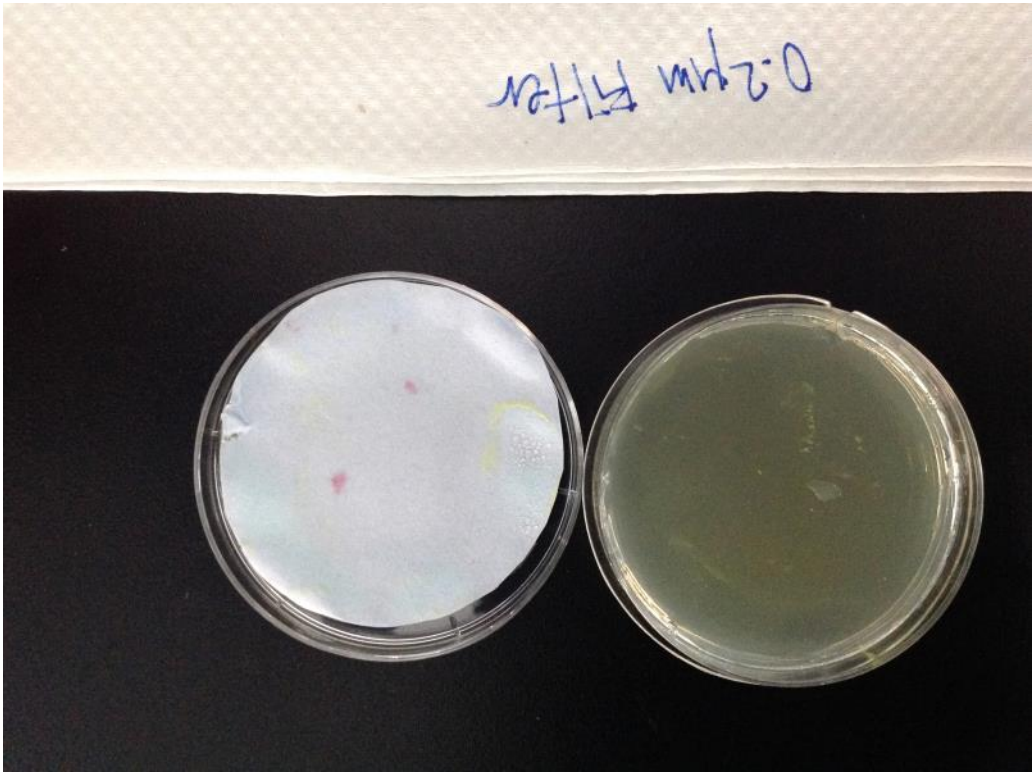


Figure #7: set up for new 0.2 micrometer blue membrane

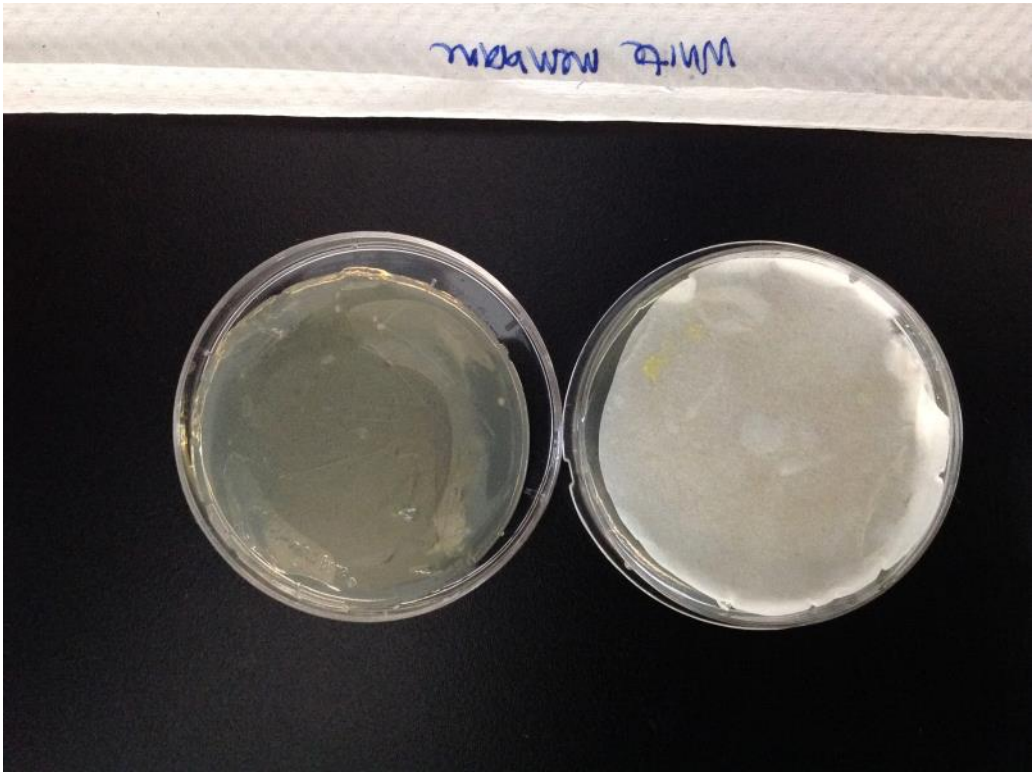


Figure #8: set up for white membrane

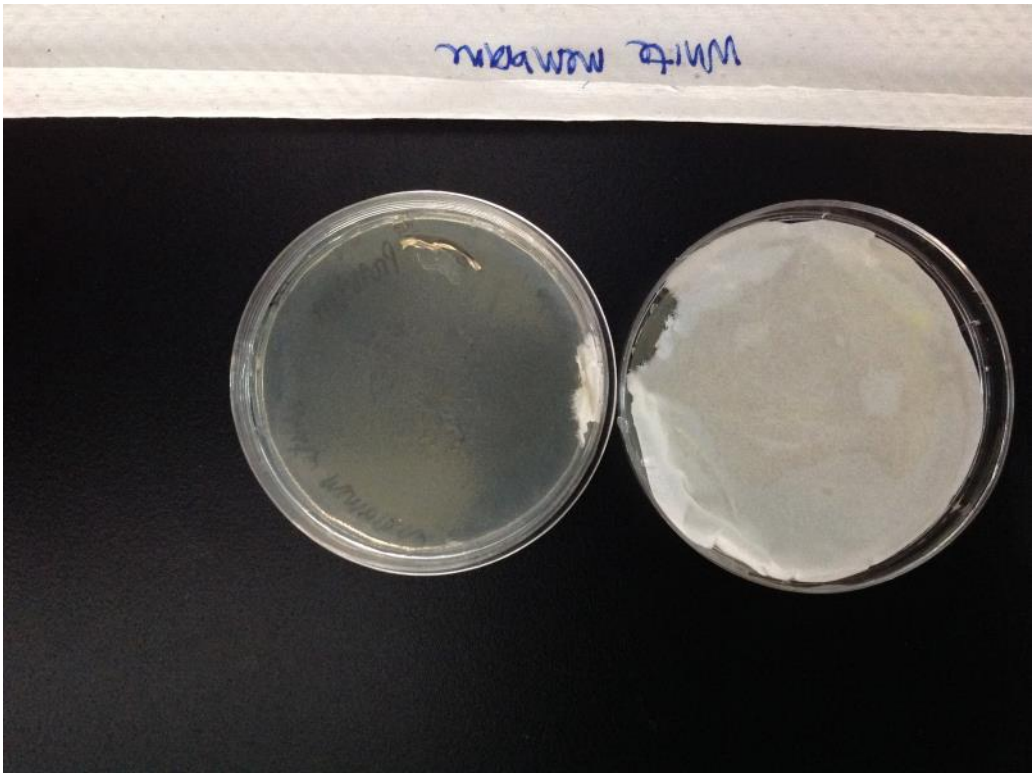


Figure #9: set up for white membrane (bottom plate)

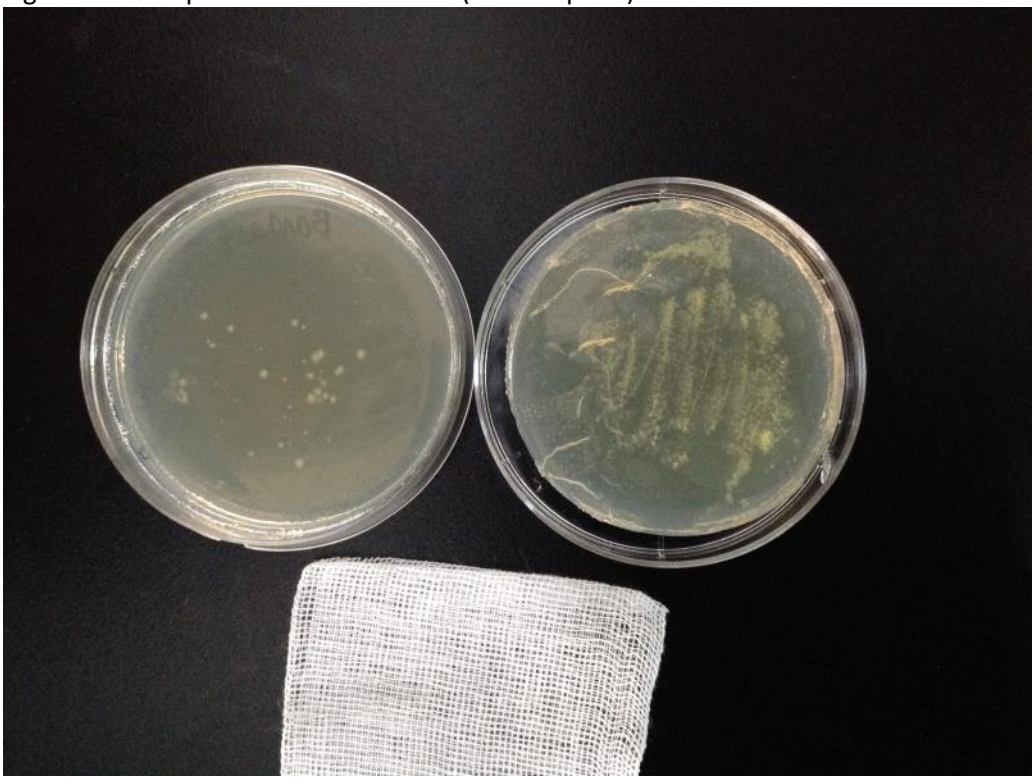


Figure #10: set up for prototype (bandage method; positive control)

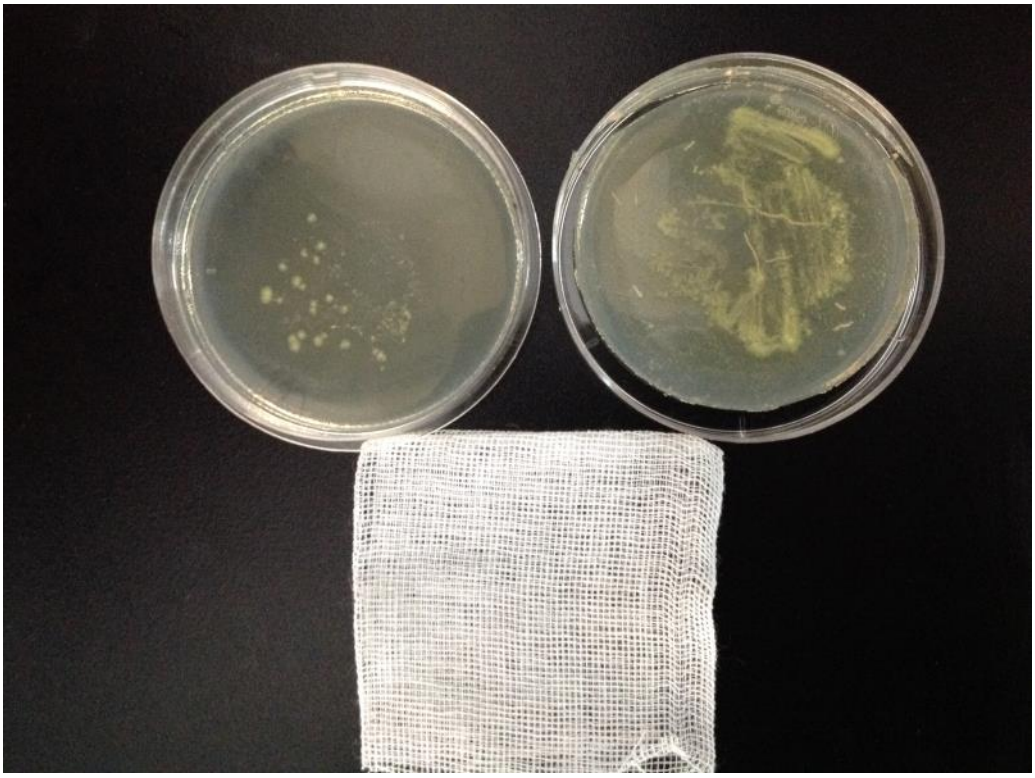


Figure #11: set up for prototype (bandage method; positive control)

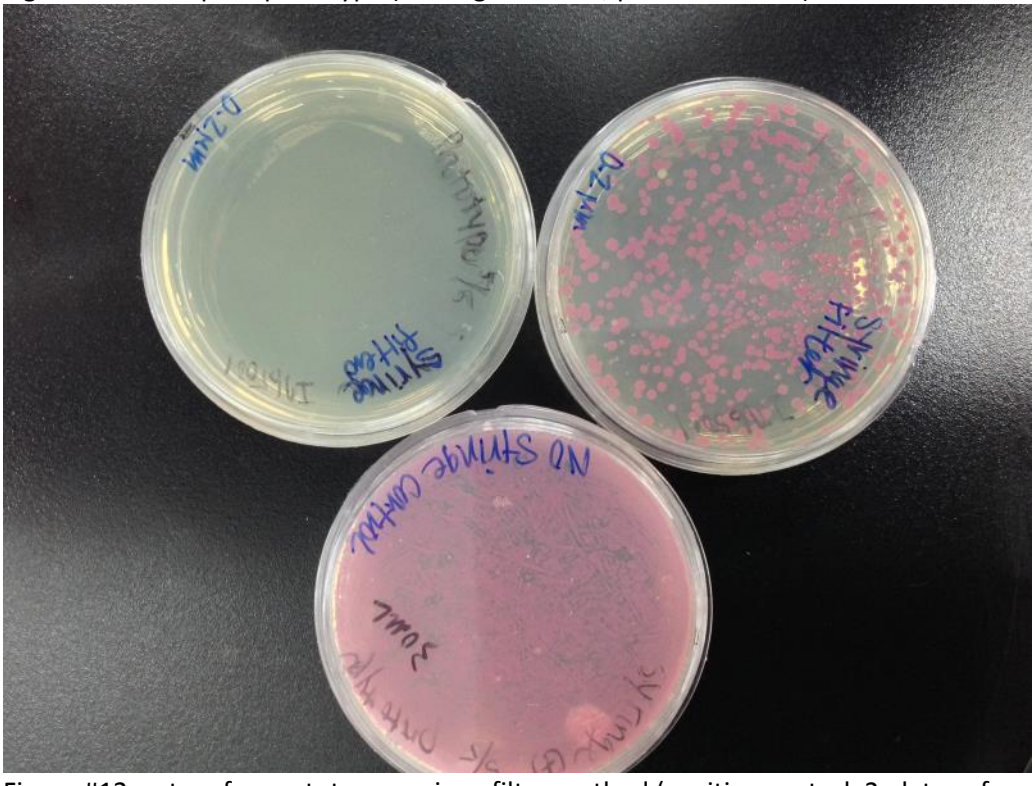


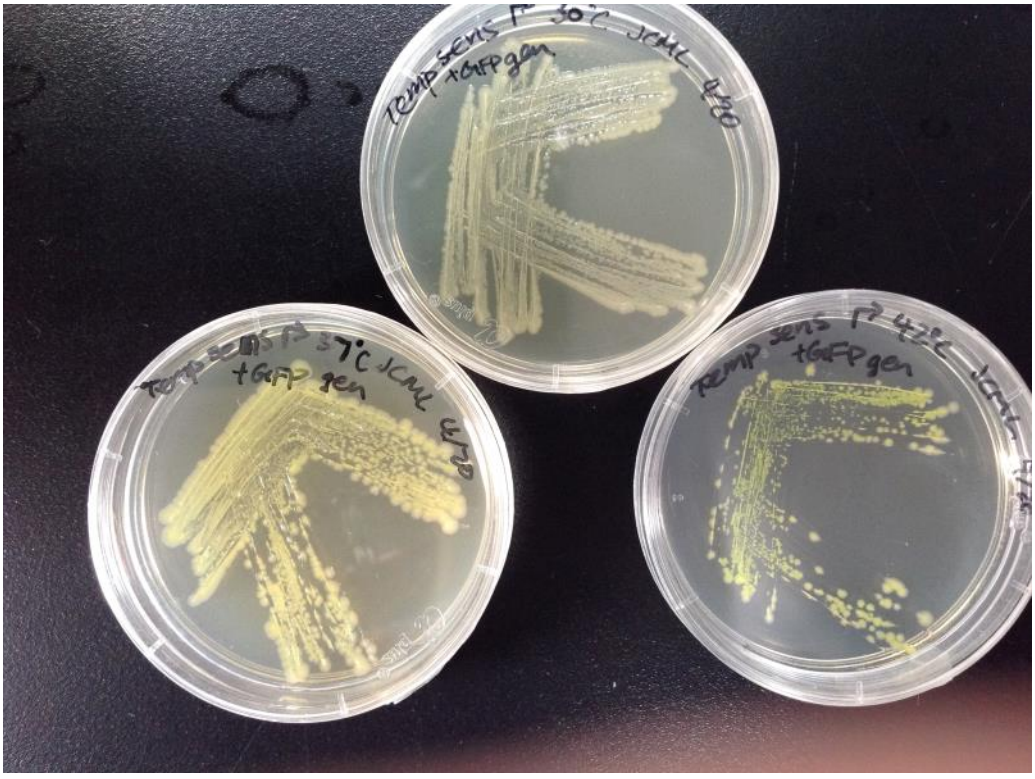
Figure #12: set up for prototype- syringe filter method (positive control, 2 plates of experimental)

Temperature Sensitive Promoter

Monday, April 27, 2015
2:24 PM

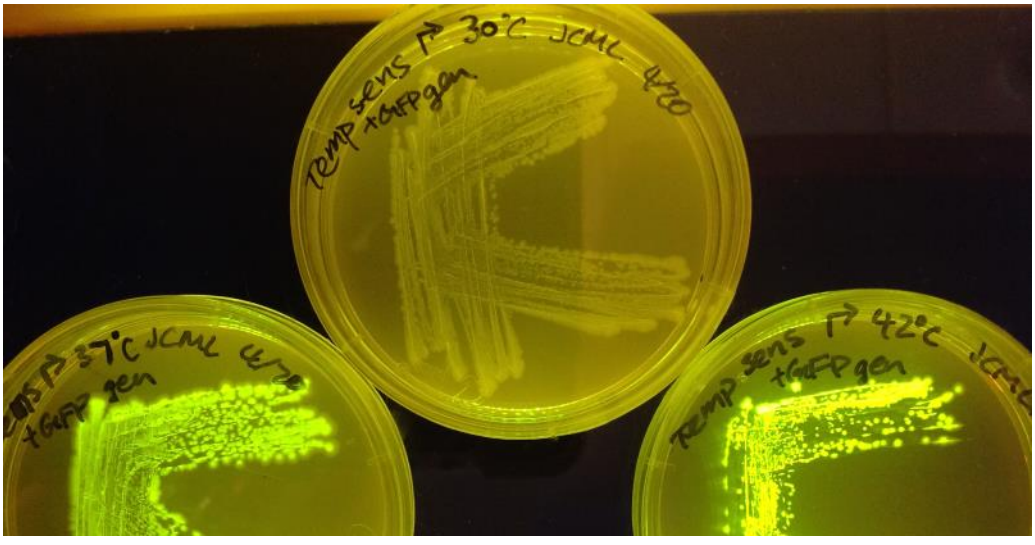
Purpose: testing sensitivity of temperature sensitive promoter

Figure 1. Bacteria with GFP gen driven by temperature sensitive promoter cultured in 30, 37, 42 degrees C



Plates in all the temperatures grew. There were fewer colonies in the 42 degrees C due to the high temperature.

Figure 2. Bacteria with GFP gen driven by temperature sensitive promoter cultured in 30, 37, and 42 degrees C under blue light



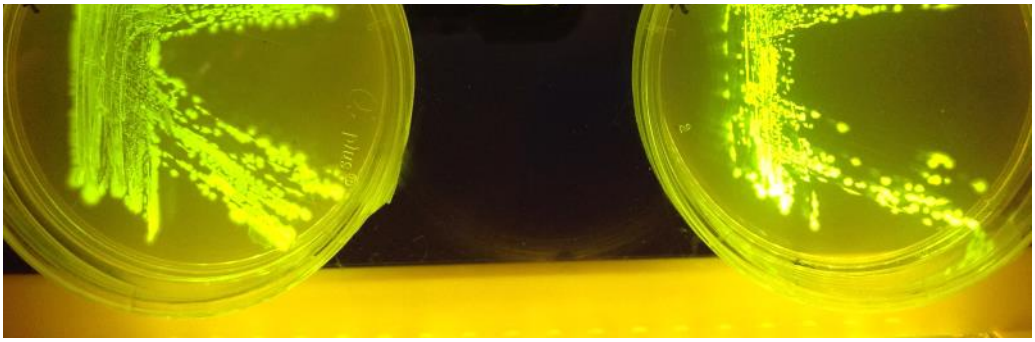
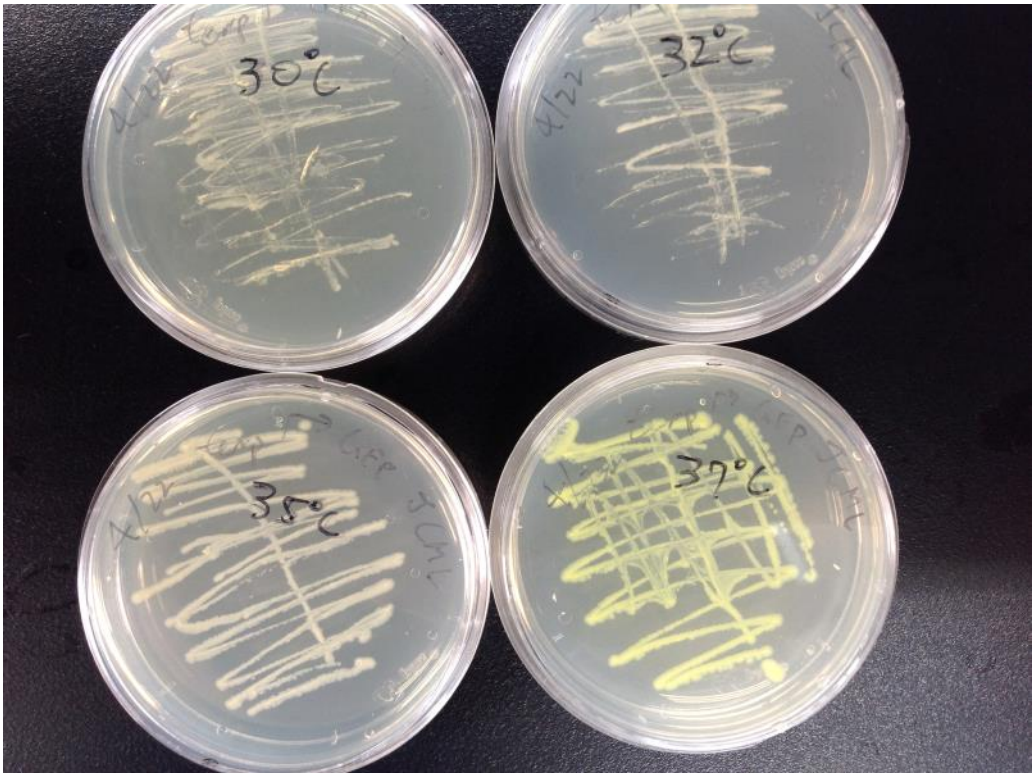
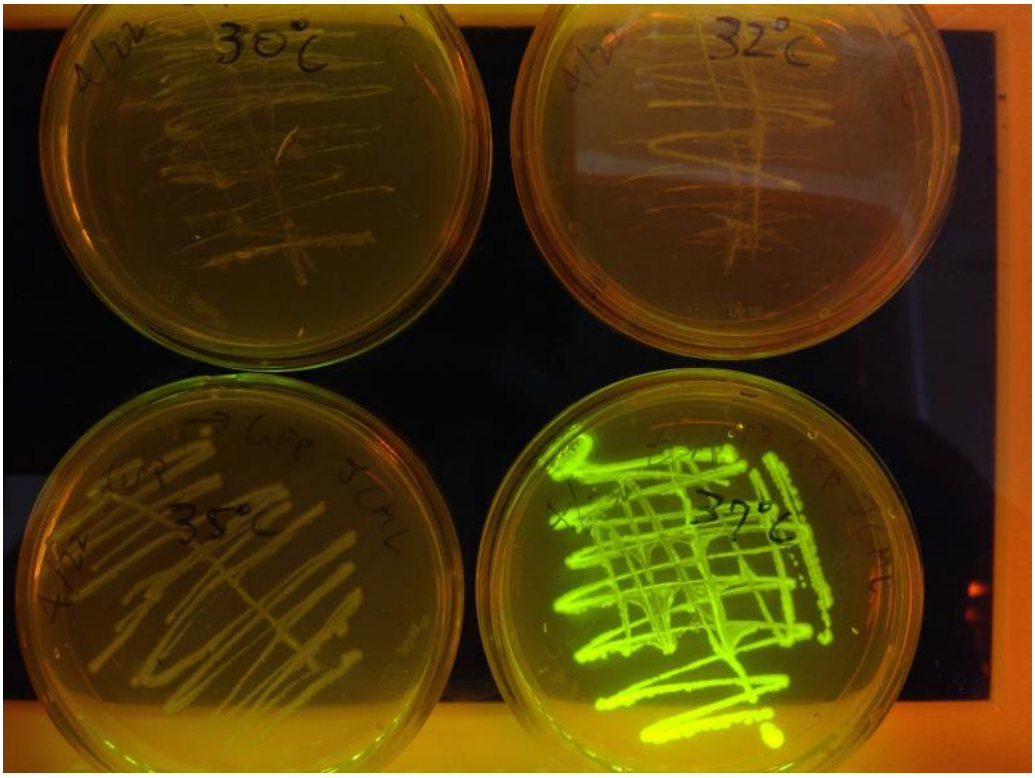


Figure 3. Bacteria with GFP gen driven by temperature sensitive promoter cultured in 30, 32, 35, and 37 degrees C



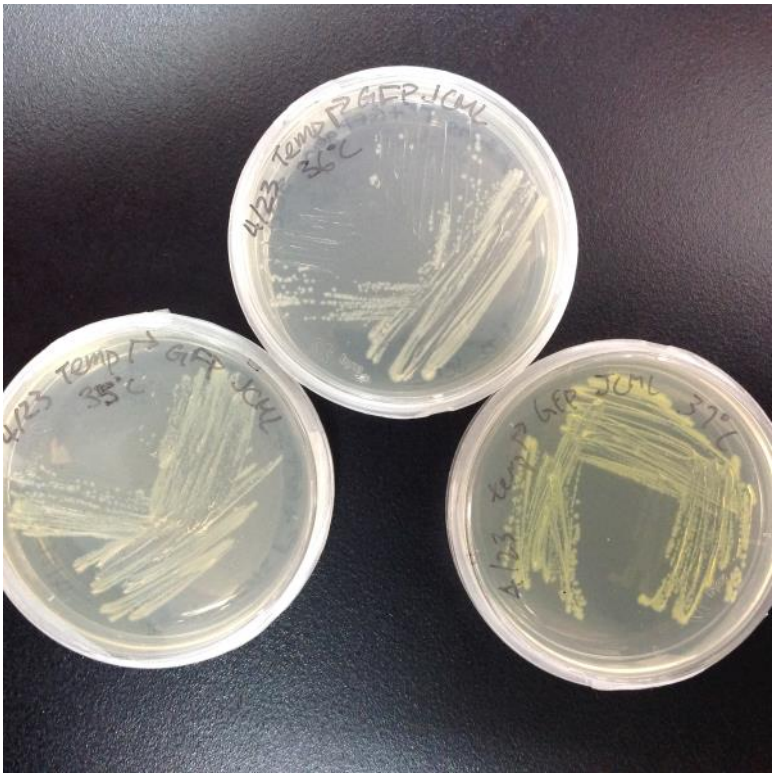
Colonies grew in all the temperatures.

Figure 4. Bacteria with GFP gen driven by temperature sensitive promoter cultured in 30, 32, 35, and 37 degrees C under blue light



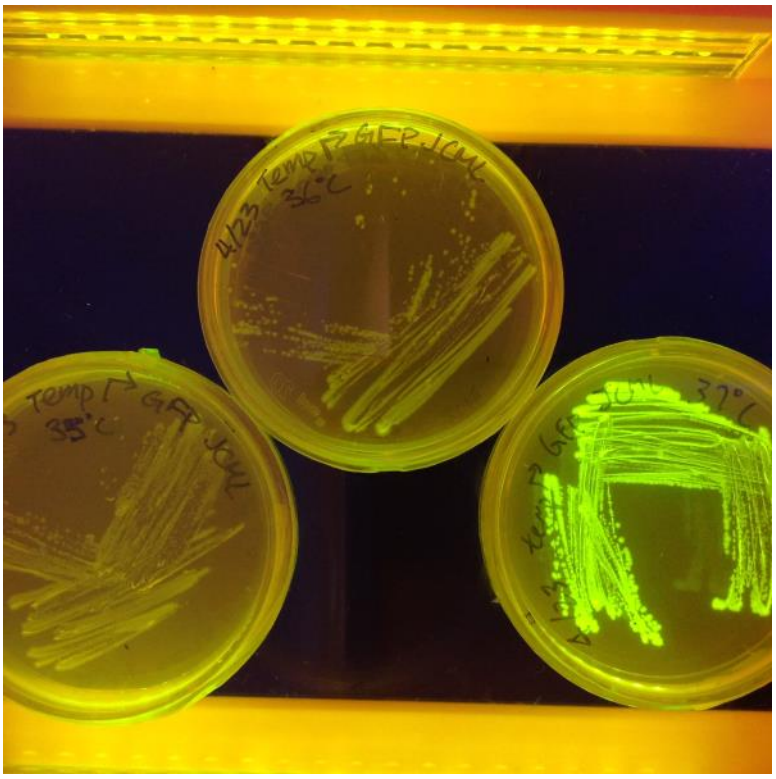
Only the plate grown at 37 degrees C glowed.

Figure 5. Bacteria with GFP gen driven by temperature sensitive promoter cultured in 35, 36, and 37 degrees C



Colonies grew at all temperatures.

Figure 6. Bacteria with GFP gen driven by temperature sensitive promoter cultured in 35, 36, and 37 degrees C under blue light



36 degrees C plate glowed faintly. 37 degrees C plate glowed.

Conclusion: Temperature sensitive promoter is activated at 37 degrees C.

Summary

2015年6月4日
上午 11:51

Temperature sensitive promoter

After successfully testing the temperature sensitive promoter and GFP gen construct, this week, we collected the temperature sensitive promoter and GFP gen plasmids for future uses.

Experiments

Wednesday, April 29, 2015
2:46 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Temp promoter	Jo and Monica	Miniprep - grow cultures	Let it grow	Let it grow	Can't hold it back anymore
ACT	Leon	Grow some cultures	Cultures grew	They're ready	Miniprep them

Pictures

Wednesday, April 29, 2015
3:24 PM

Experiments

Wednesday, April 29, 2015
2:46 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Temp promoter	Jo and Monica	Miniprep - continue	142.7 ng/ul for temp promoter 133.4 ng/ul for temp+GFP		
ACT	Leon	Miniprep some DNA for mutagenesis	Good conc high 100s and low 200s	We have plasmid DNA ready	Miniprep was good Send them out

Pictures

Wednesday, April 29, 2015
3:24 PM

Experiments

Wednesday, May 06, 2015
7:26 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Organization	Leon	Make LB agar plates with chloramphenicol resistance	Made a lot of plates	We got plates	Use the plates

Wednesday, May 06, 2015
2:03 PM

This is just for the searching function for one note.

The biobrick number for the temperature promoter is K608351.

Summary

2015年5月17日
下午 07:21

SOS promoter

This week we miniprepped SOS promoter to use it in place of the UV promoter as we were having problems with it. We ran the gel check and the parts (SOS promoter and GFP gen) were the right sizes.

Experiments

Friday, May 15, 2015
12:41 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
SOS GFP plasmid	Andrew and Phillip	- Miniprepped SOS Promoter - Digested SOS Promoter at SP - Digested GFP at XP - Ran Gel and Gel extraction (the results are under the pictures tab)	Gel picture under UV is put under pictures tab. Please refer to figure 1. The gel results suggest that the GFP is correct because we see a faint band at the 800 bp mark. The SOS promoter also seems to be good enough to continue because it includes the backbone length.	This is going to work. As today is a Friday, we will not be able to do transformations.	- Ligation+Transformation

Picture

Friday, May 15, 2015
2:55 PM

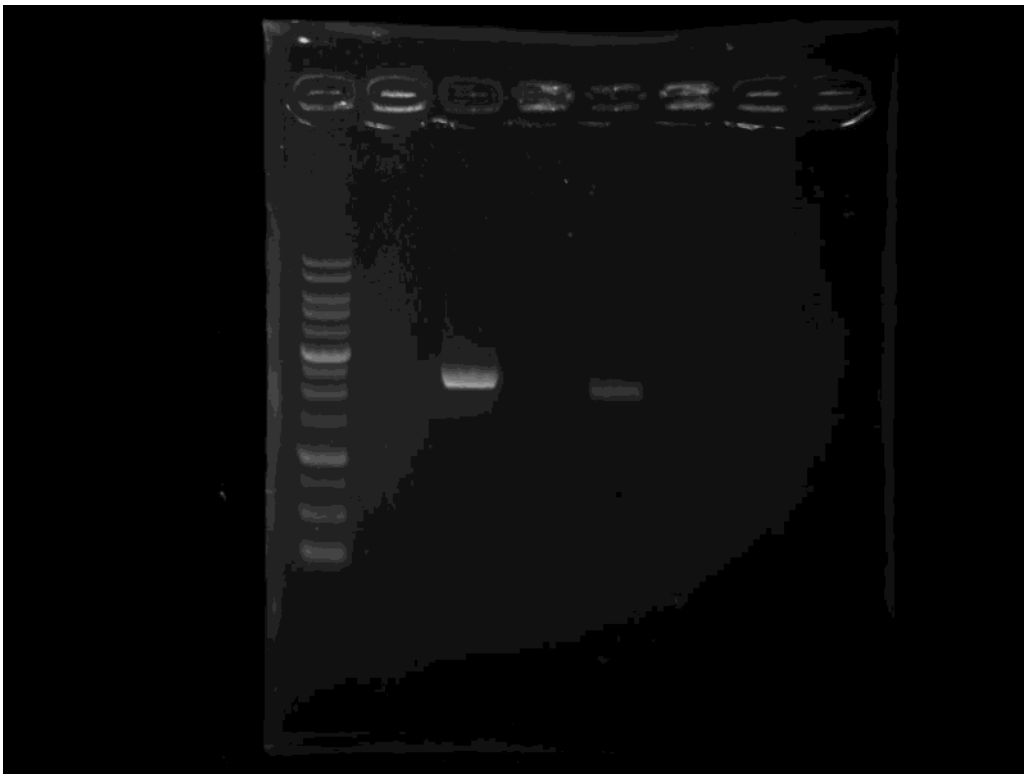


Figure 1: Order from left to right.	Ladder	Blank	SOS Promoter cut at SP	Blank	GFP cut at XP

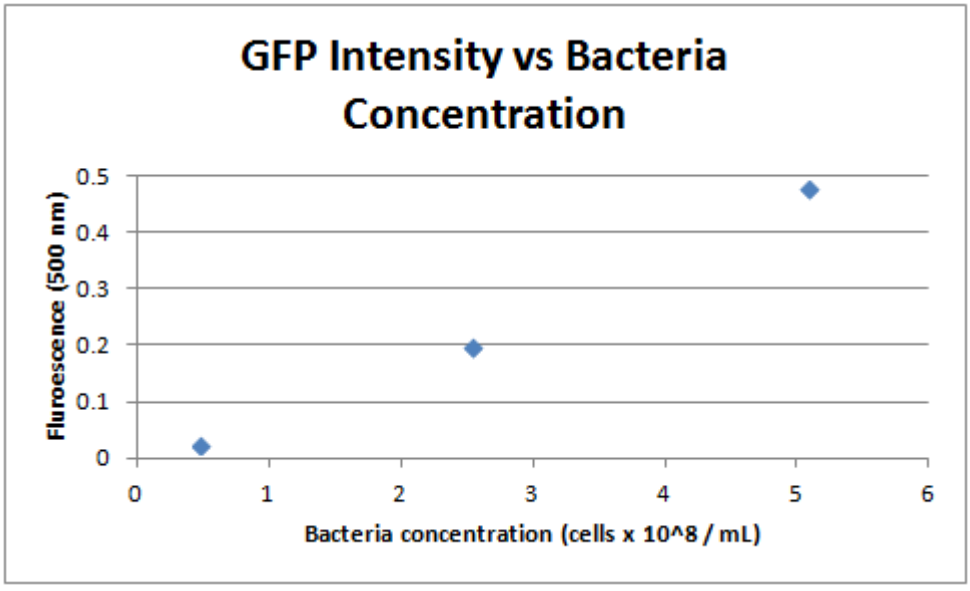


Figure 2. Measure of GFP intensity using spectrophotometer for different concentrations of bacteria culture

Experiments

Monday, May 18, 2015
3:05 PM

12:41 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
PCR for PFU and TAQ	Jon	- PCR for PFU and TAQ	- Created the mixture of PCR for PFU and TAQ for Leon.	- Wait for the result from PCR to test if the polymerase is functioning properly	- Leon takes over

Photos

Monday, May 18, 2015
3:05 PM

Experiments

Wednesday, May 20, 2015
3:24 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
3 in 1 for SOS + GFP	Andrew	Did restreak, liquid culture and PCR of SOS promoter + GFP			Do Gel Check of PCR nad miniprep of SOS GFP

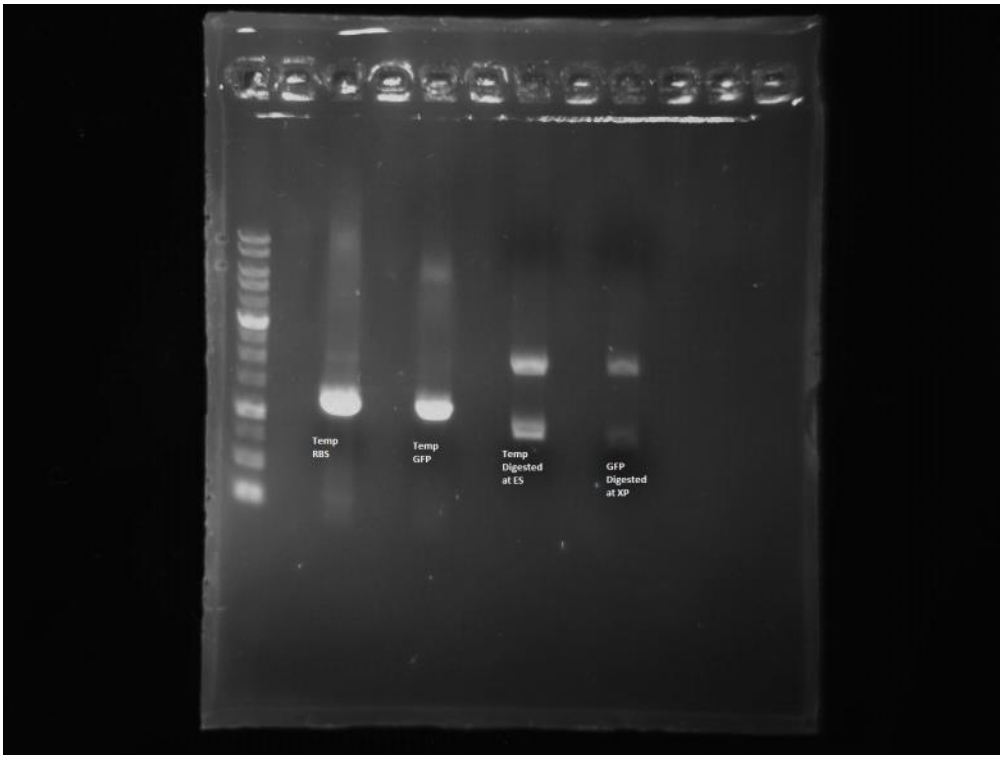
Experiments

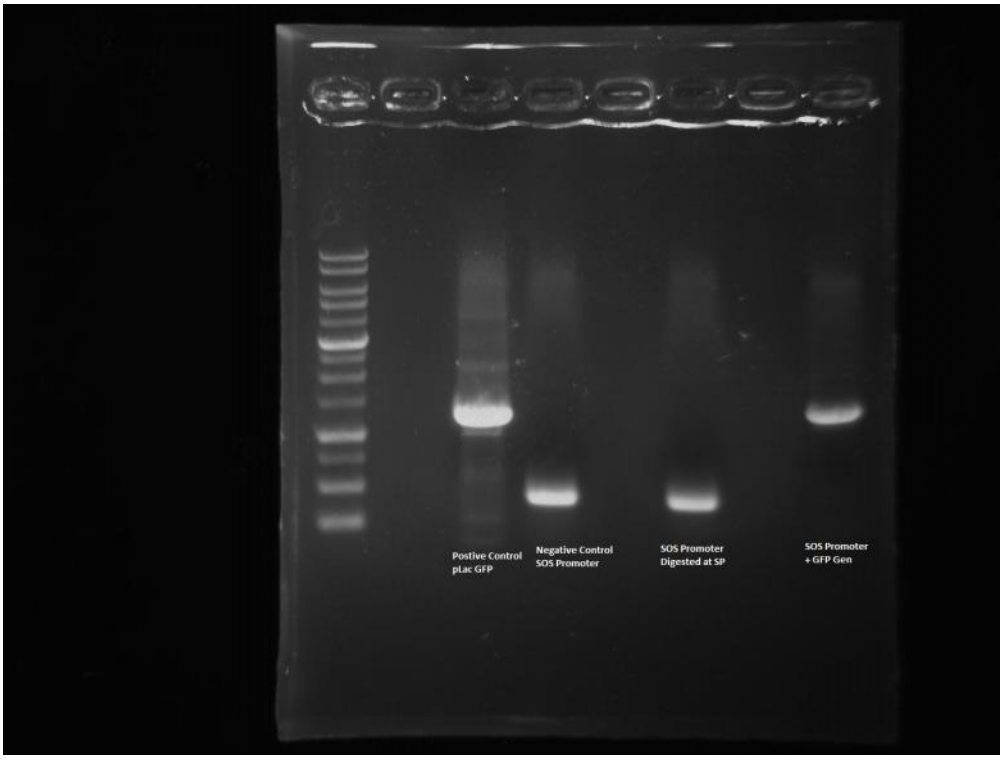
Wednesday, May 20, 2015
3:24 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Presentation Picture	Andrew	Ran Gel for Temp RBS, Temp GFP, Temp promoter cut at ES and GFP cut at XP			
PCR gel check of SOS GFP	Andrew	PCR checked SOS promoter + GFP			
SOS GFP miniprep	Andrew	Miniprep of SOS promoter + GFP with elution			

Pictures

Wednesday, May 20, 2015
3:25 PM





Tuesday, May 26, 2015
12:33 PM

12:41 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
plac	Leon Yim	Grow cultures 2x5mL	Cultures grew	good	miniprep
CFP	Leon Yim	Grow cultures 2x5mL	Cultures grew	good	miniprep

Tuesday, May 26, 2015
12:33 PM

12:41 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
plac	Leon Yim	miniprep	100+ conc	good	digest
CFP	Leon Yim	Miniprep	100+ conc	good	Digest
Terminator/rbs	Leon Yim	Transformation	Not sure yet		Liquid culture + miniprep
PCR gelcheck: PEV, TAQ	Jo Chuang	gel	good	Polymerase works	
Temp promoter timelapse	Jo Chuang	Growing at 33C	growing	growing	Record timelapse

Summary

2015年6月4日
下午 12:39

TAQ polymerase
This week we ran a PCR and gel check for TAQ polymerase. We found that the polymerase works.

Temperature sensitive promoter timelapse

Interval test of GFP expression

Wednesday, May 27, 2015
7:33 AM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps

Thursday, May 28, 2015
2:16 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Intrv Test	JC	Checking GFP expression of TMP-GFP at 37C	Nothing so far		

Experiments

Monday, June 22, 2015
4:14 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
ACT	Leon	Gel check PCR of ACT (Pst1)EXP	Perfect	Cloning confirmed use COLONY 8	Picture!
ACT	Leon	Gel check PCR of ACT(muta)+term	Legendary	Cloning confirmed use COLONY 6	Picture!
ACT cloning	Leon	3 In 1 ACT(muta)+term	Bacteria grew	Pcr worked parts confirmed	
Bacteria Growth	Bryan	Check the plates and take pictures	5µl: Overgrew 1µl: Overgrew 0.1µl: Overgrew 0.01µl: Overgrew 0.001µl: Ok	Keep 0.01µl as an example of overgrow Keep 0.001µl for observation	Take pictures next time

Pictures

Tuesday, June 23, 2015
2:11 PM

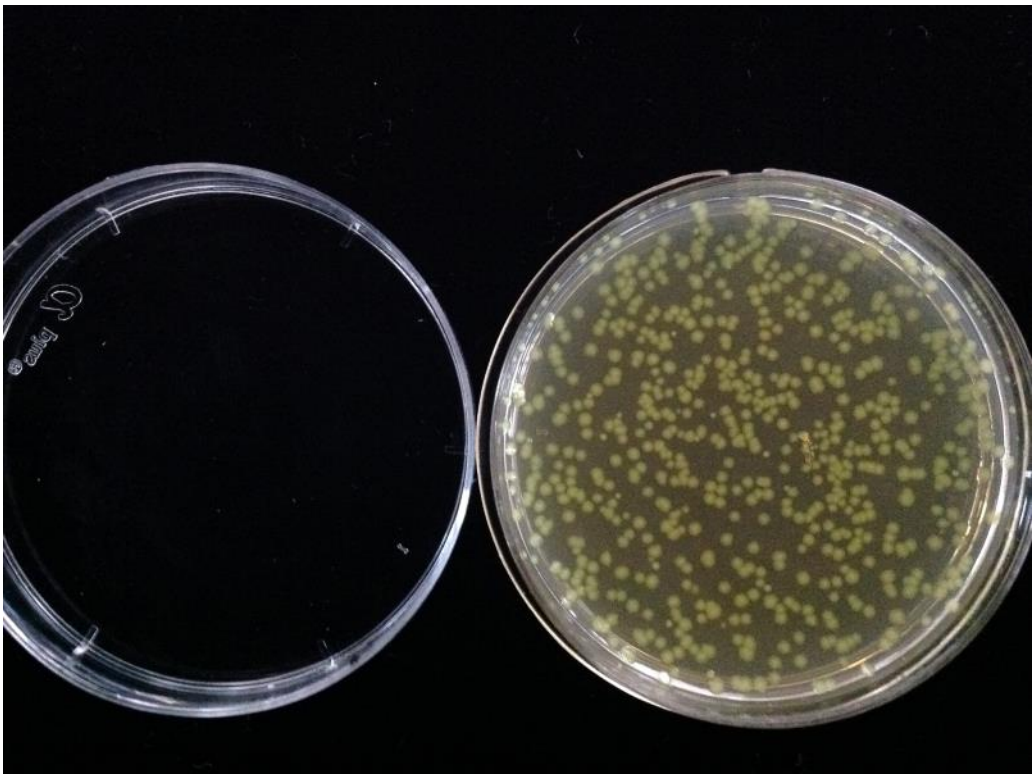


Figure 1: Plate with 0.001µl Liquid Culture

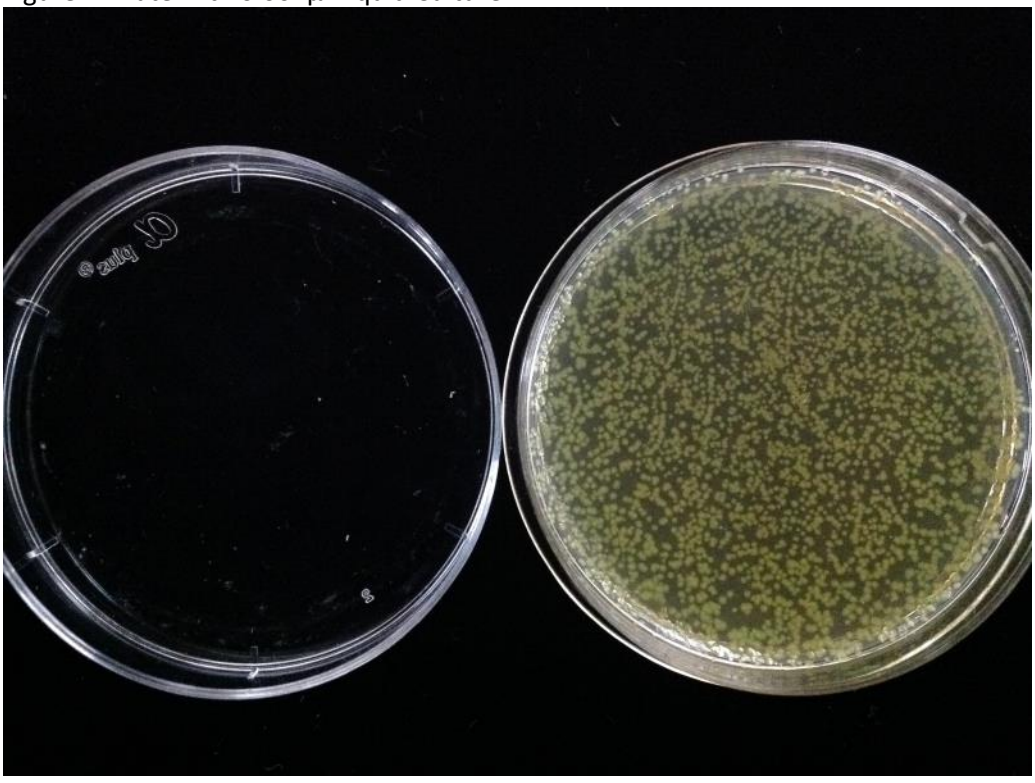


Figure 2: Plate with 0.01µl Liquid Culture

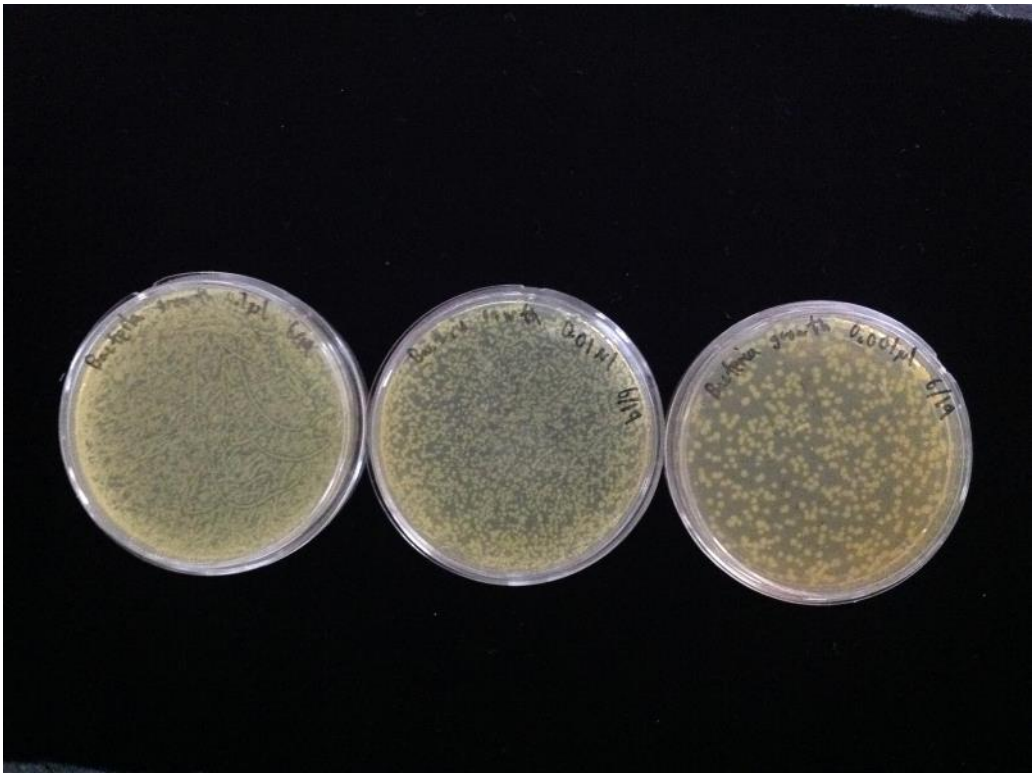


Figure 2: Comparison for Bacteria growth

Experiments

Tuesday, June 23, 2015
11:00 AM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
ACT cloning	Leon	Miniprep	High concentration	Good	Digest
ACT cloning	Leon	Digestion 25uL mix	Check with gel extract		Gel RUN
ACT cloning	Leon	Run gel with 1Kb ladder 100volt	Waiting		Gel extract
Bacteria Growth	Bryan	Check the plates and take pictures	0.01µl: Colonies enlarged 0.001µl: Colonies enlarged	Refer to pictures	Take pictures next time

Pictures

Wednesday, June 24, 2015
4:20 PM

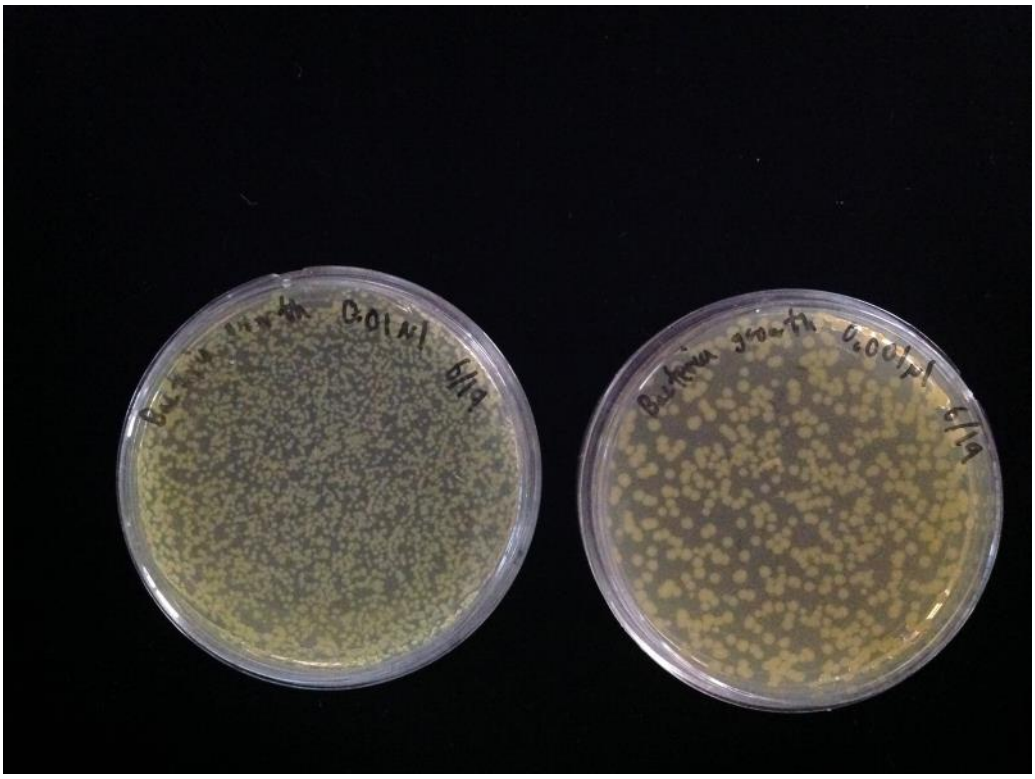


Figure 1: Comparison

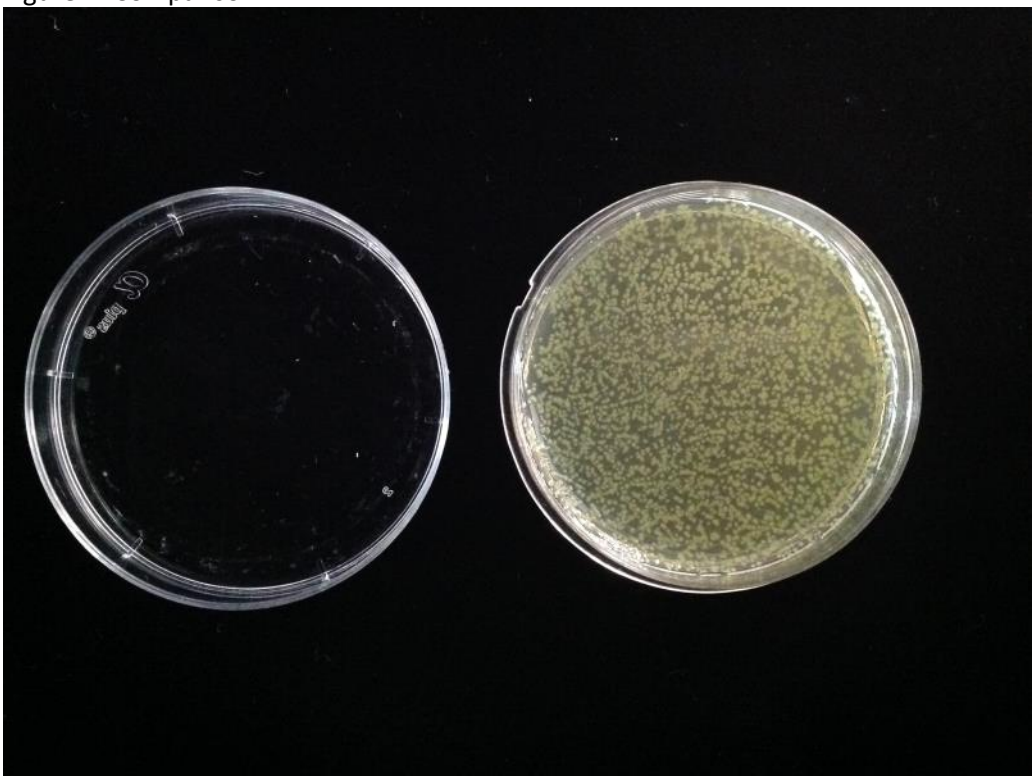


Figure 2: Plate with 0.01µl Liquid Culture

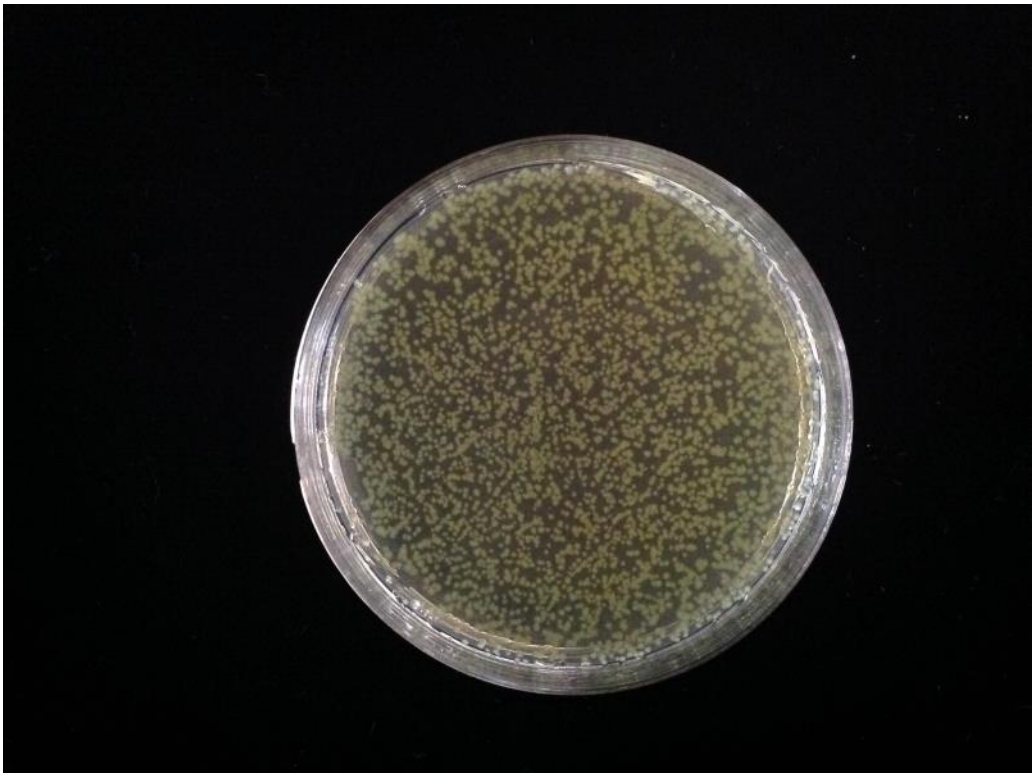


Figure 3: Plate with 0.01µl Liquid Culture (Detailed)

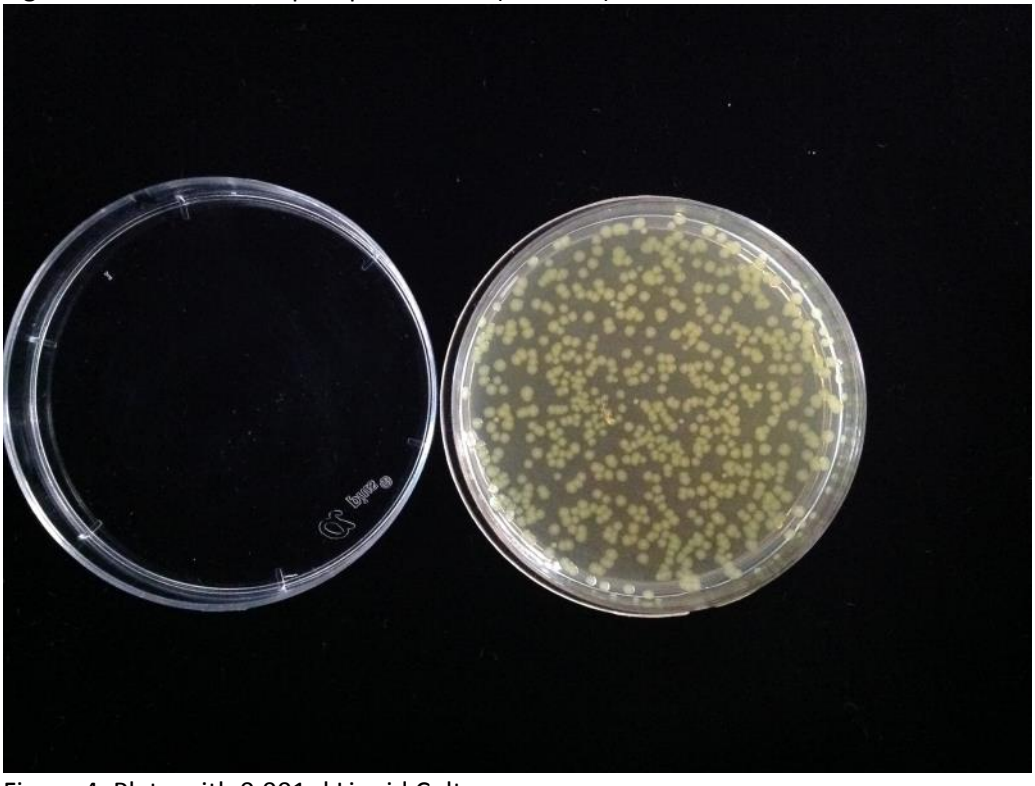


Figure 4: Plate with 0.001µl Liquid Culture

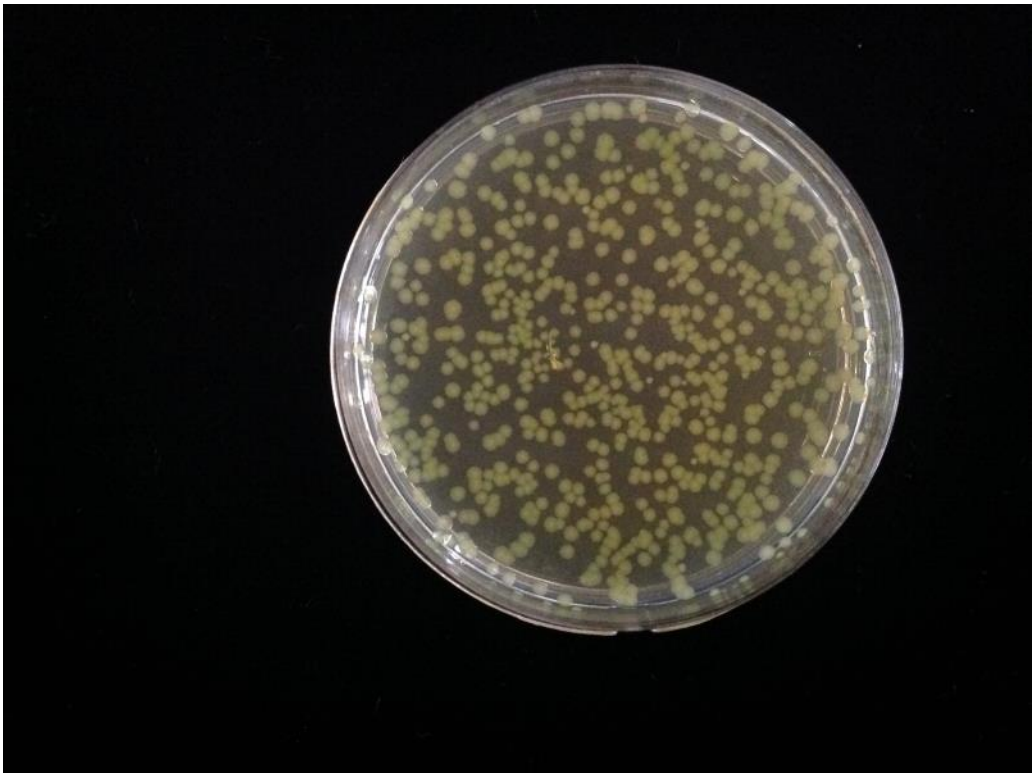


Figure 5: Plate with 0.001µl Liquid Culture (Detailed)

Experiments

Wednesday, June 24, 2015
4:20 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Bacteria Growth	Bryan	Check the plates and take pictures	0.01µl: Colonies enlarged 0.001µl: Colonies enlarged	Refer to pictures	Take pictures next time
ACT Cloning	Leon	Miniprep Digest Gel extract Ligate Transform pTS + RBS + ACT + Term	Good	Good	done

Pictures

Wednesday, June 24, 2015
4:20 PM

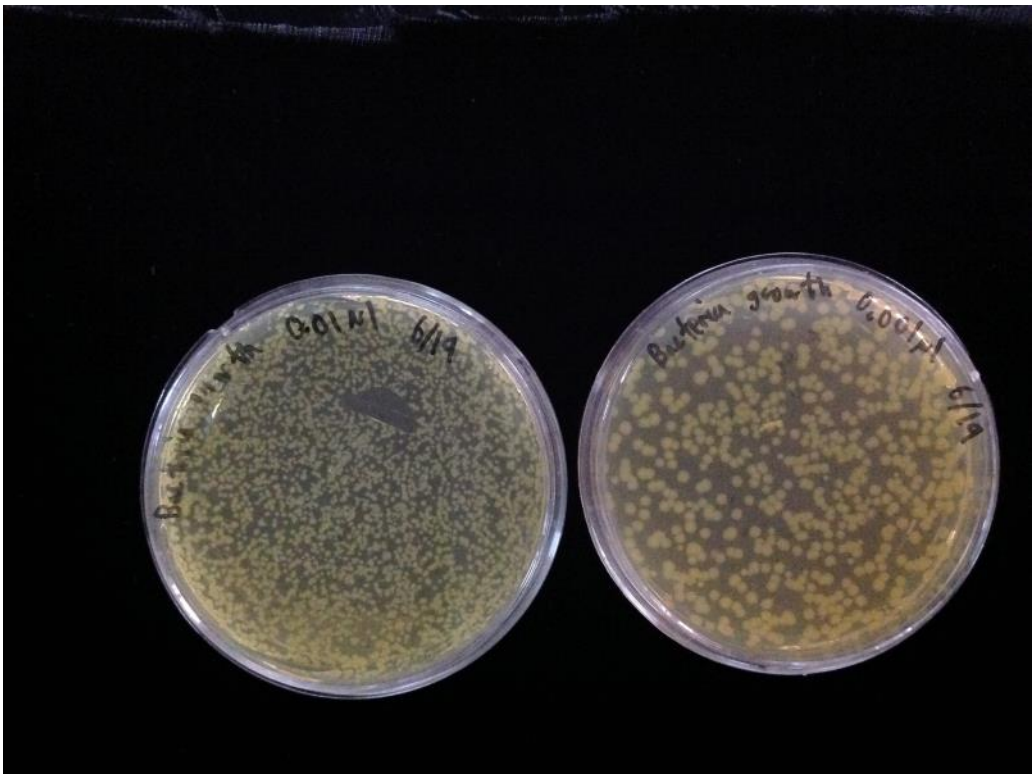


Figure 1: Comparison

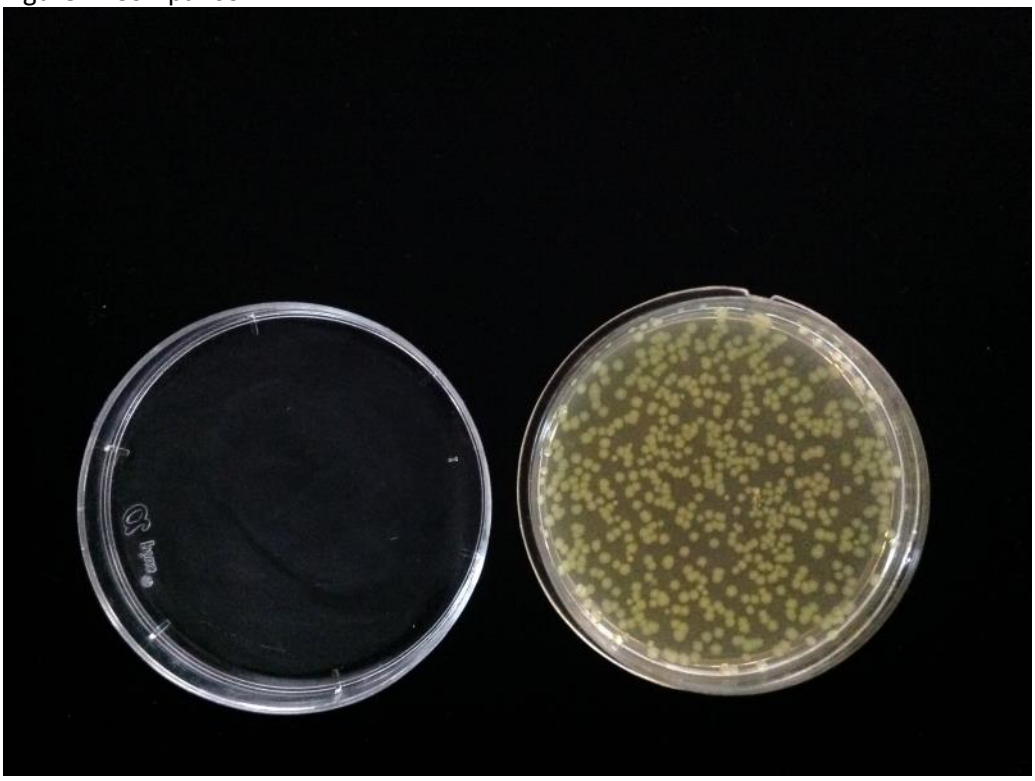


Figure 2: Plate with 0.01µl Liquid Culture

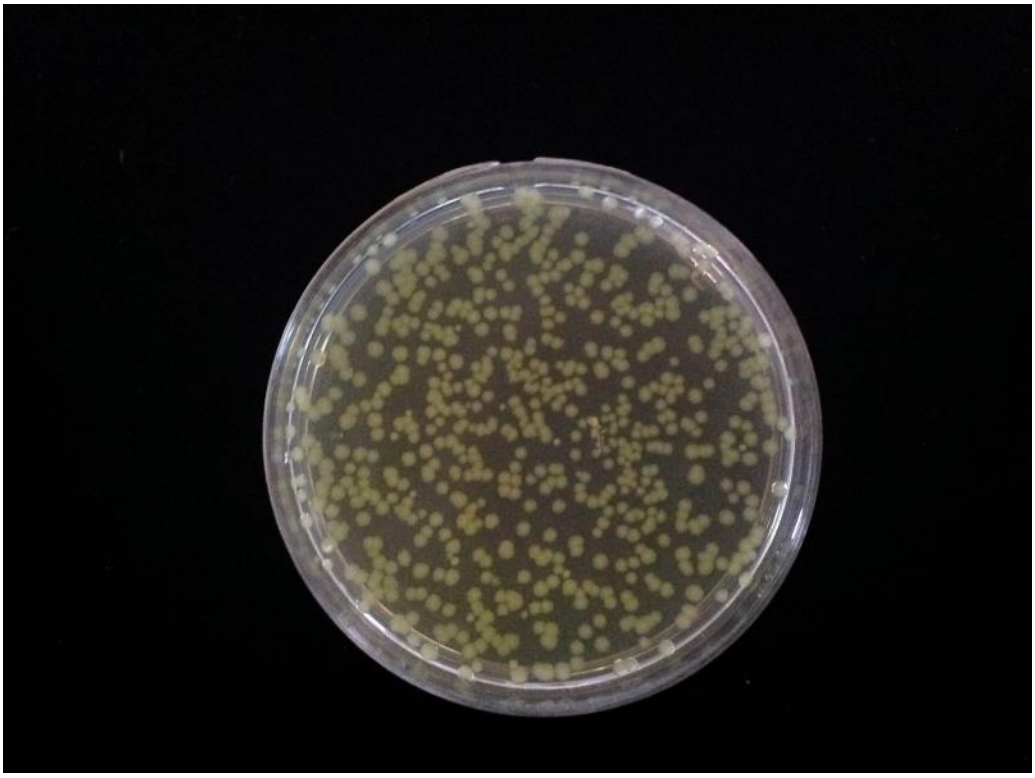


Figure 3: Plate with 0.01µl Liquid Culture (Detailed)

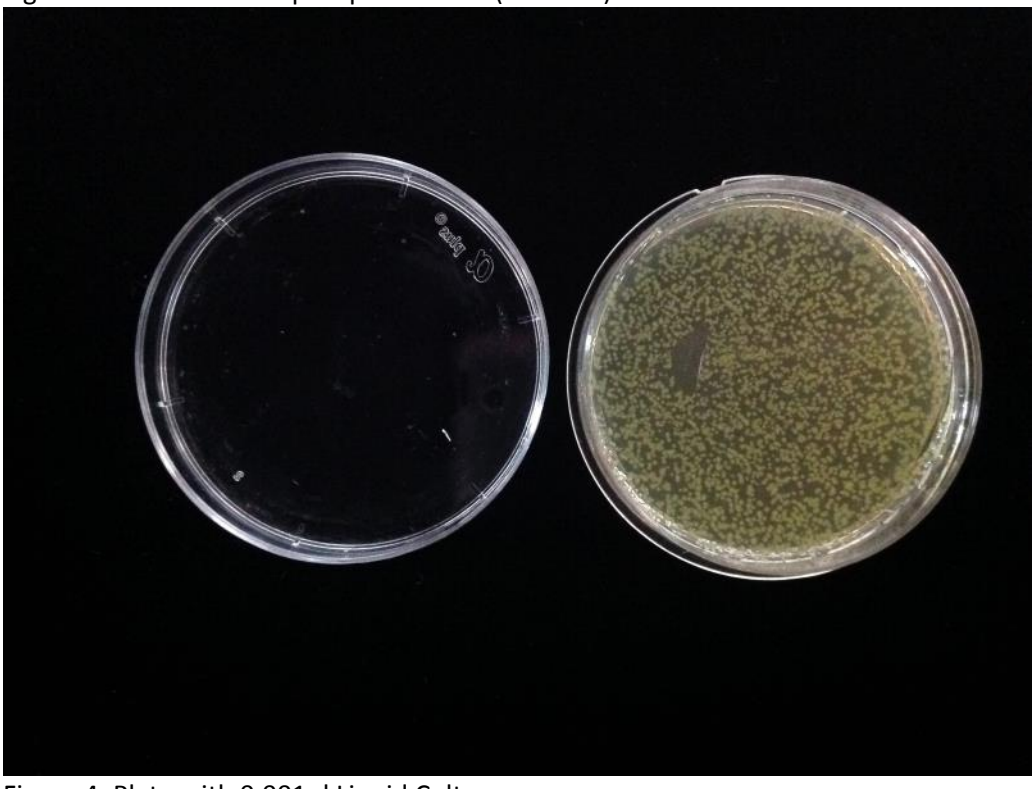


Figure 4: Plate with 0.001µl Liquid Culture

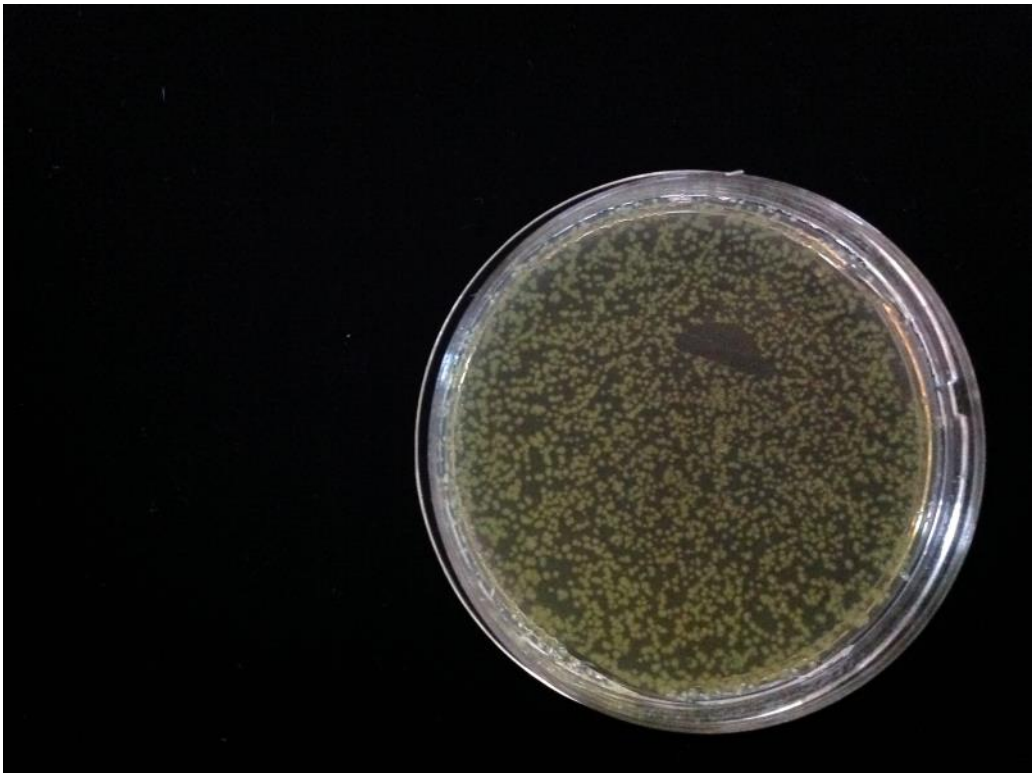


Figure 5: Plate with 0.001µl Liquid Culture (Detailed)

Experiments

Tuesday, June 30, 2015
3:23 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
ACT clonign	Leon	3 in 1 pTS + RBS + ACT + term	Did 20 trials only 3 PCR looks good	Need to sequence for final check	Send for sequencing

Experiments

Tuesday, June 30, 2015
3:23 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
ACT cloning	Leon	Miniprep digest	Can't seem to get yebF out	Need to make pTS + RBS + yebF + ACT + term	Pcr yebf

Thursday, May 28, 2015
2:16 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Intrv Test	JC	Growing new batch of temp-gfp for interv test, need to determine time range (between 8 to 24 hours)			

Thursday, May 28, 2015
2:16 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Intrv Test	JC	Still growing			

Thursday, May 28, 2015
2:16 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Intrv Test	JC	Started 37C yesterday at 5PM, by 7:30 AM, plate was glowing			

Experiments

Monday, June 08, 2015
4:23 PM

Purpose	Group	Experiments	Results	Conclusion/Notes	Next Step
UV promoters	Leon	Restreaked all UV promoters J22106 1 J22106 2 I765001 A I765001 B	They should all grow	They grow?	Miniprep + sequence
ACT	Leon	Ordered new EX primer for ACT	Waiting	Waiting	PCR into EX SP format and front insert parts
ACT	Leon	M1T5 Mutagenesis sequencing	Failed, no mutation occurred	Mutagenesis doesn't work	Dr. Chiang's friends
Prototype	Phillip	Prototype testing of filter sandwich	Check pictures for tomorrow 6.9	Check pictures for tomorrow 6.9	Check pictures for tomorrow 6.9
SOS Test on/off	Daphne	Streaked 4 plates of SOS GFP. 2 plates are covered in foil (1 layer of cloth and 3 of foil to be safe)and 2 without foil. Left in incubator for overnight.	After 22 hours of incubation, 2 plates that are not covered glowed. Foil is removed from a covered plate and it glowed. All 4 plates are moved to the fridge.	SOS plates glow in the presence and absence of UV. Something's wrong with SOS.	Need to send off for sequencing.
Bacteria Growth	Bryan	Added one colony into 30 microliter of water and streaked it on a thin plate for to resemble a bandage. Left for growth in 37 degrees	The experiment should grow to reach a maximum or keep on growing	Waiting	Check and take pictures after a 20 hour growth period

Photos

Tuesday, June 09, 2015
3:25 PM

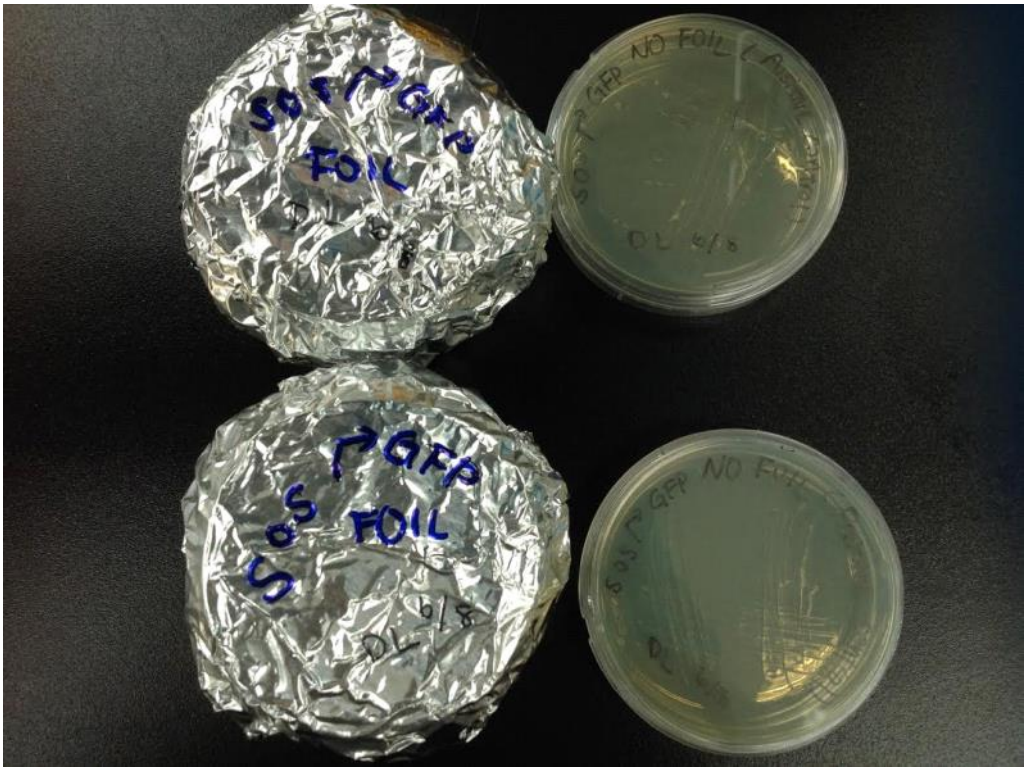


Image 1. SOS testing on/off experiment setup, 2 plates on left are covered with cloth and 3 layers of foil. 2 plates on the right are not covered.

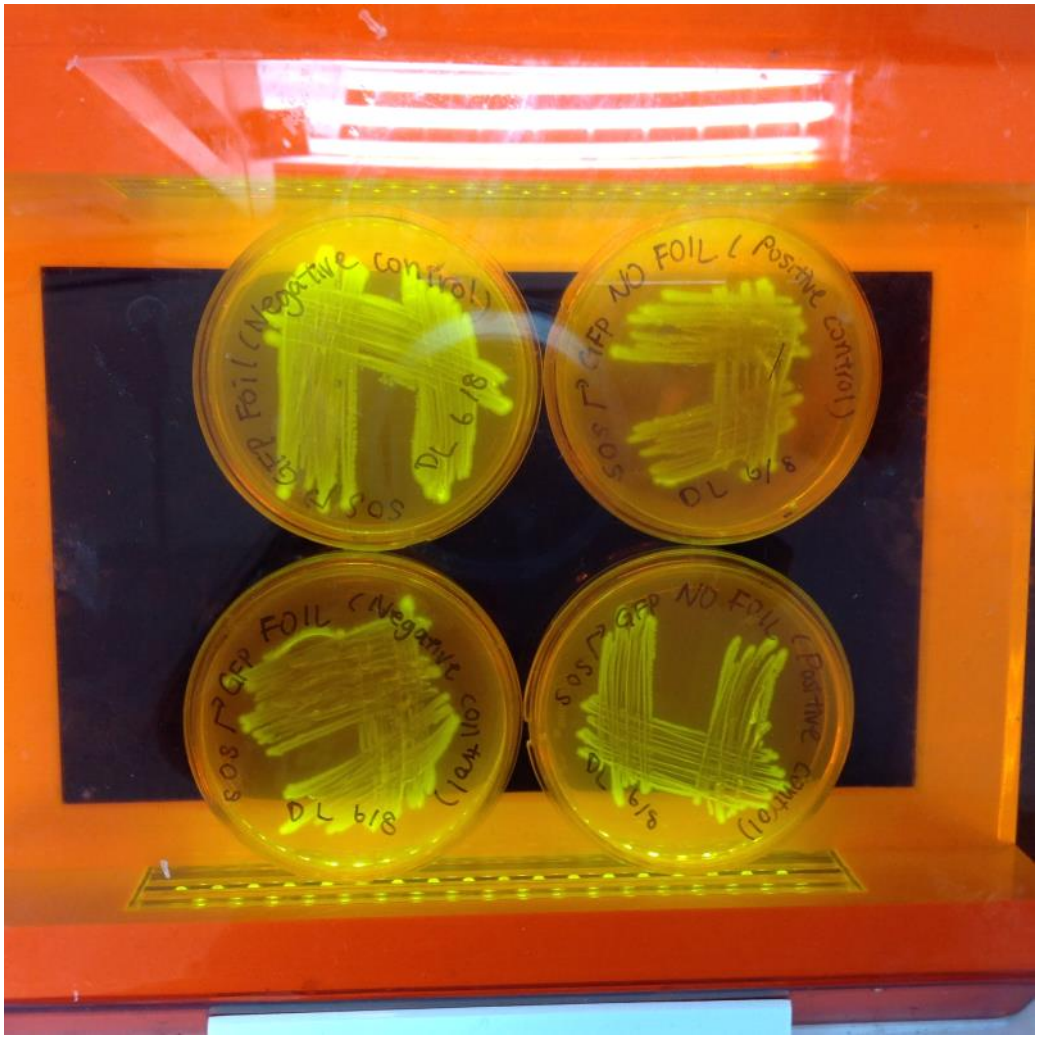
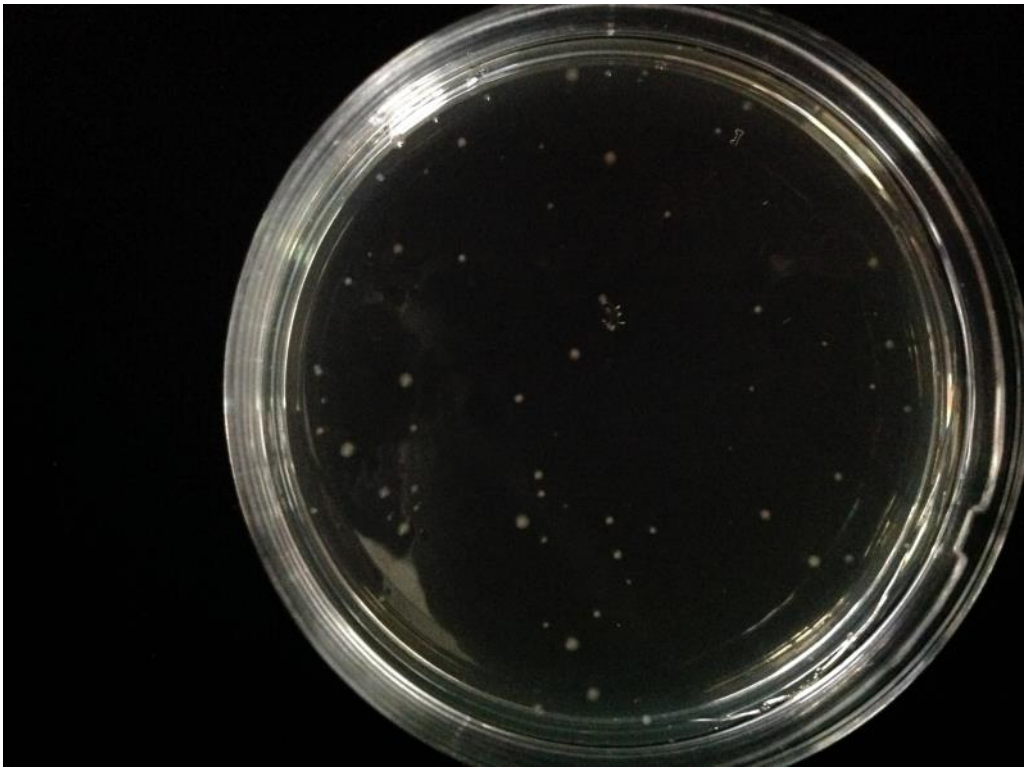


Image 2. SOS testing on/off Result, all 4 of them glowed.



Experiments

Monday, June 08, 2015
4:25 PM

Purpose	Group	Experiments	Results	Conclusion/Notes	Next Step
Temp sense GFP intrv test	**Jo Chuang**	**Starting 8-14 hour trial 9:08 start**			
UV Promoters	**Leon**	**2 in 1 culture + restreak I765001 J22106 4/17 J22106 4/24**	**Not sure**	**Not sure**	**Miniprep + sequeunce**
ACT	**Leon + Phillip**	**PCR with EX ACT and ACT SP**	**Not sure**	**Not sure**	**Sequence eventually**
Prototype	**Phillip**	**Looked at results**			
Bacteria growth	**Bryan**	**Took pictures for the result and record time Continue to leave it in 37 degrees**	**Small colonies**	**Growth takes more than 20 hours for thin plates**	**Check and take pictures after a 24 hour growth period**

Photos

Tuesday, June 09, 2015
6:33 PM

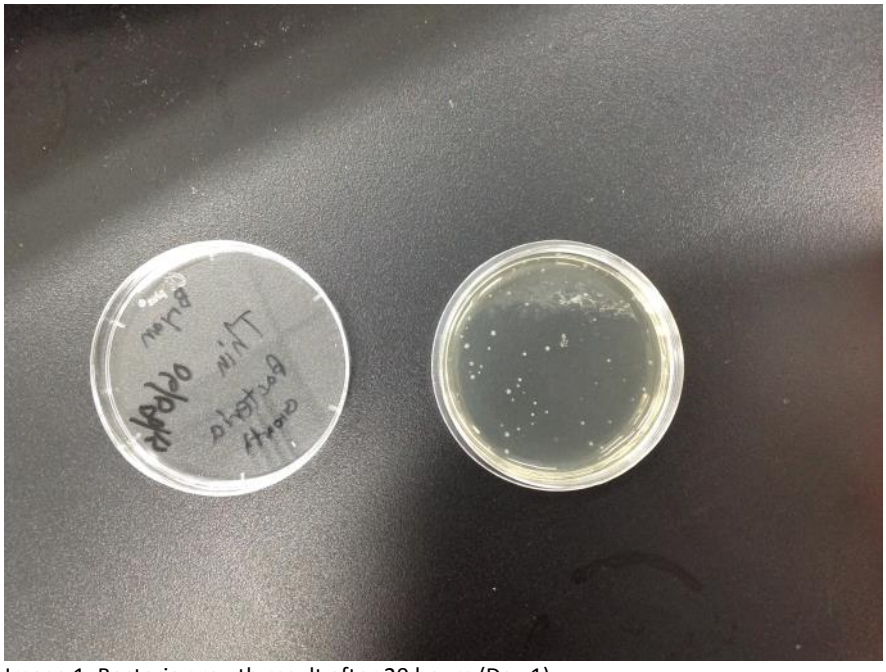


Image 1. Bacteria growth result after 20 hours (Day 1)

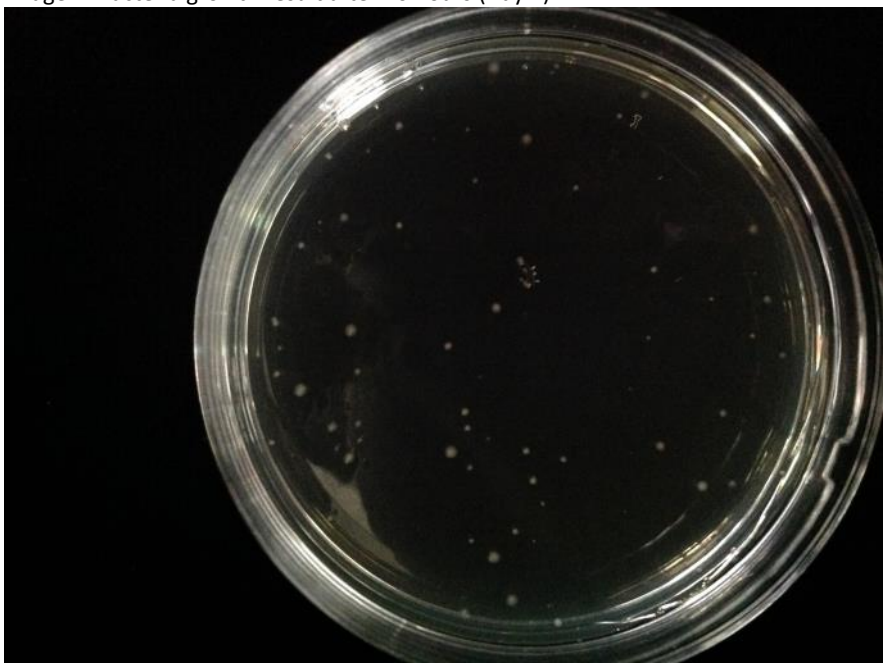
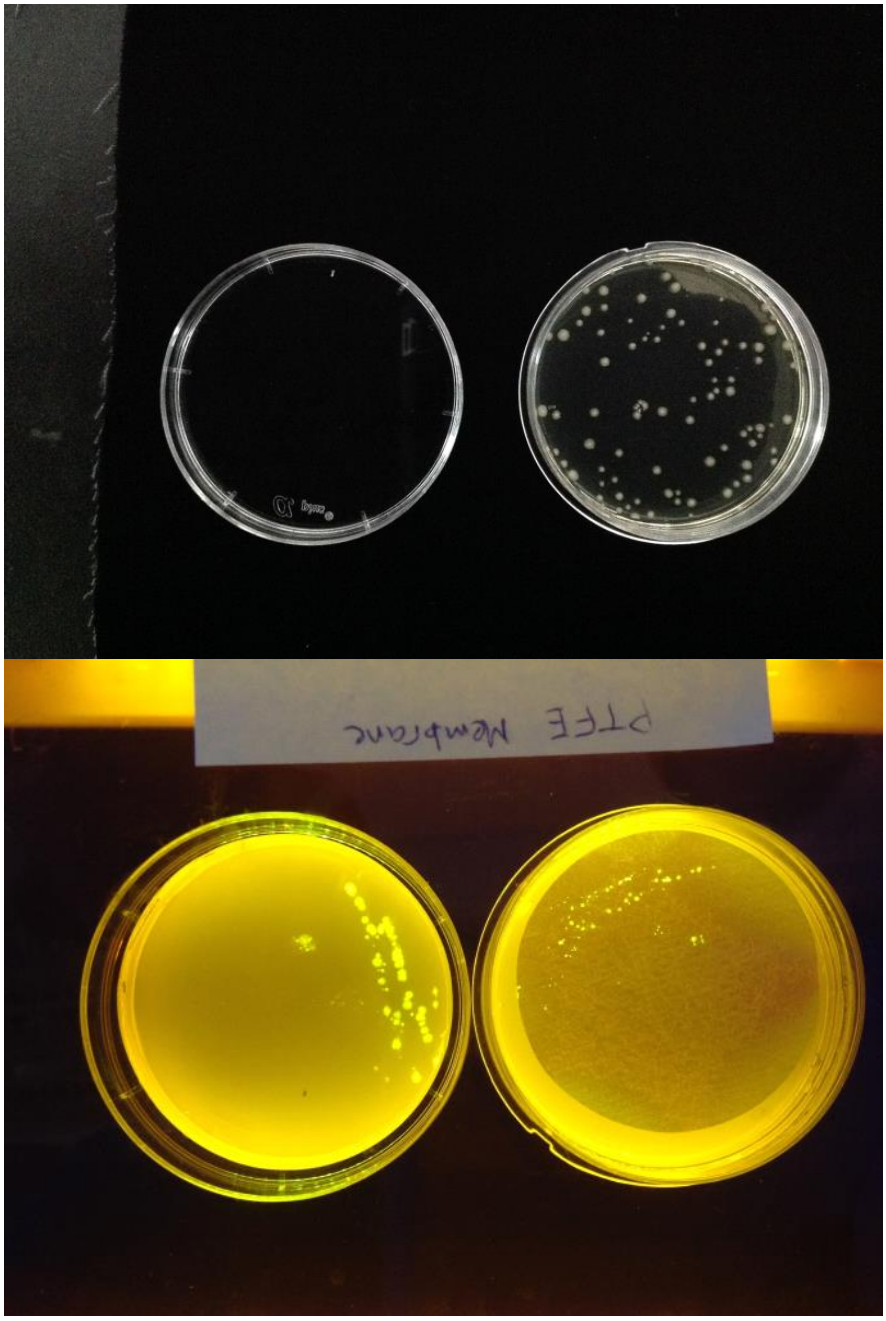
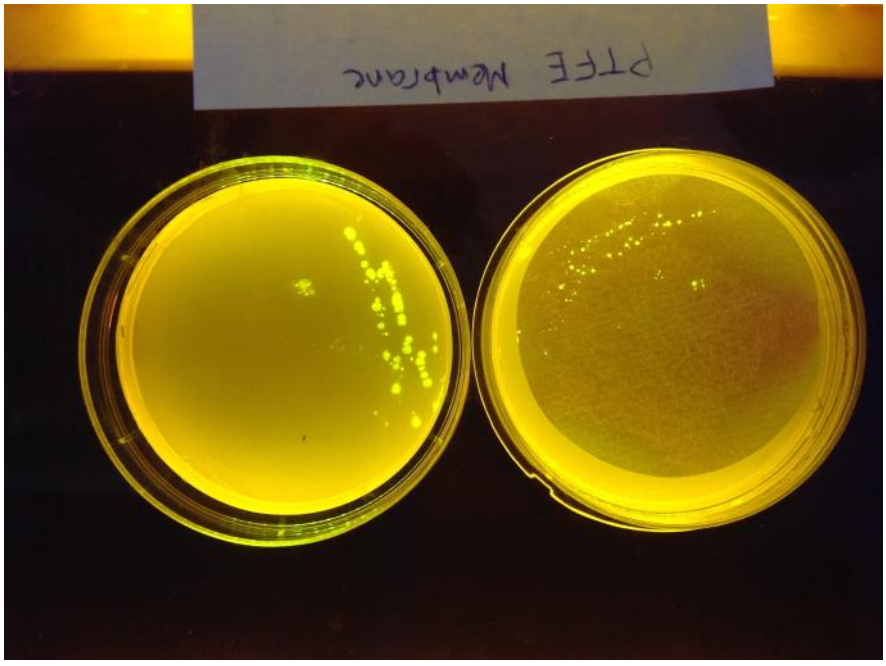
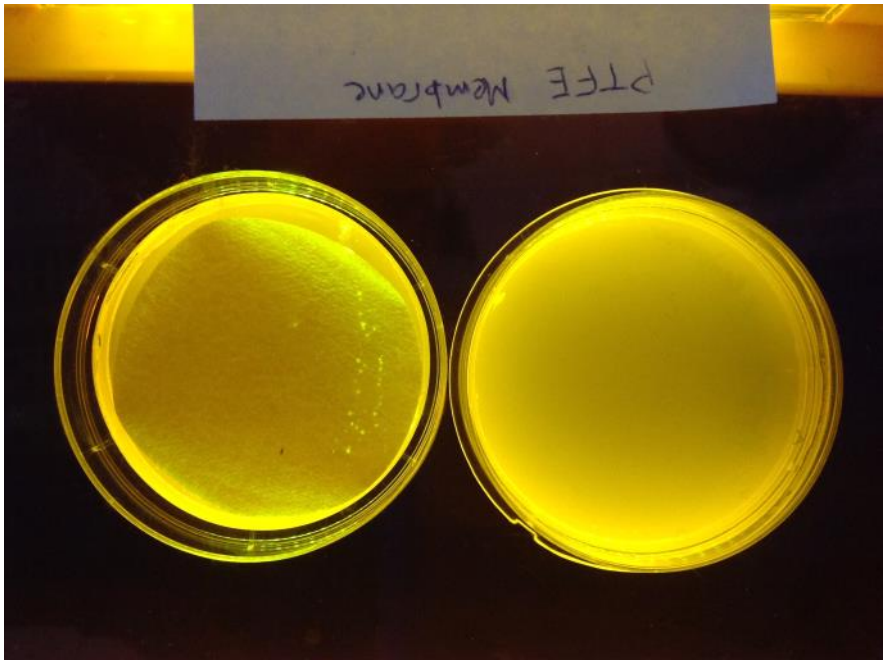
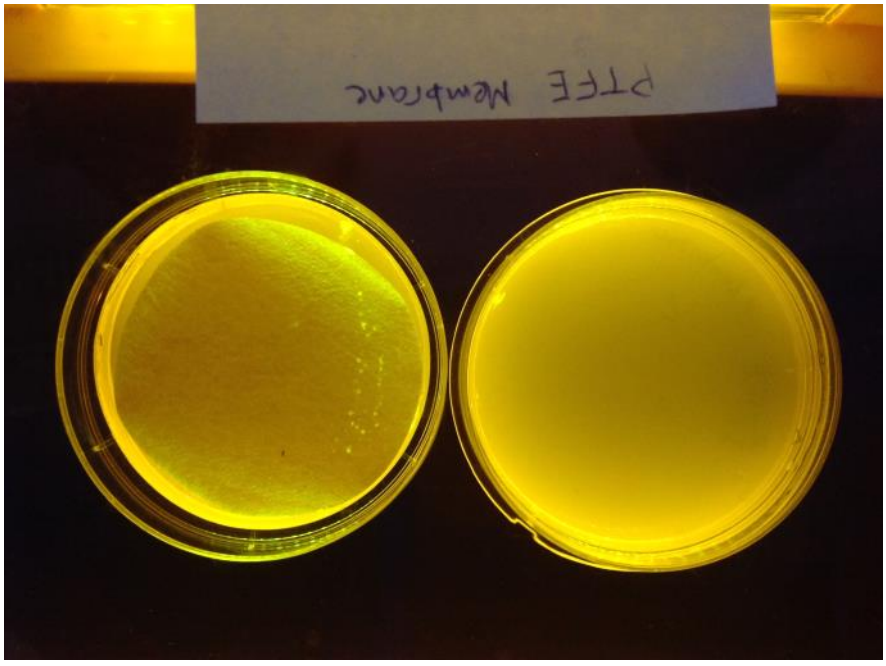


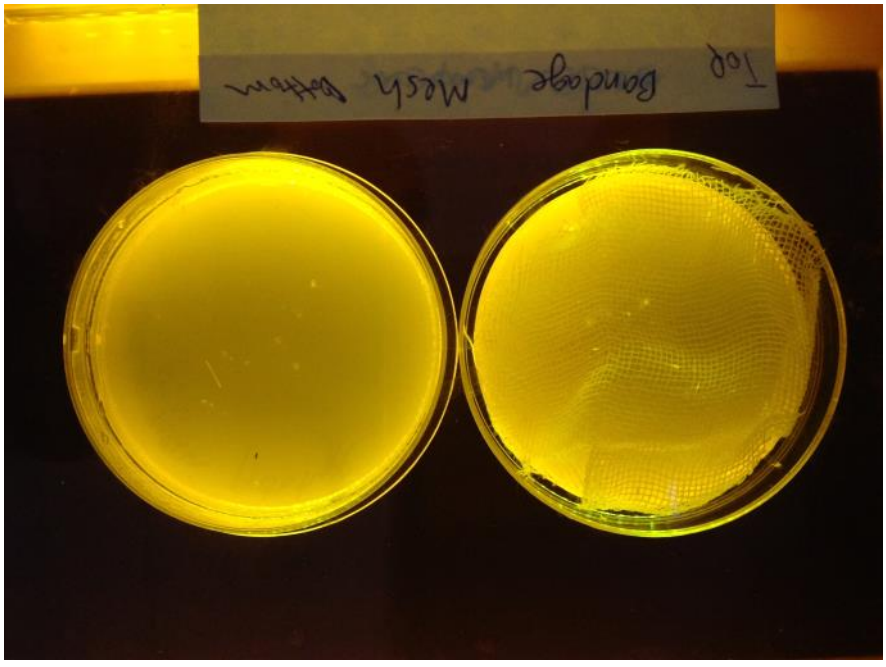
Image 2. Bacteria growth result after 20 hours detailed (Day 1)

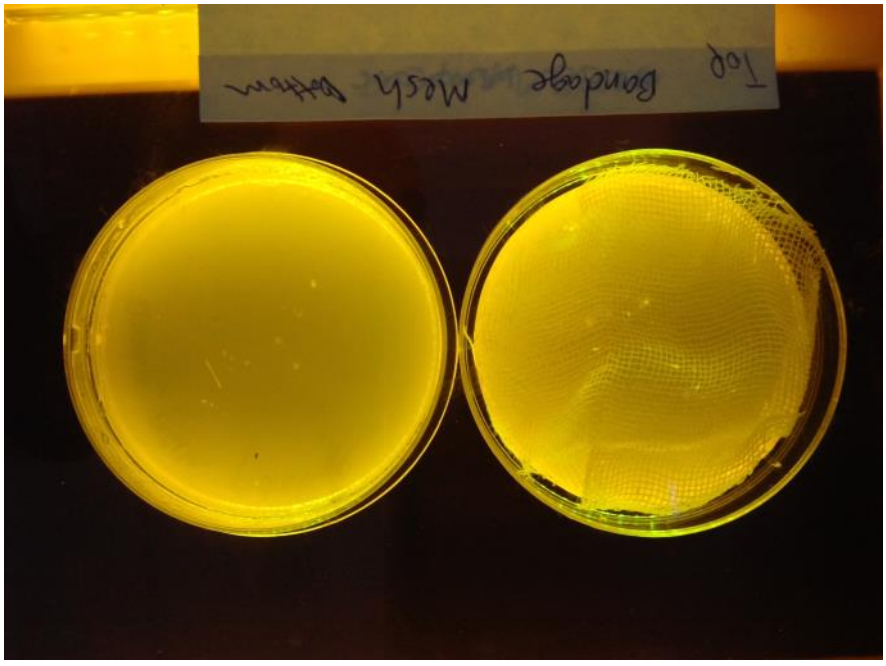

PTFE Membrane

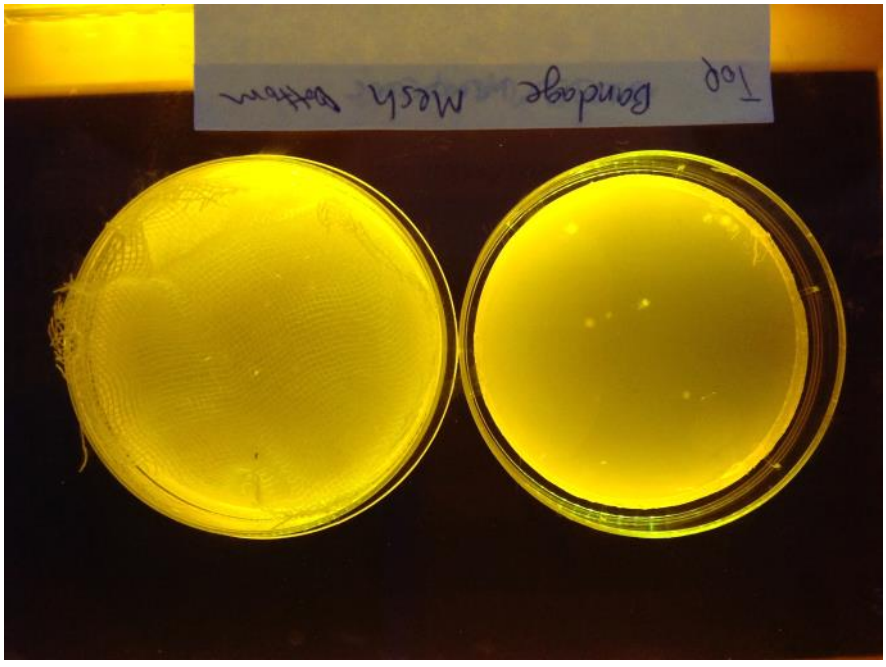

PTFE Membrane

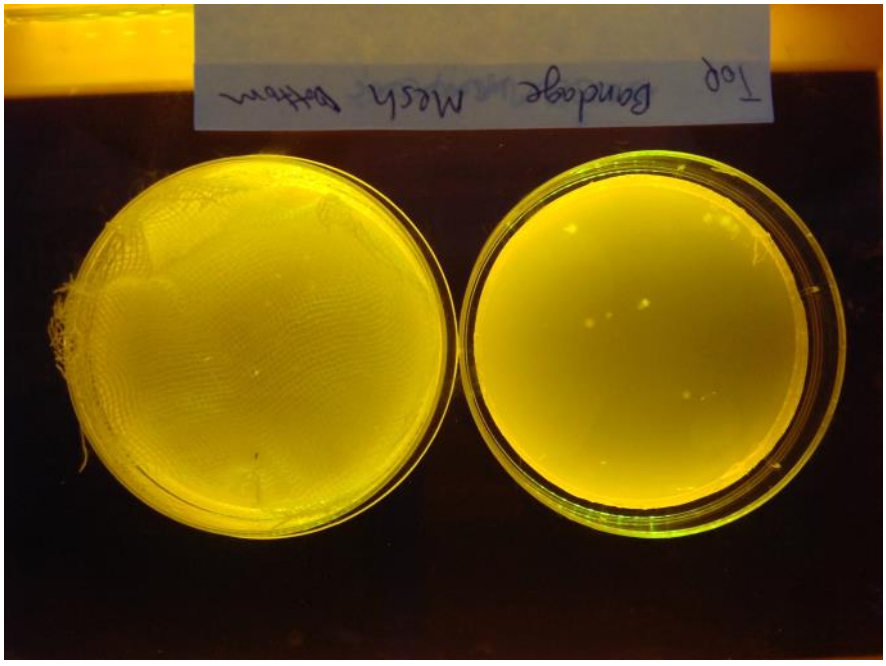

PTFE Membrane


PTFE Membrane

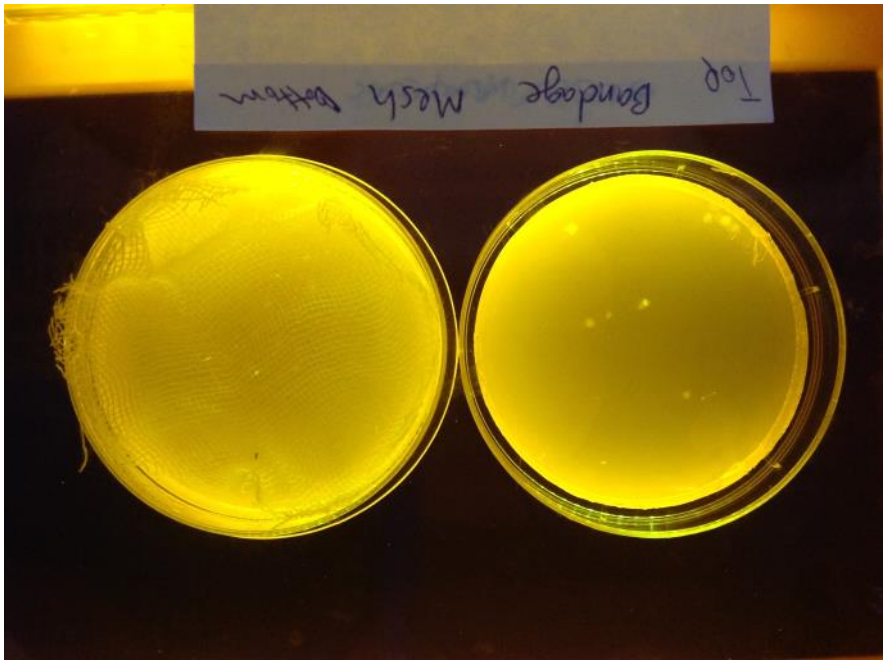

Top Bandage Mesh Pattern


Top Bandage Mesh Pattern

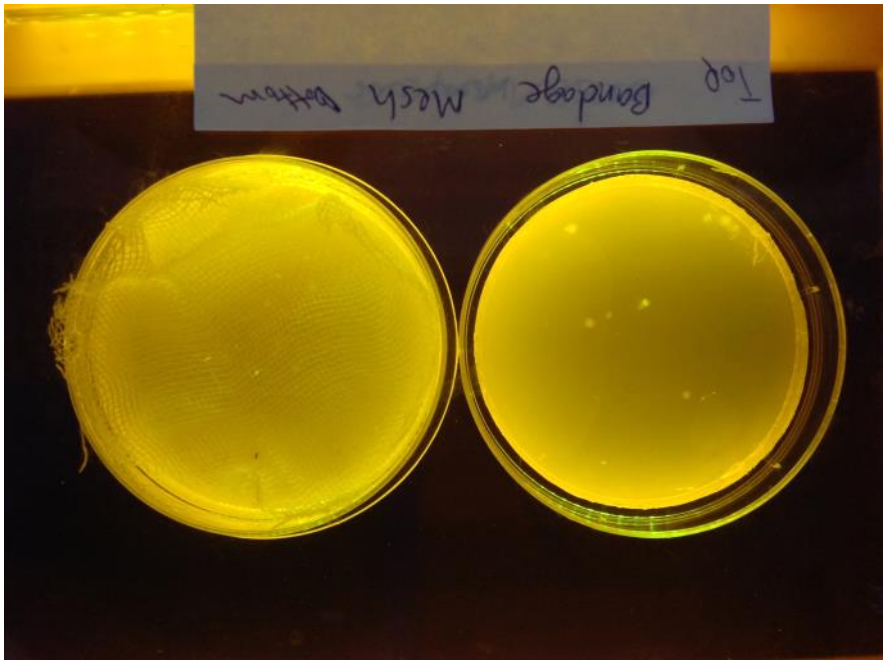

Top Bandage Mesh Pattern



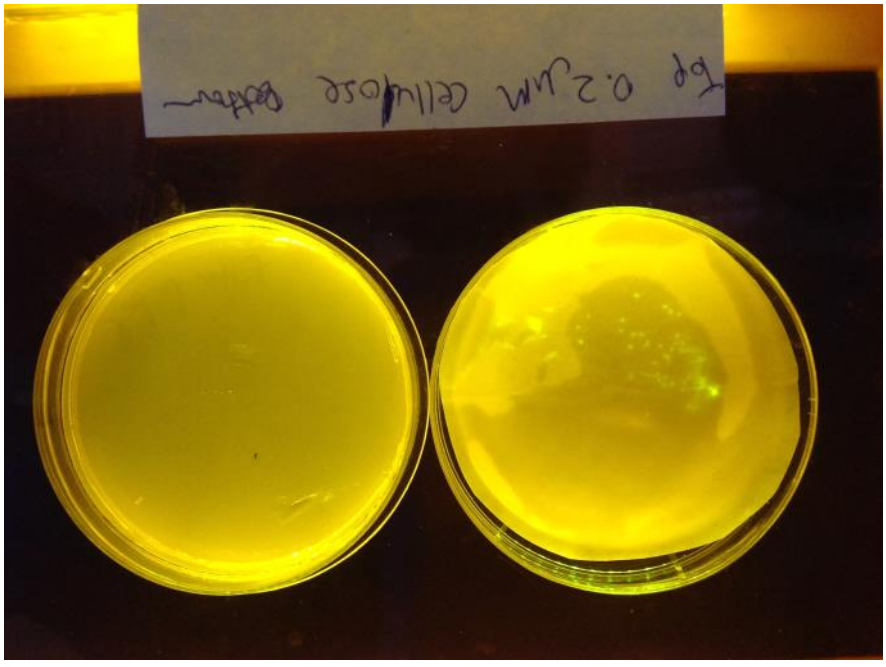
NOT CELLULOSE BUT .45 PTFE



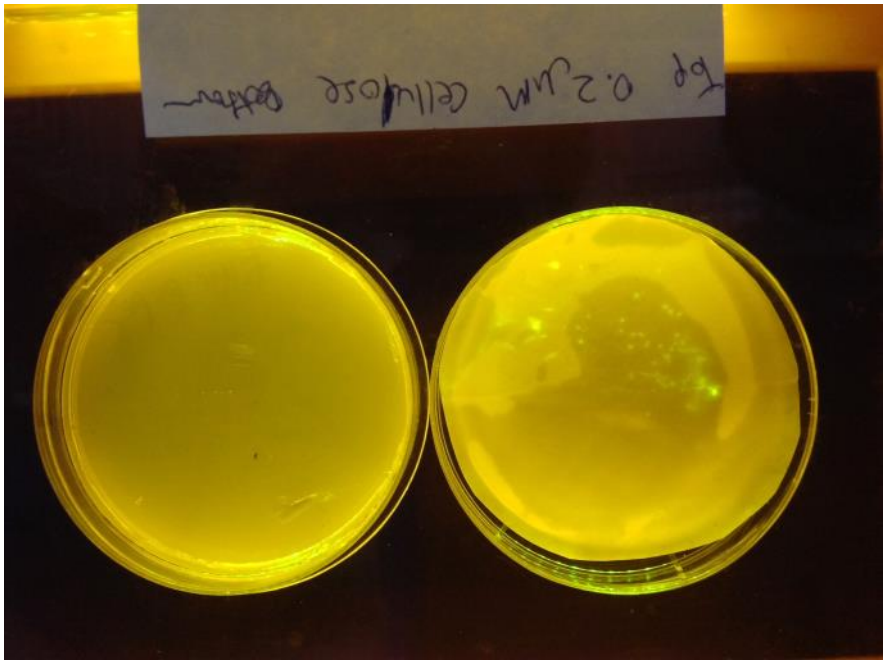
NOT CELLULOSE BUT .45 PTFE

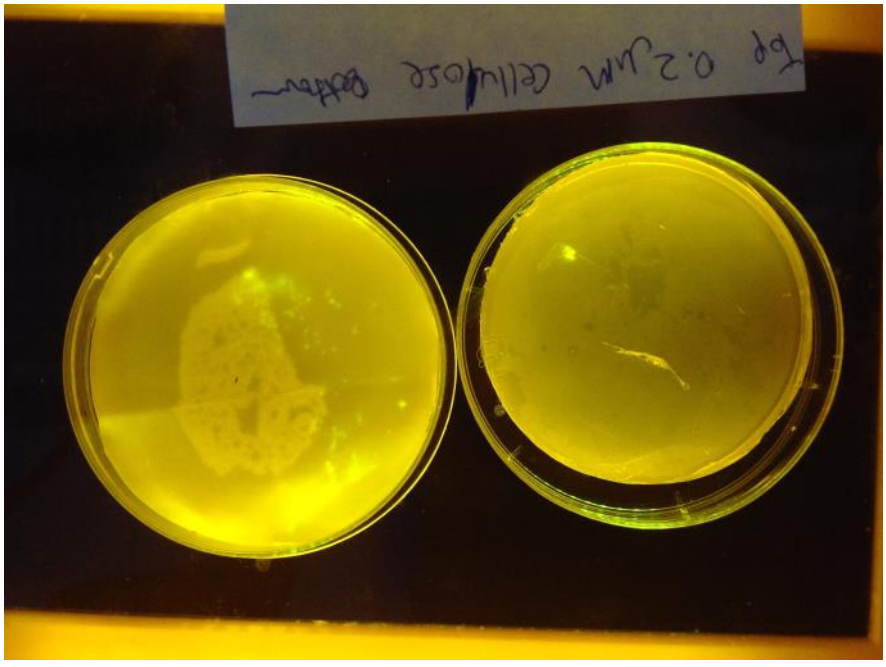


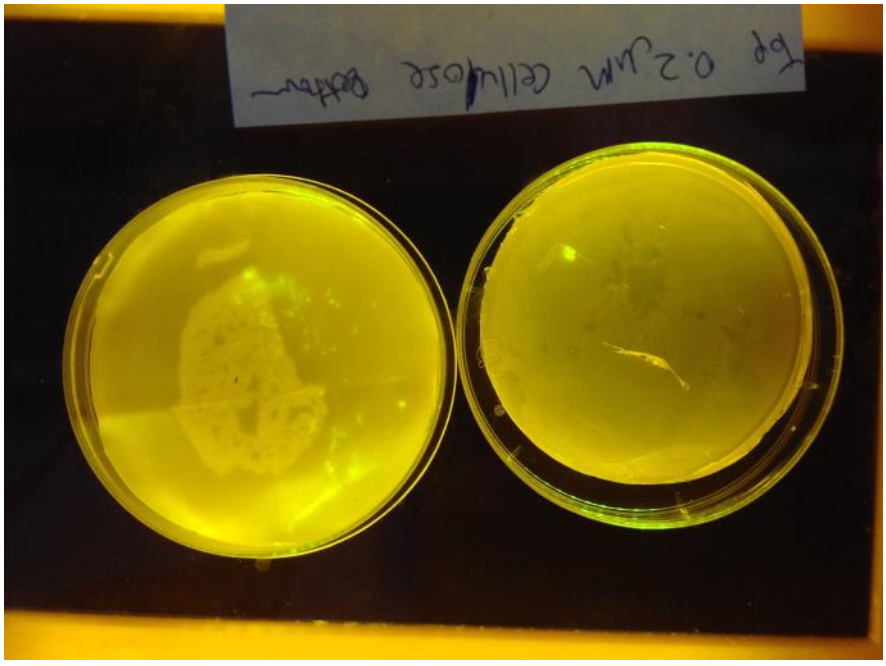
NOT CELLULOSE BUT .45 PTFE

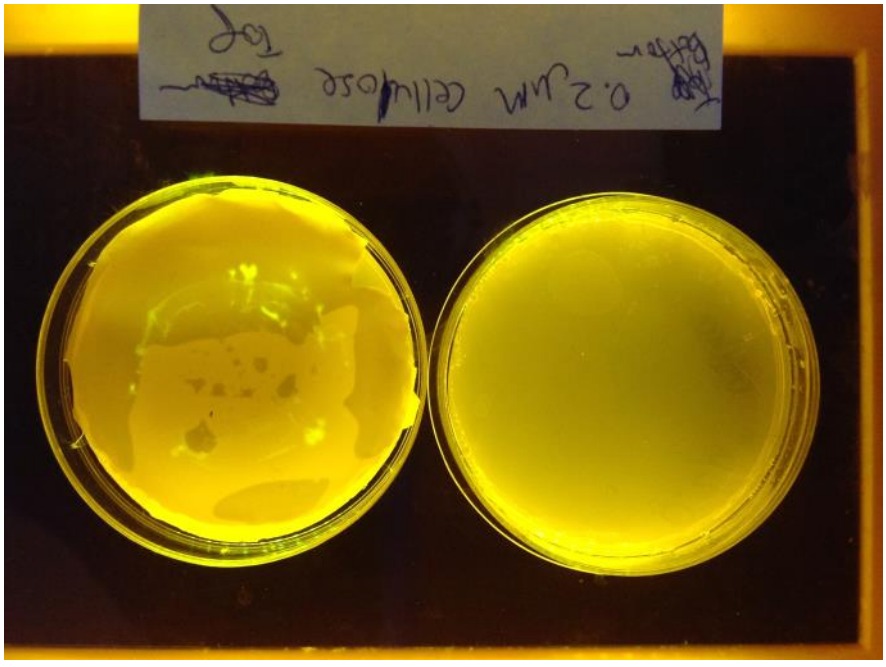


NOT CELLULOSE BUT .45 PTFE


Bp 0.2um cellulose


Bp 0.2um cellulose


0.2 µm cellulose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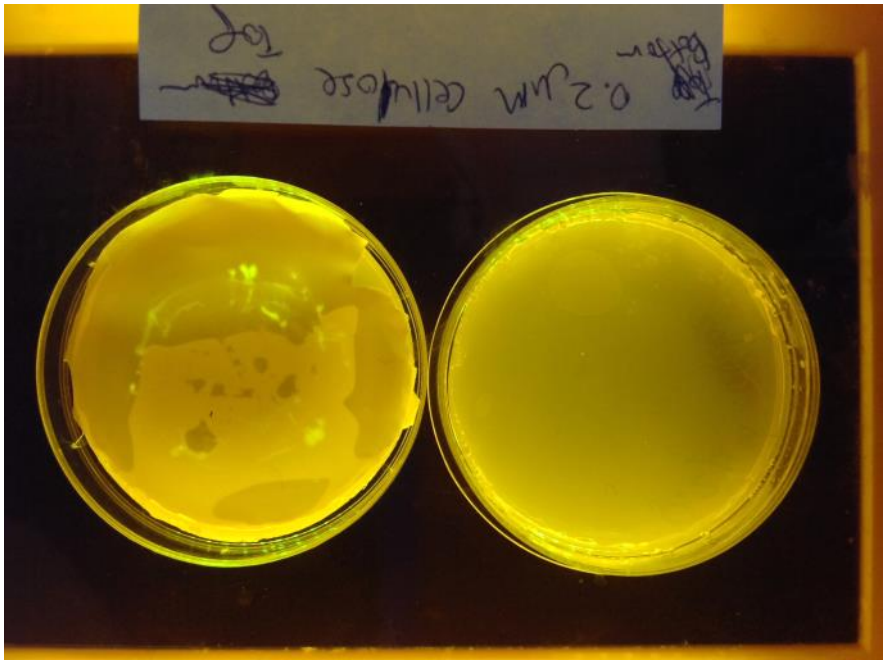
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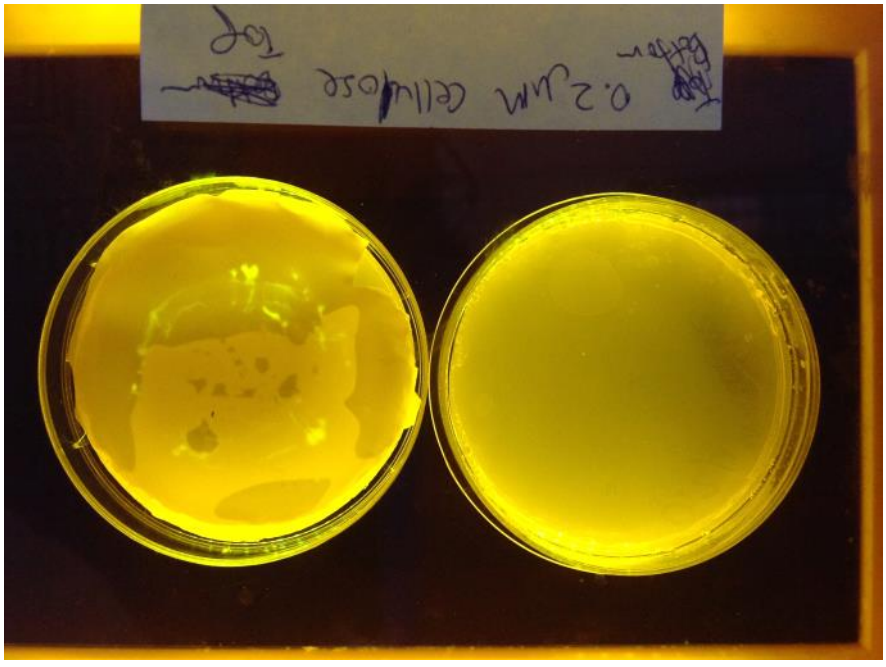
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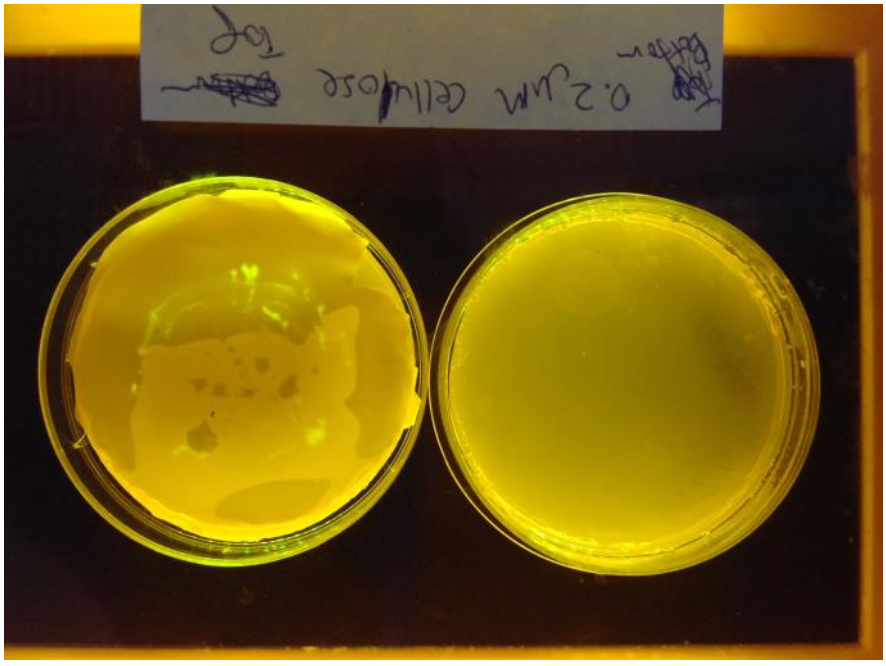
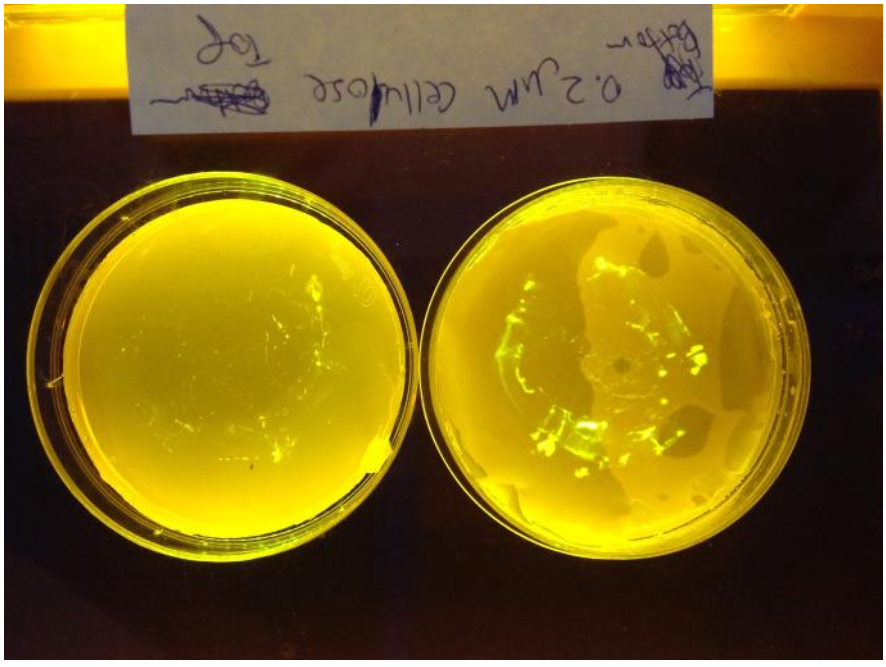

0.2µM cellulose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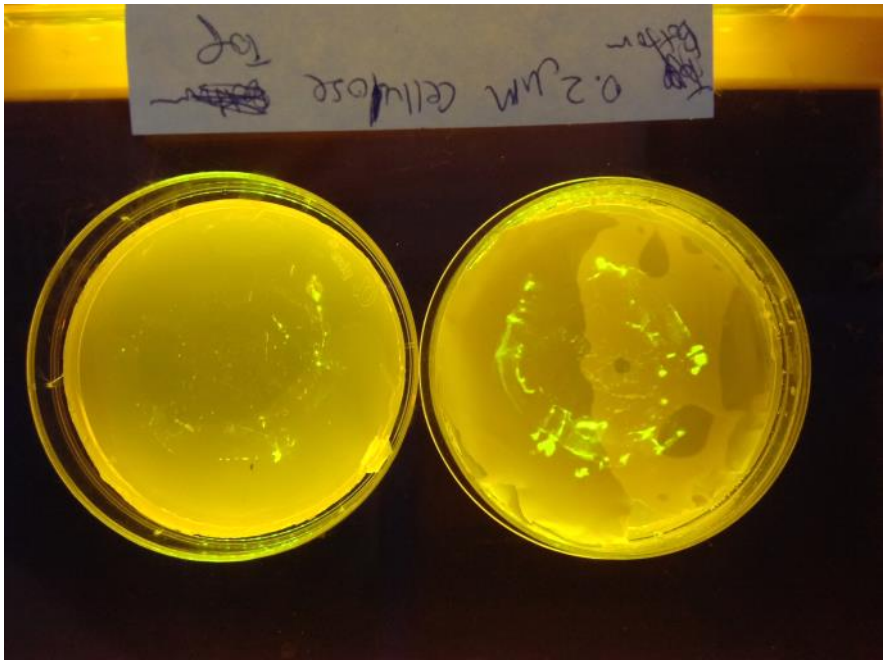
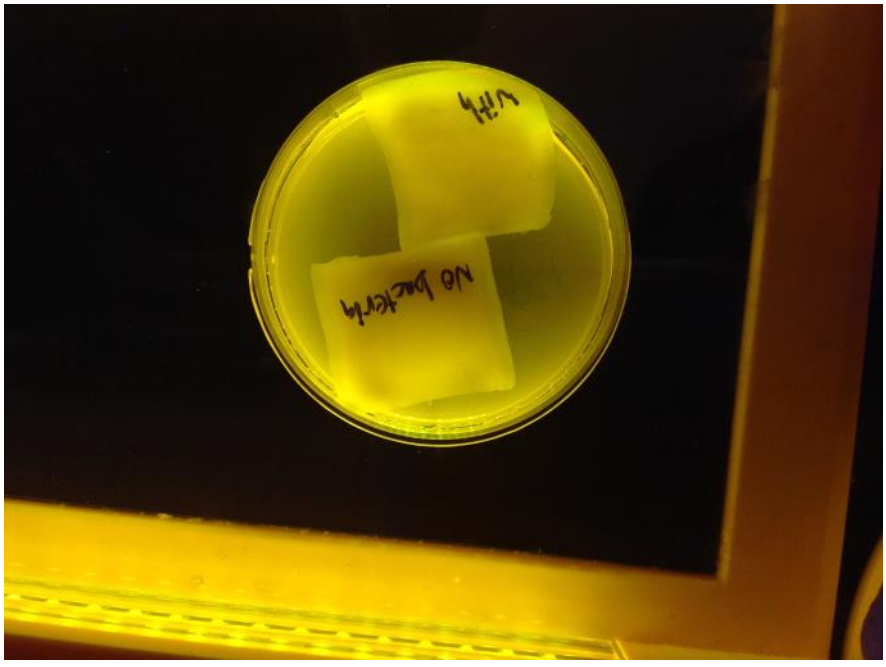
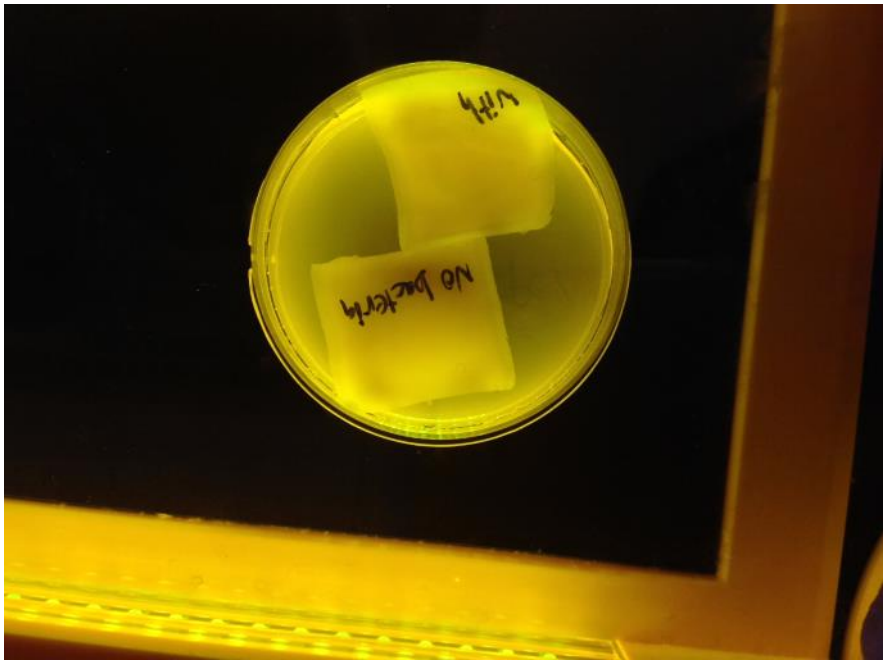
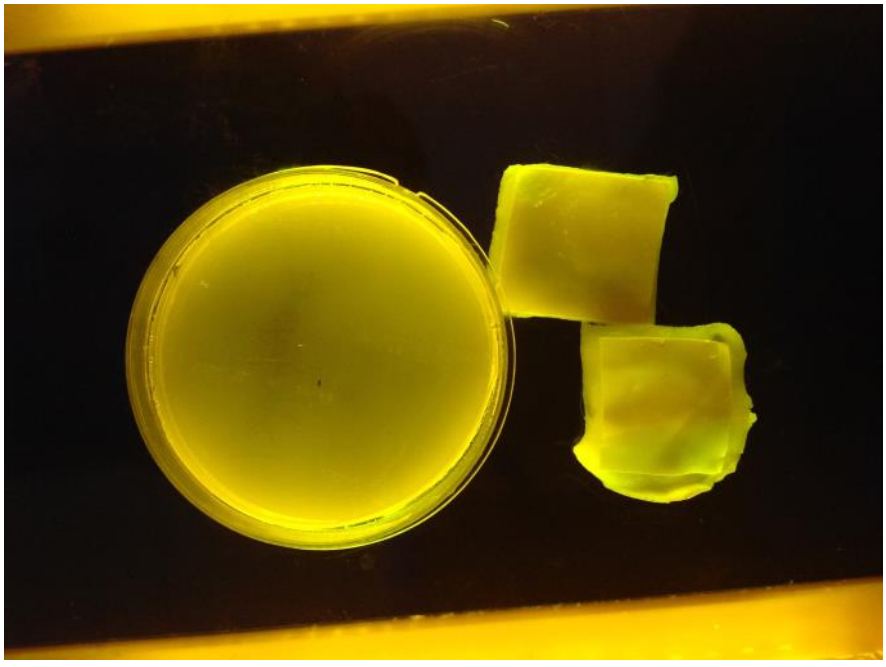
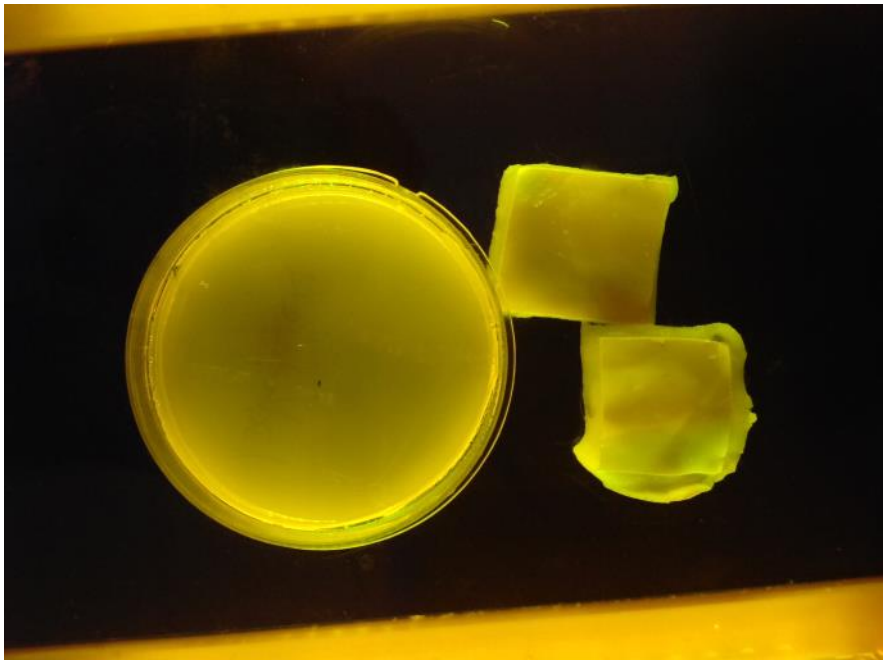
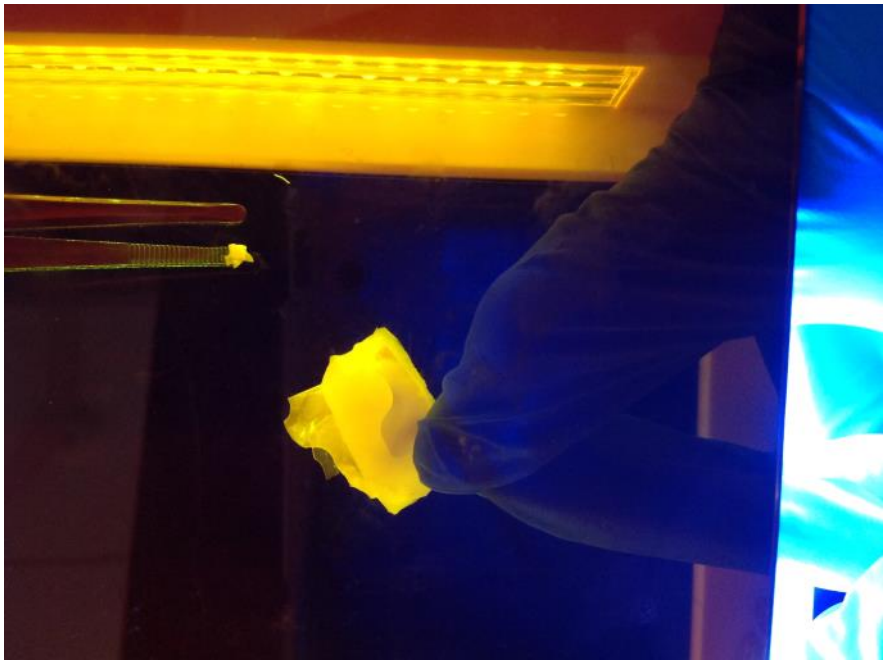

0.2 µM cellulose

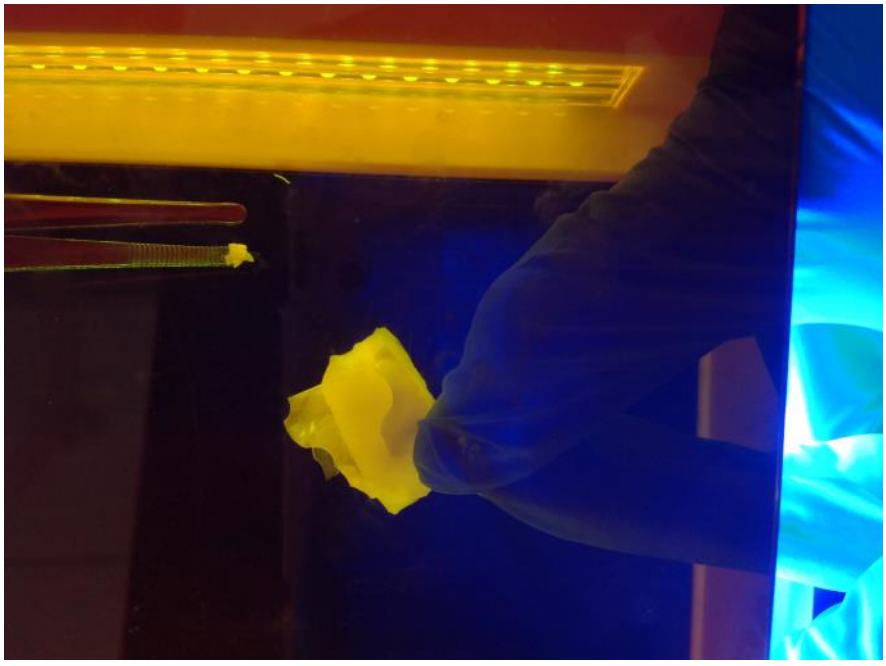

No bacteria
With

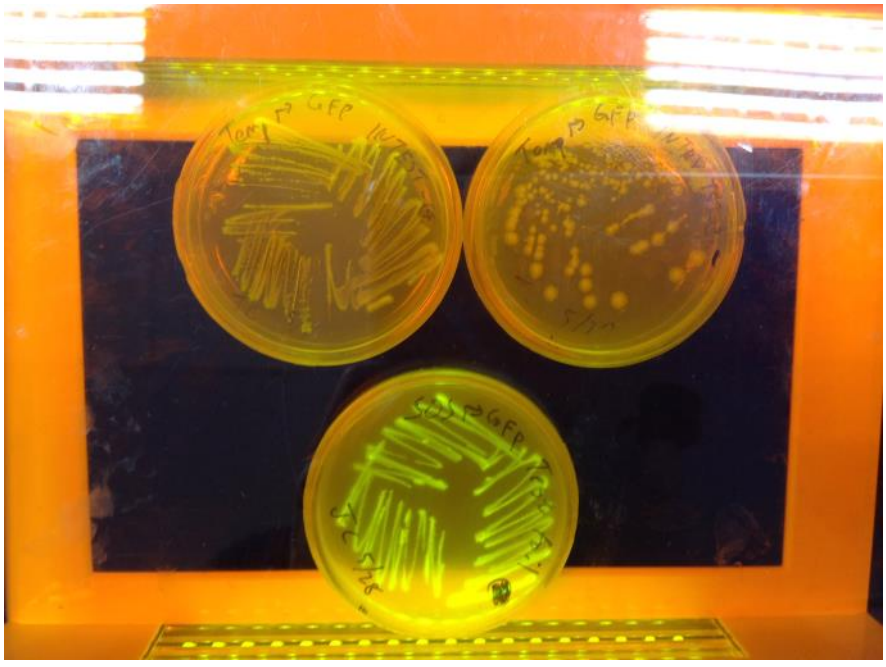

V.h
No bacteria

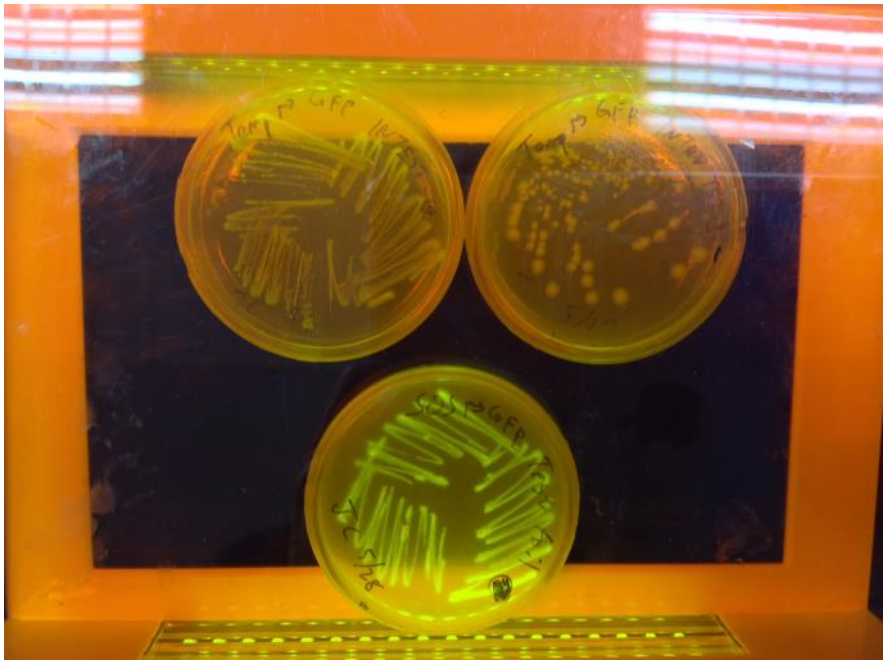












Experiments

Monday, June 08, 2015
4:25 PM

Purpose	Group	Experiments	Results	Conclusion/Notes	Next Step
Bacteria growth	**Bryan**	**Took pictures for the result and record time Moved the colony to 33 degrees**	**Larger colonies**	**Starting to observe what happens under average skin temperature**	**Check and take pictures after a 24 hour growth period**
Prototype	**Phillip**	**Did redid the experiments this time with the filter only covering half of the plate. Providing a control to contrast the results if the filter actually succeeded in blocking the bacteria**	**Photos are in 6.11**		
ACT	**Leon**	**PCR started**	**Low concentration**	**Made changes during step 2 lower temperature to allow primers to bind**	
UV Promoter	**Leon**	**4-16 SOS miniprep 4-24 SOS miniprep**	**2 UV promoters grew and minipreped**	**Ready for sequencing**	

Photo

Wednesday, June 10, 2015
4:05 PM

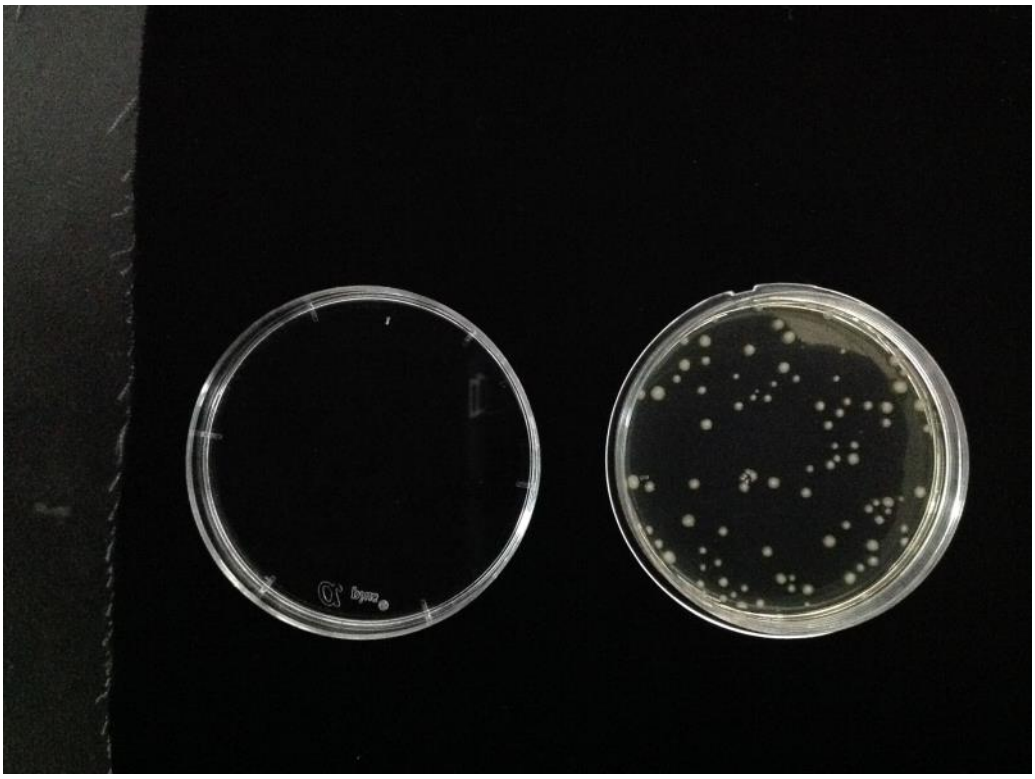


Image 1. Bacteria growth result after 44 hours (Day 2)

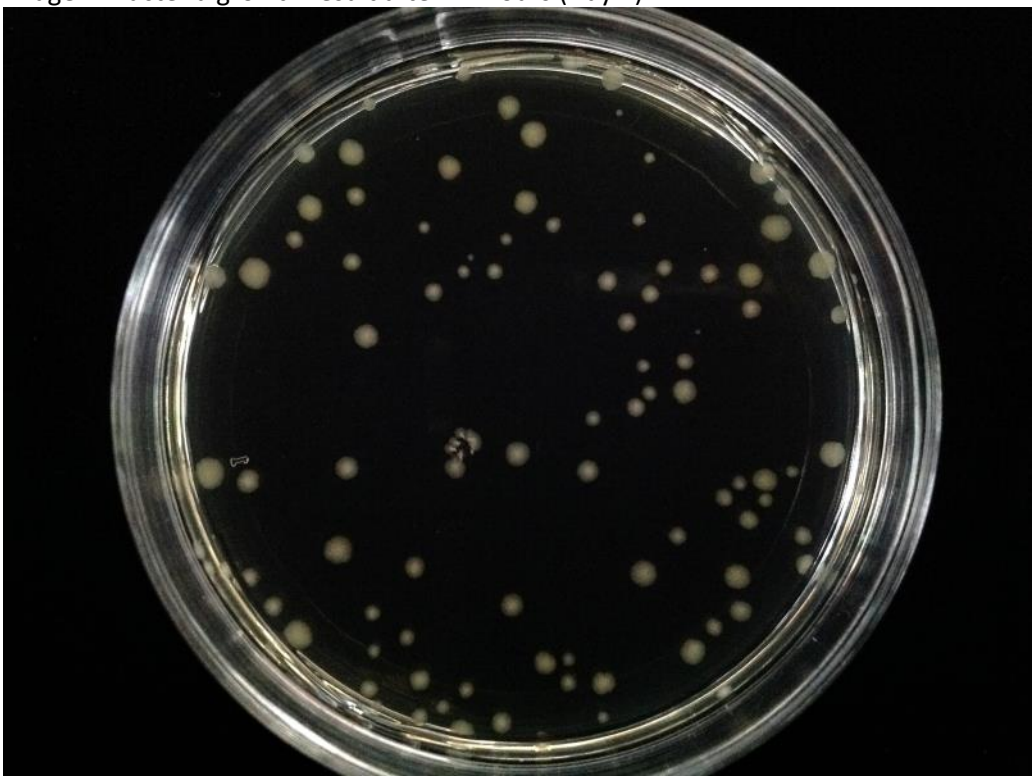


Image 2. Bacteria growth result after 44 hours detailed (Day 2)

Experiments

Wednesday, June 10, 2015
3:09 PM

Purpose	Group	Experiments	Results	Conclusion/Notes	Next Step
Prototype	**Phillip**	**None**	**Checked from yesterday.**		
Bacteria growth	**Bryan**	**Took pictures for the result and record time Continue to leave it in 33 degrees**	**Colonies with approximately the same size**	**Starting to observe what happens under average skin temperature**	**Check and take pictures after a 24 hour growth period**
ACT	**Leon**	**PCR + digest**	**High concentration after PCR 86 ng/uL**	**Digestion looks like it is ok**	**ligation**
UV promoter	**Leon**	**Miniprep**	**Completed**	**Ready for sequencing**	

Photos

Thursday, June 11, 2015
3:43 PM

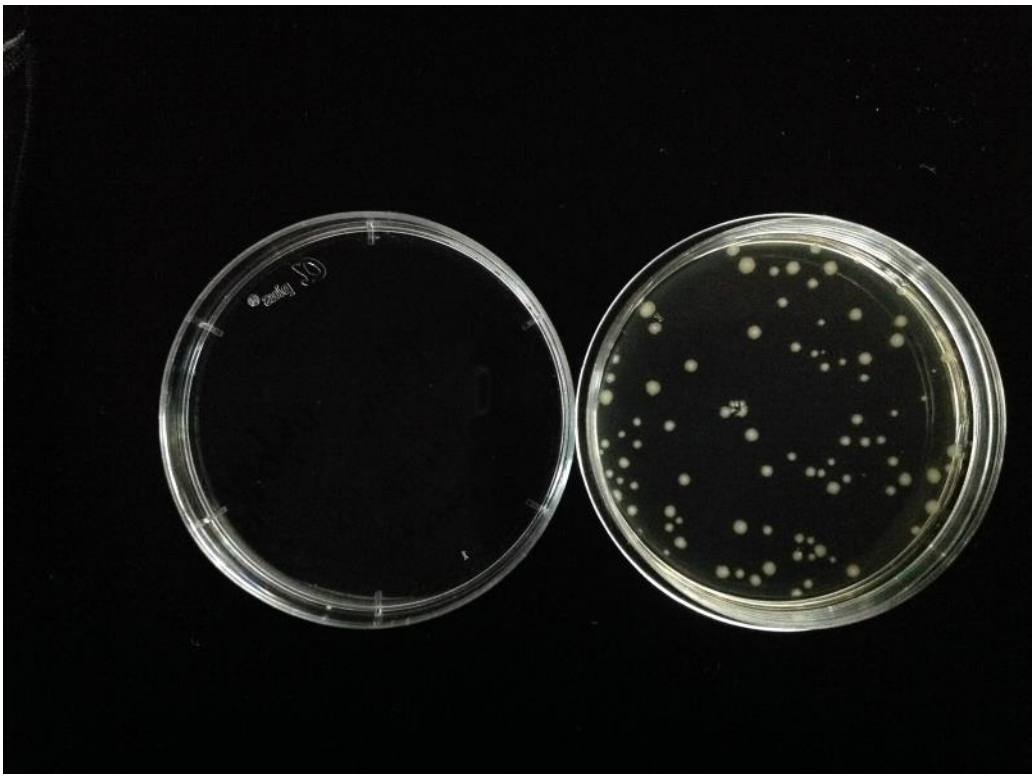


Image 1. Bacteria growth result after 68 hours (Day 3)

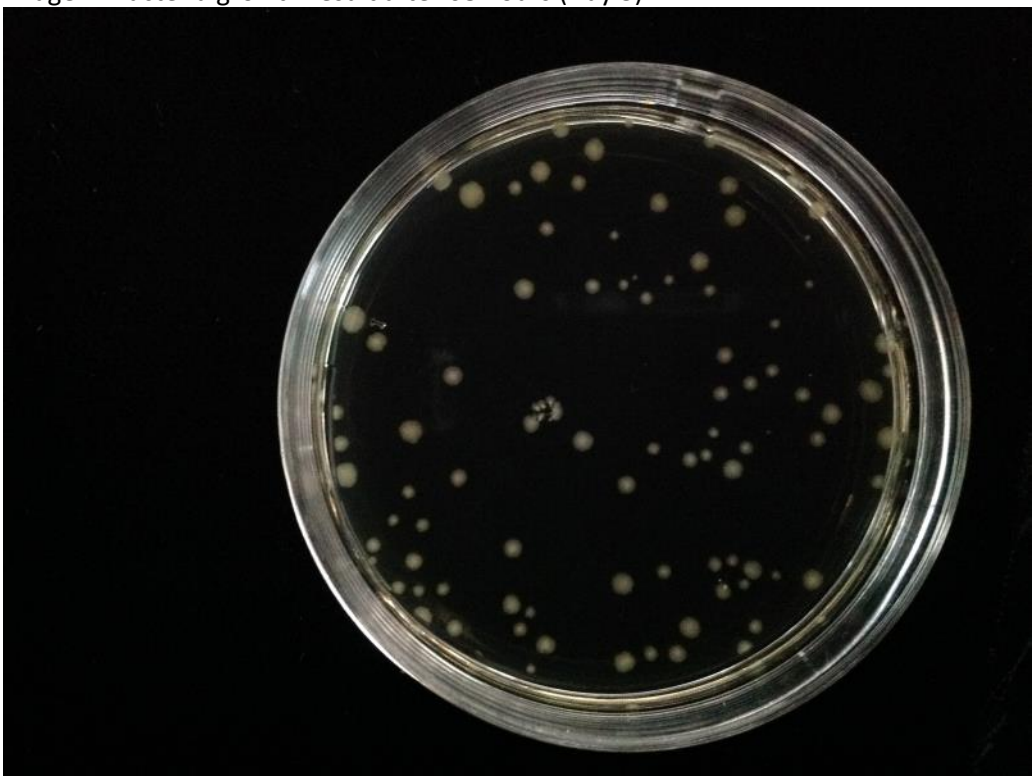
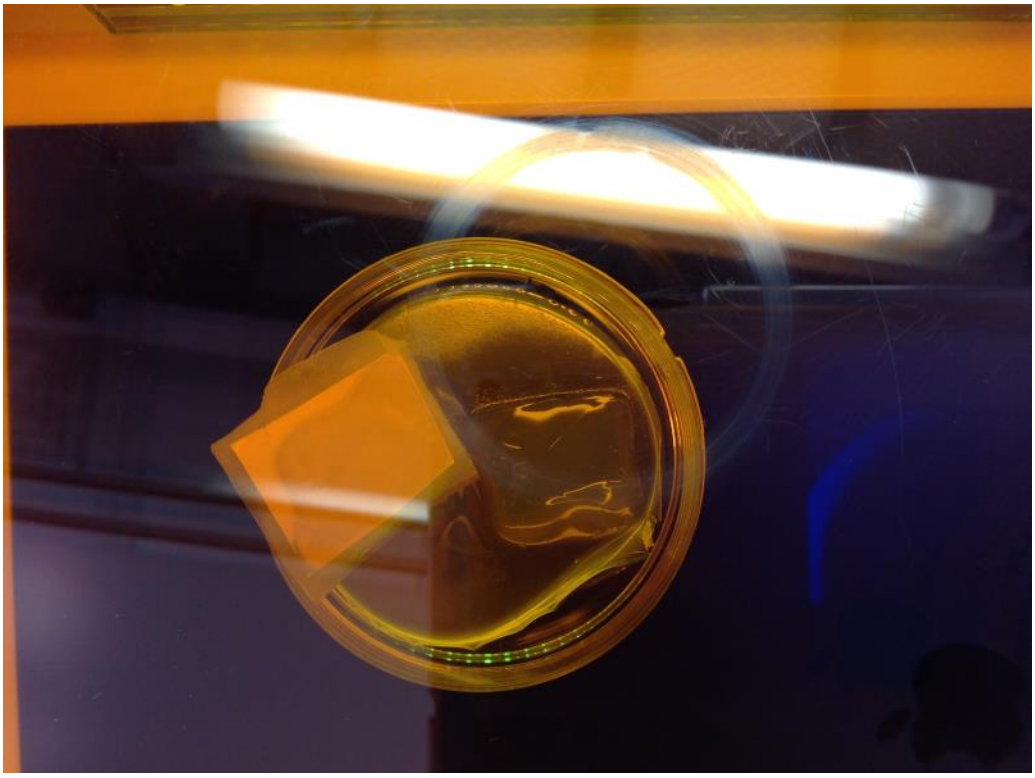
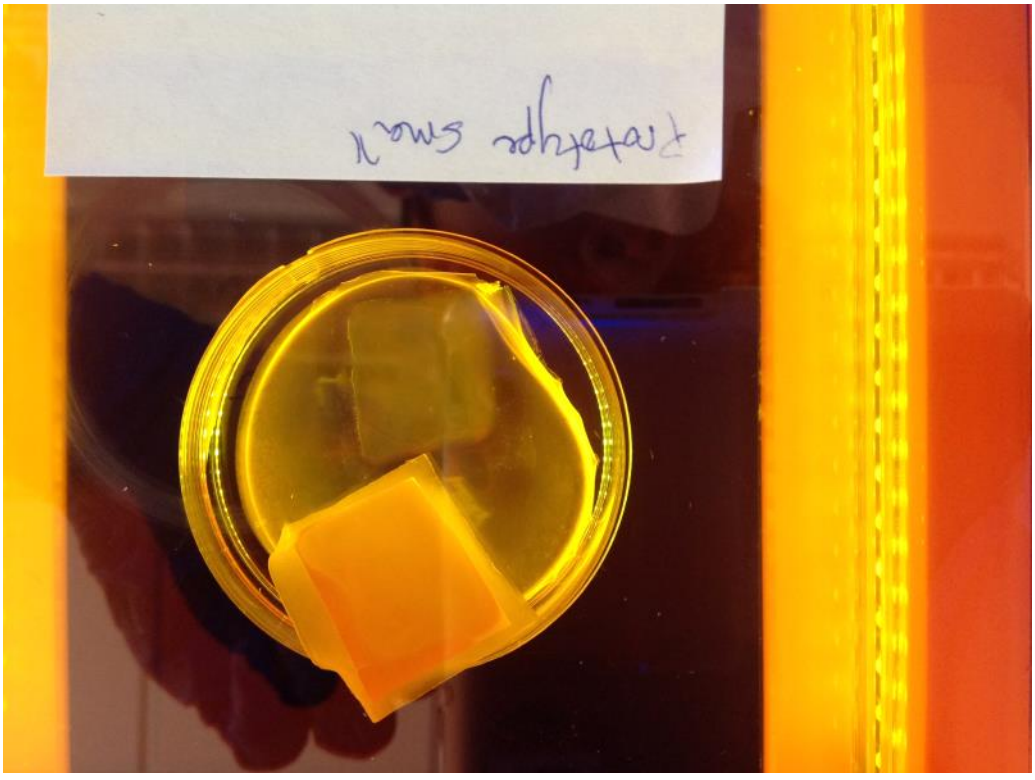
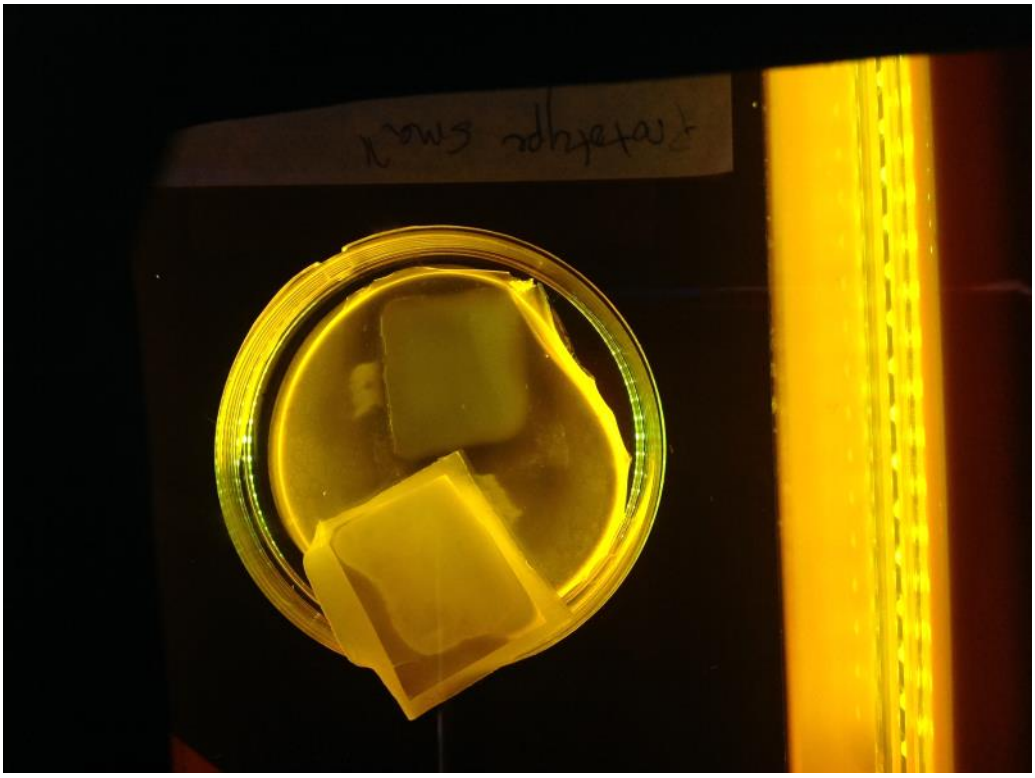
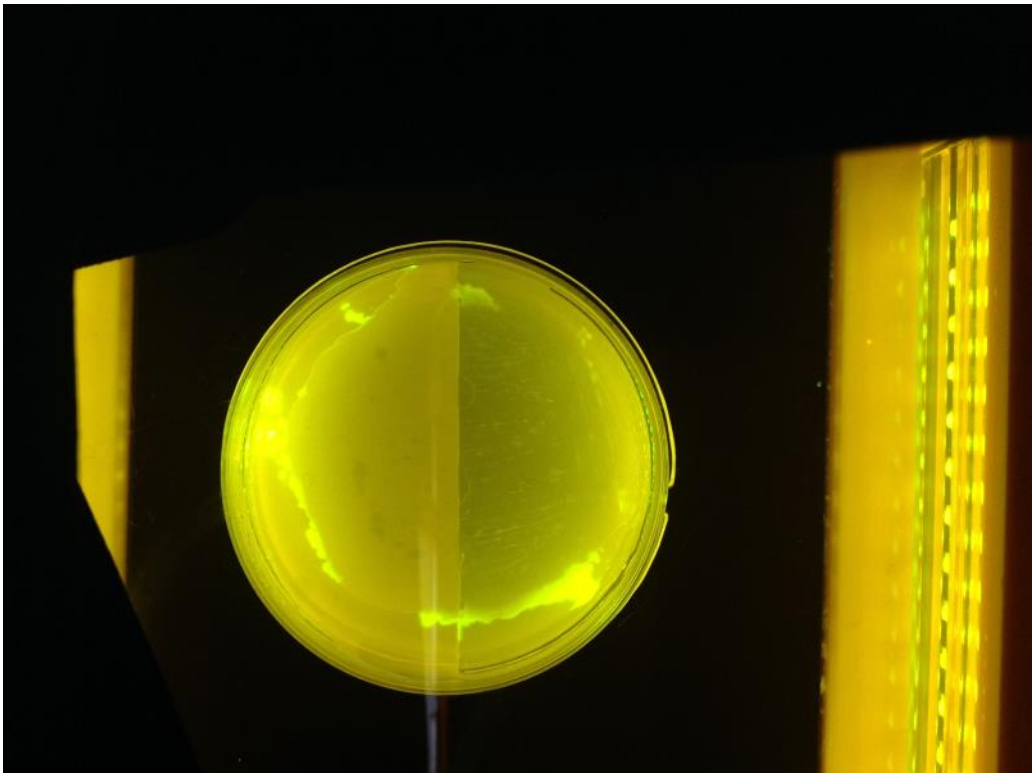


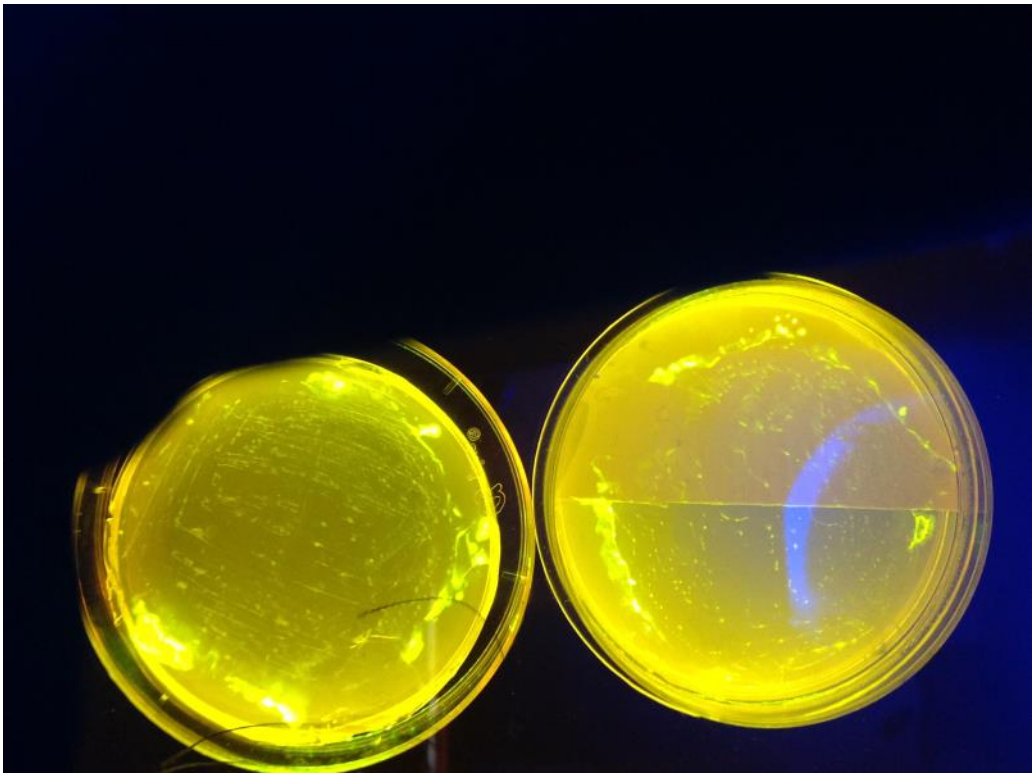
Image 2. Bacteria growth result after 68 hours detailed (Day 3)

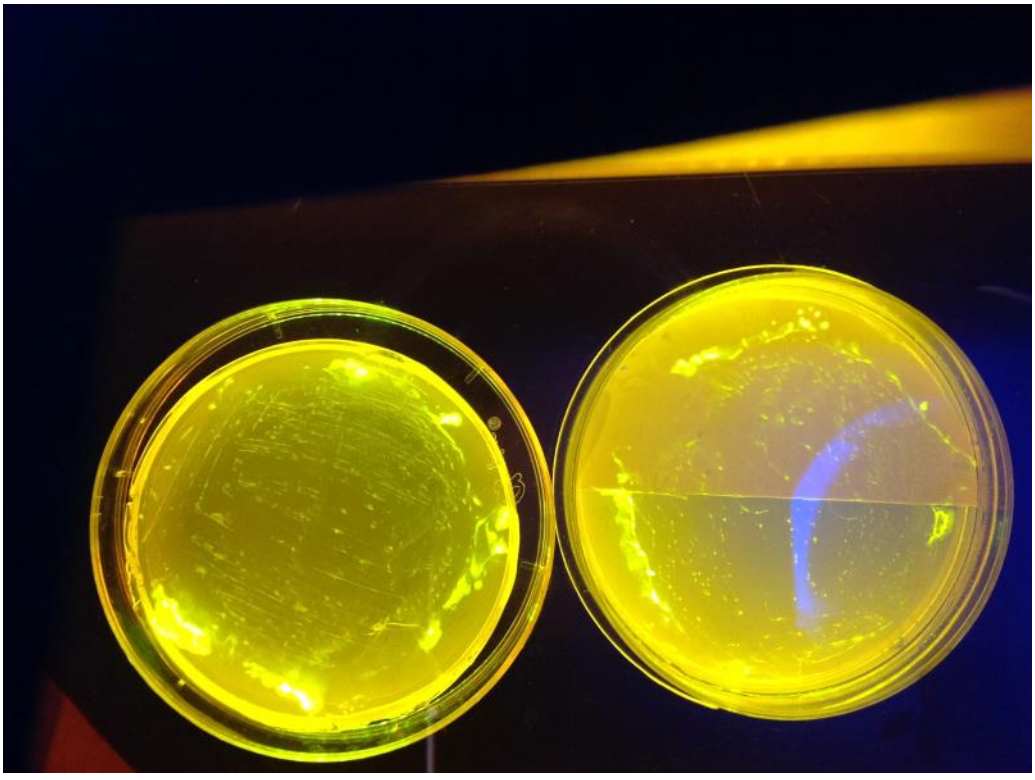


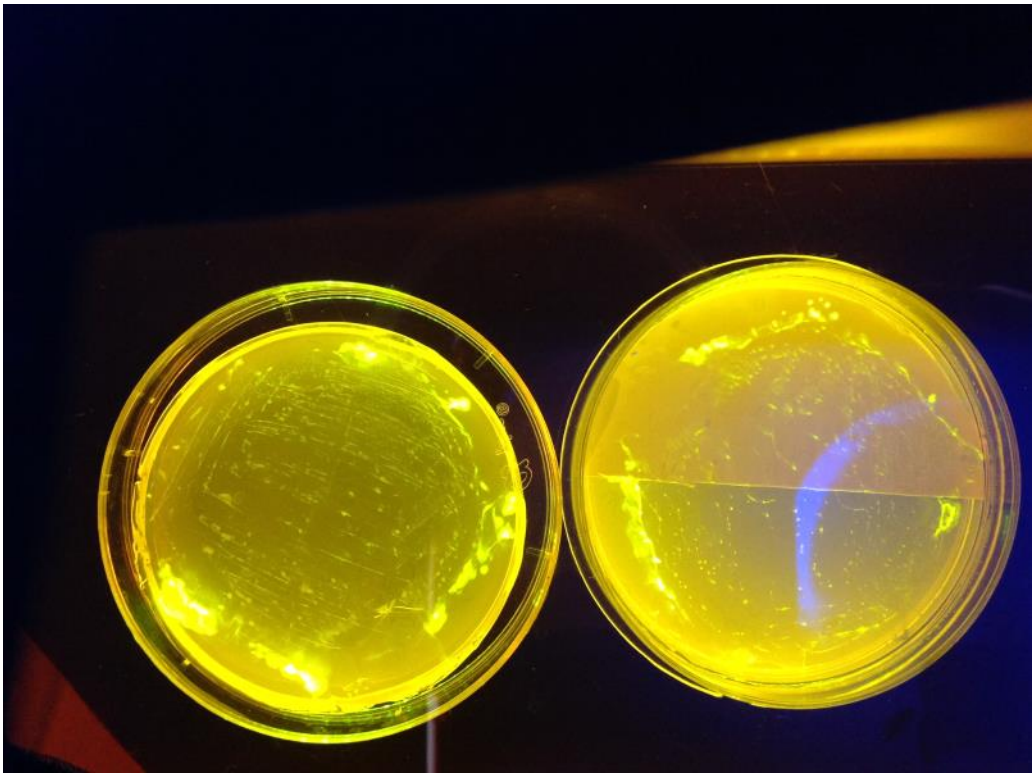


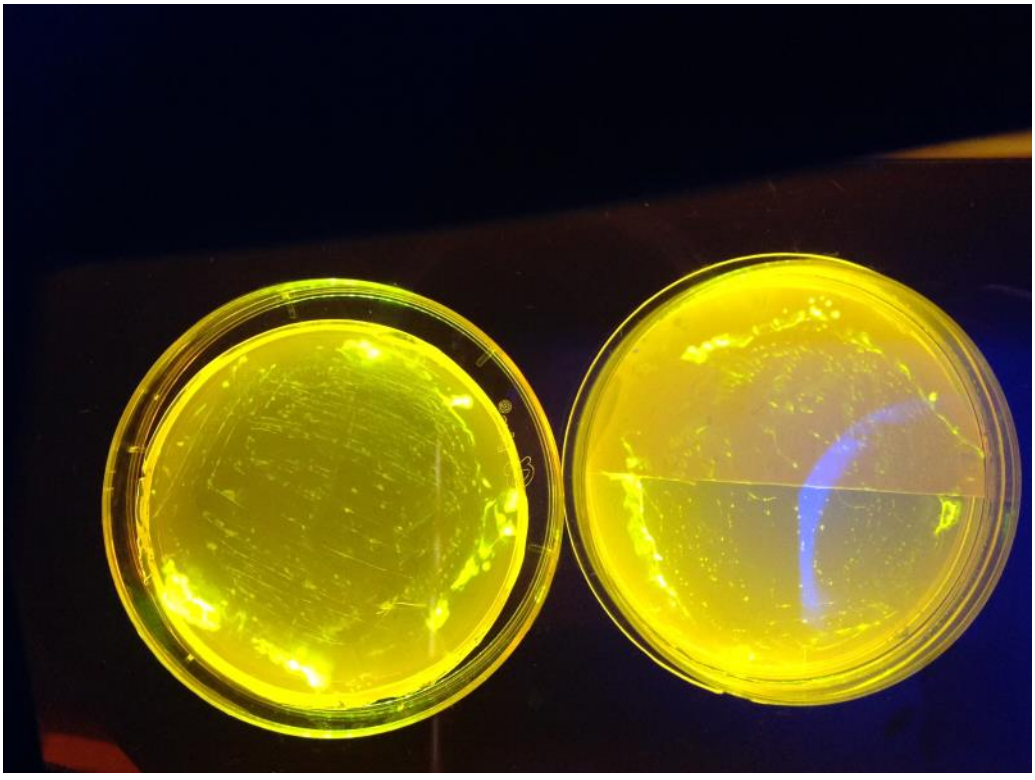


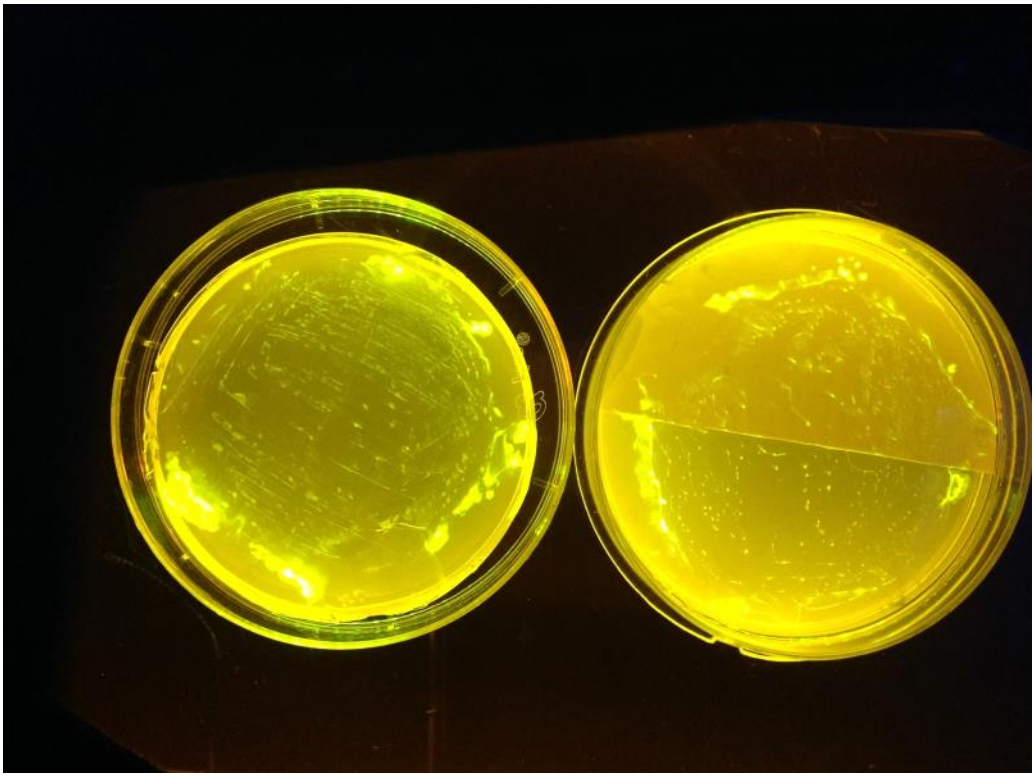


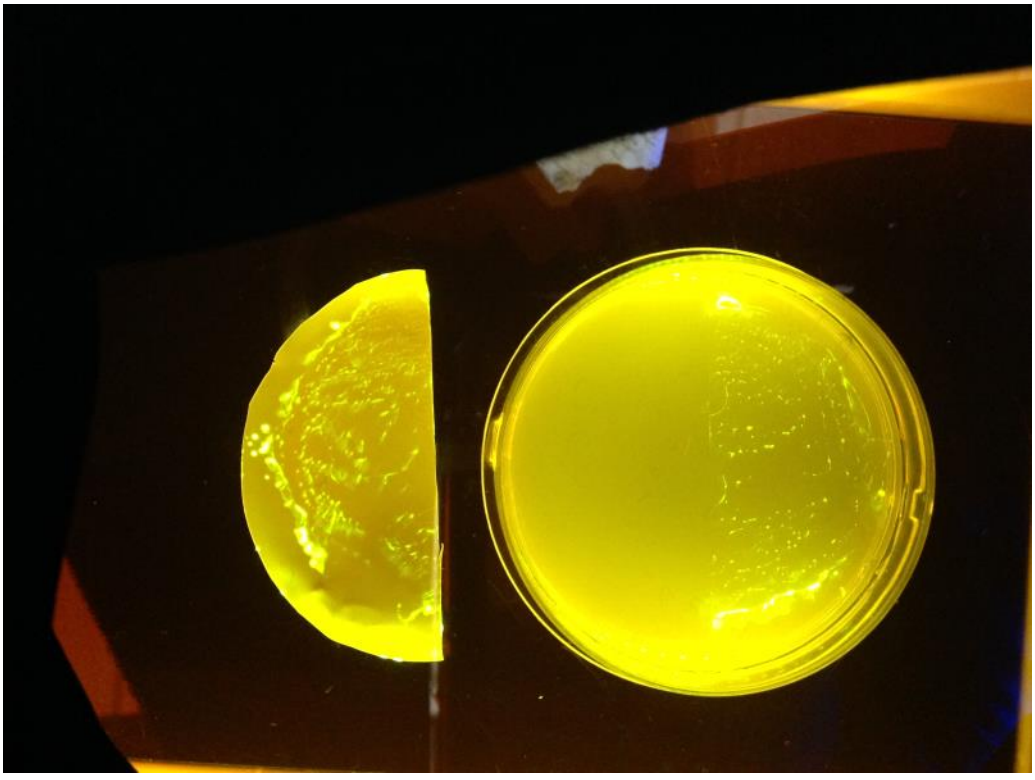


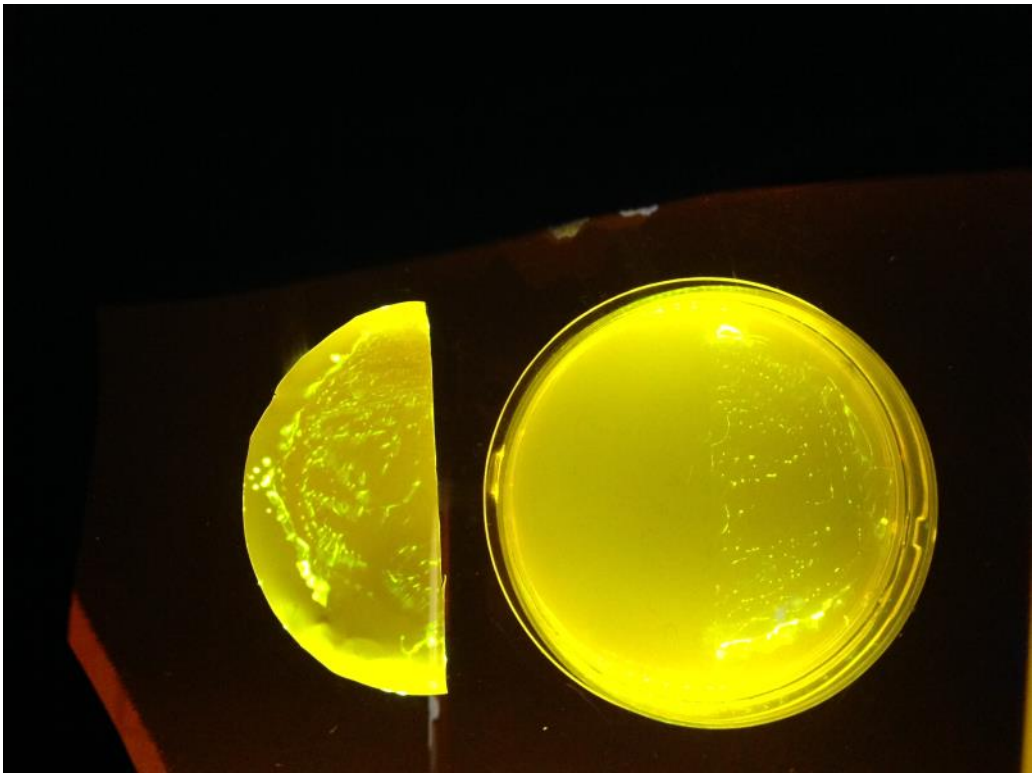


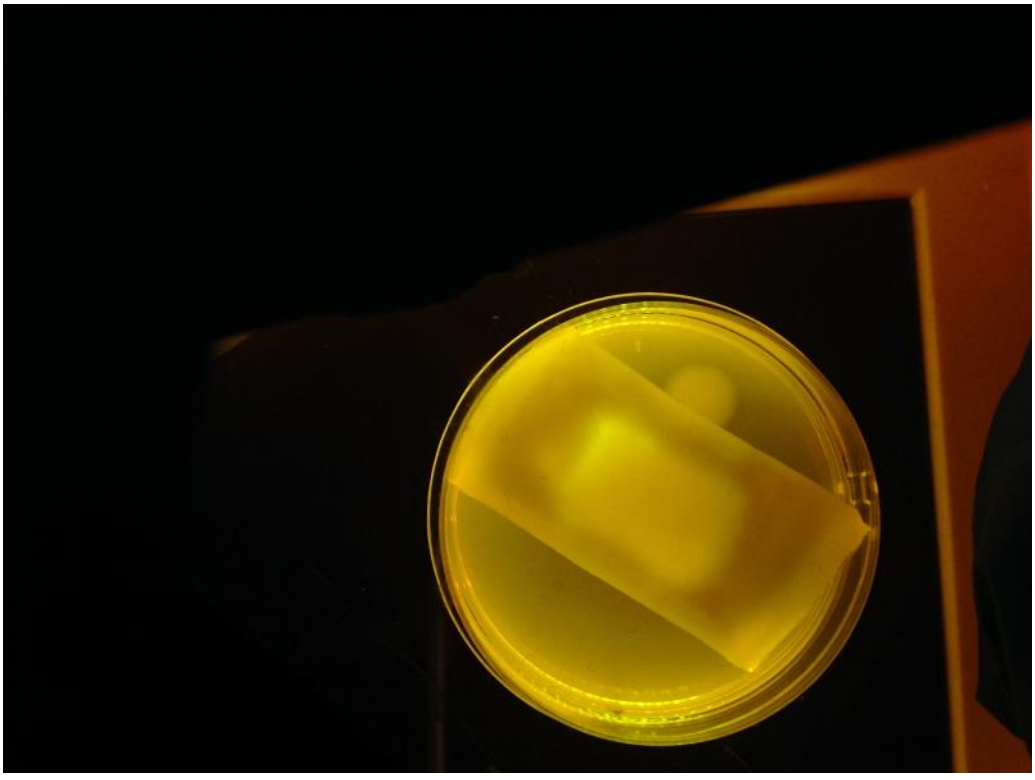


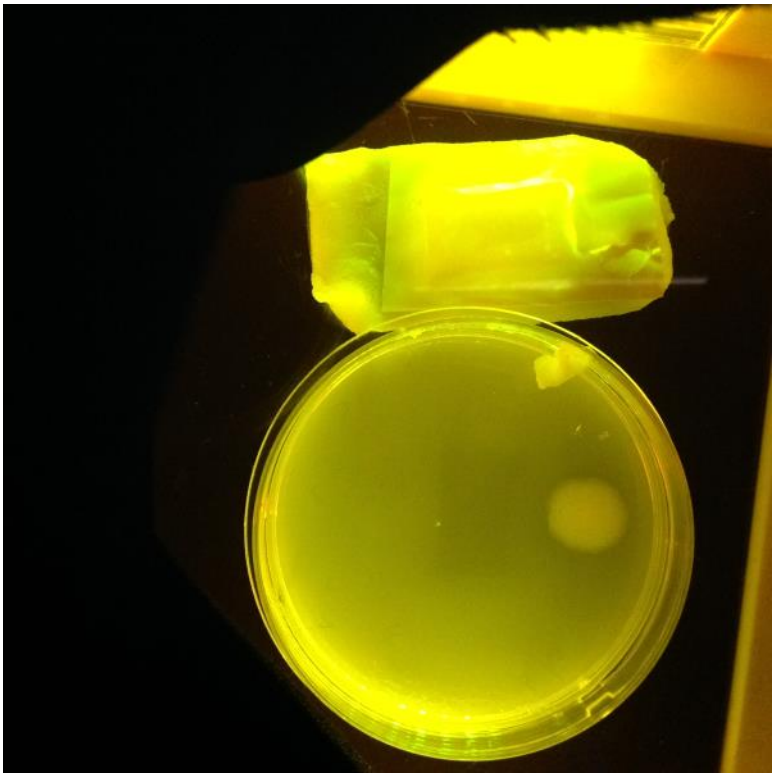


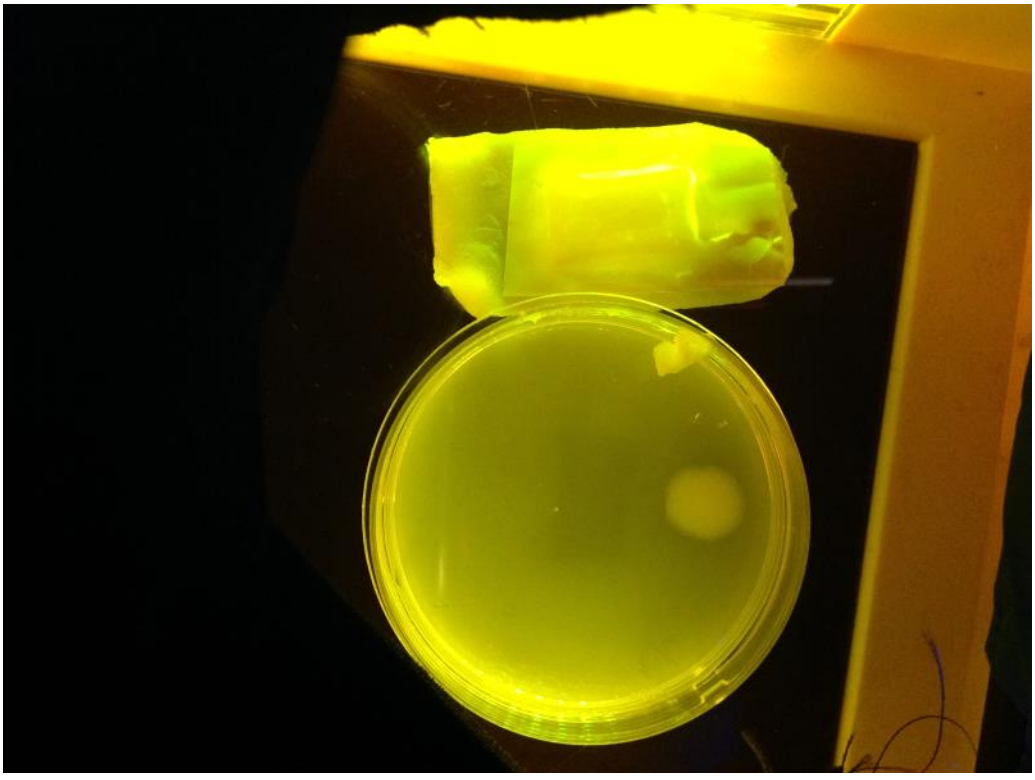


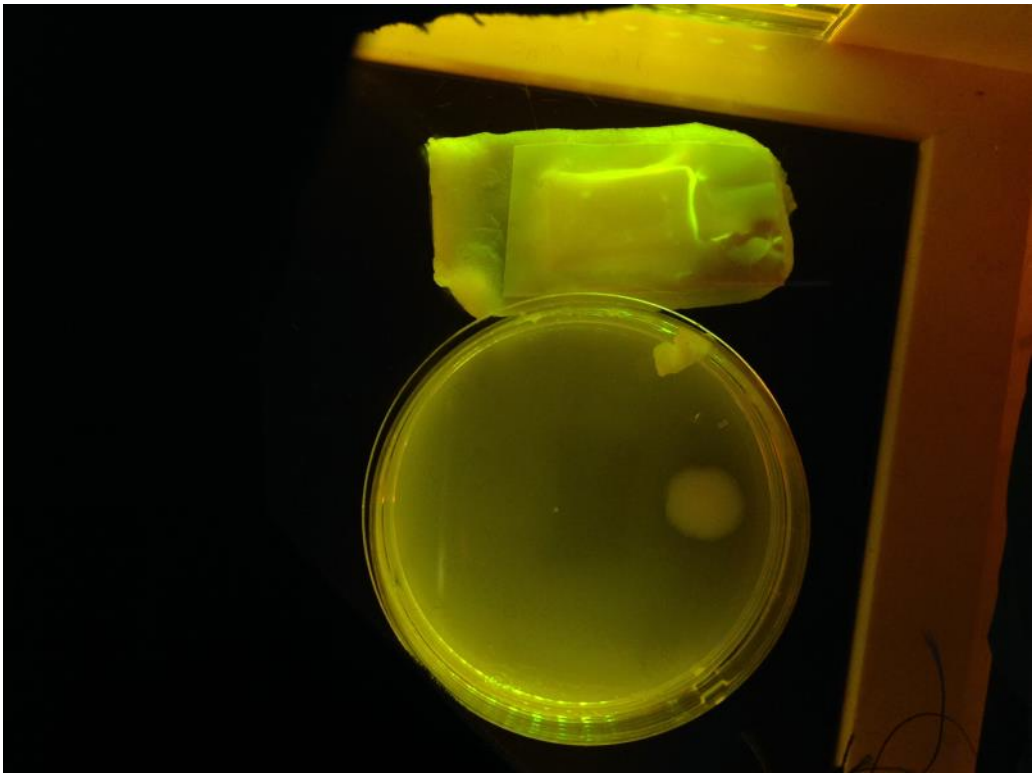


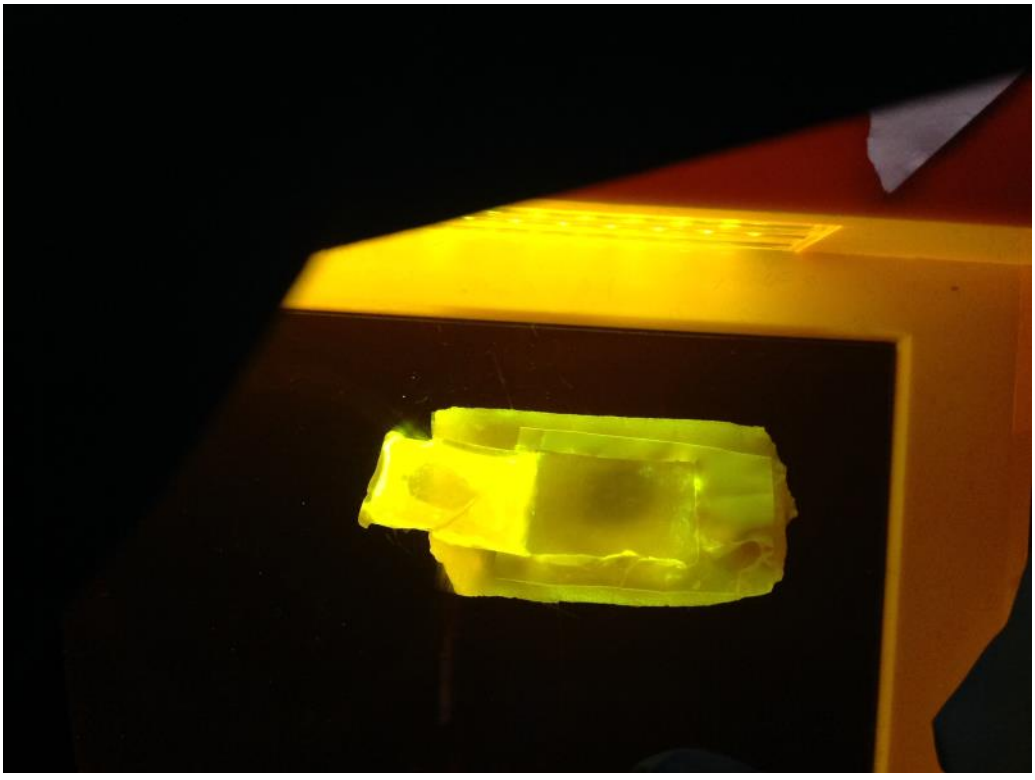


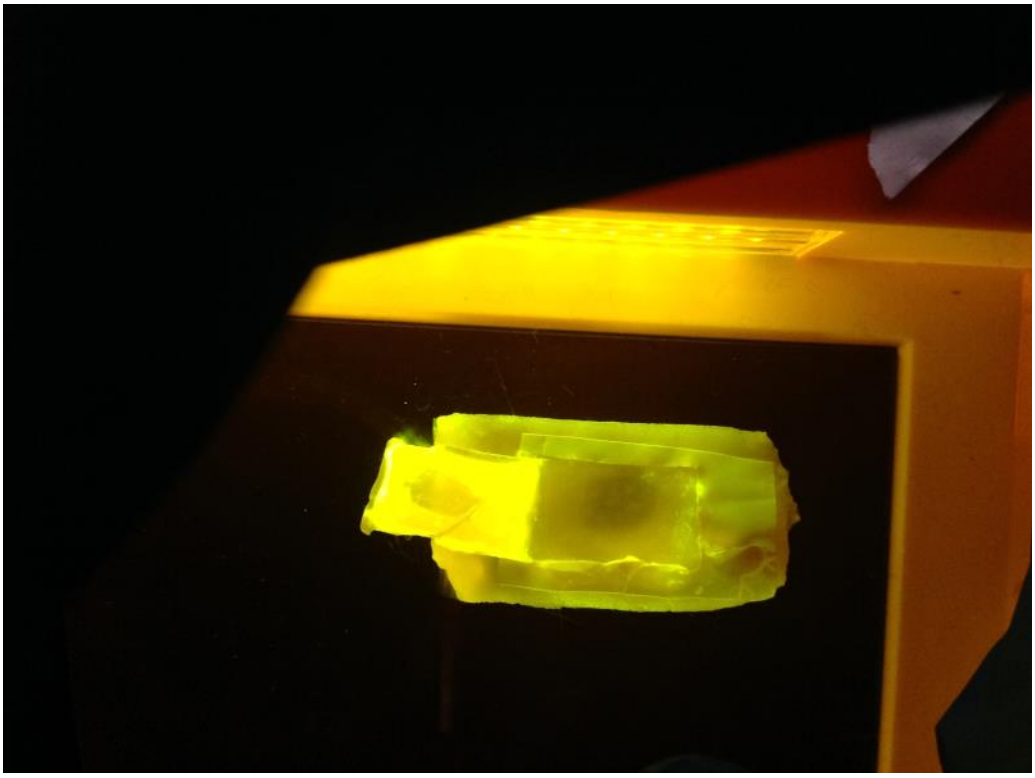












Experiments

Wednesday, June 10, 2015
3:09 PM

HELLO!!! HAVE A NICE DAY :)

Purpose	Group	Experiments	Results	Conclusion/Notes	Next Step
Prototype	**Phillip**	**None**	Checked from yesterday.		
Bacteria growth	**Bryan**	**Took pictures for the result and record time**	**Colonies became dimmer (smaller)**	**Starting to decline on day 4. The bacteria grew on thin plates had its peak growth around 2nd to 3rd day. Therefore, the bandage should be delivered around 1 and a half day after the plating of the bacteria.**	
ACT	**Leon**	**Digest - gel purify - ligation**	**Gel purified DNA conc is low**	**Not sure yet, ran ligation waiting for transformation**	

Photos

Thursday, June 11, 2015
3:43 PM

Image 1. Bacteria growth result after 92 hours (Day 4)

Image 2. Bacteria growth result after 92 hours detailed (Day 4)

Experiments

Tuesday, June 16, 2015
6:23 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
ACT exp	Leon	Transformed ligation mixture	It grew	Pcr check confirmed	Mini Give tube to central Continue in part two cloning

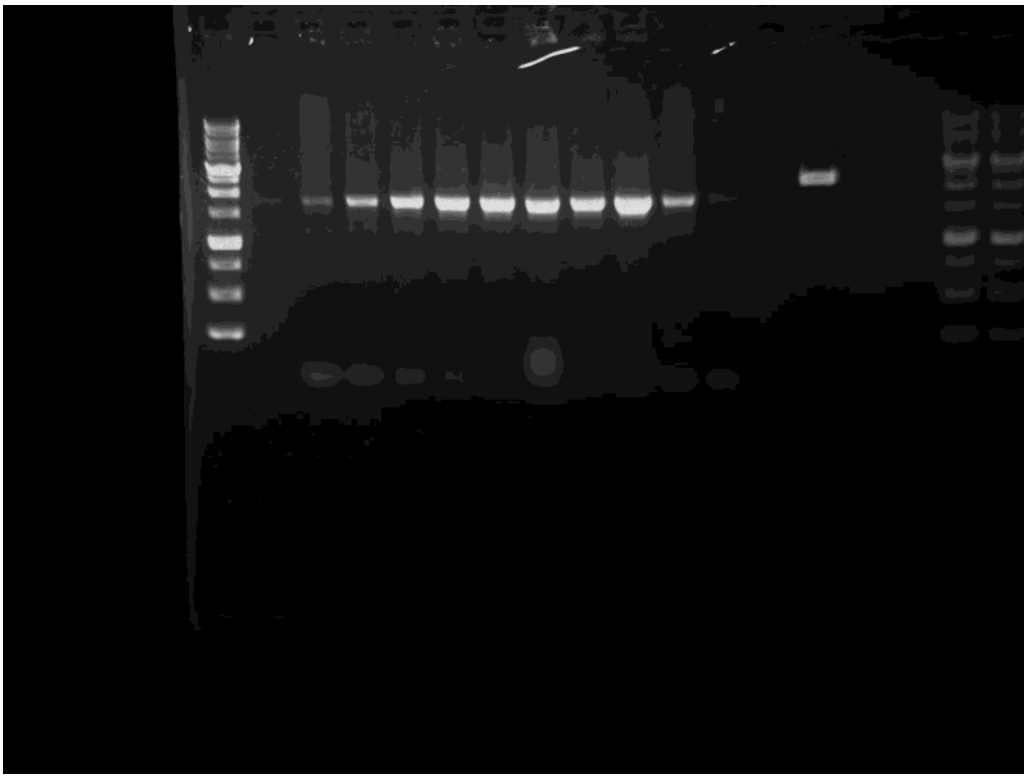
Experiments

Tuesday, June 16, 2015
6:27 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Act exp	Leon	PCR check	Good	Experimenting	Continue in cloning part ii
ACT exp and RBS and term	Leon	Grew cultures	They grew	They're good	miniprep test for term and rbs and ACT+term construct

Pictures

Tuesday, June 16, 2015
6:29 PM



This shows a successful transformation of the ligation of ACT to terminator (b0015)

According to the 1Kb ladder the ACT+term construct is found to be around 1600bp which is expected
ACT is 1200 + terminator is 100 + 300 from VF2+VR = 1600bp

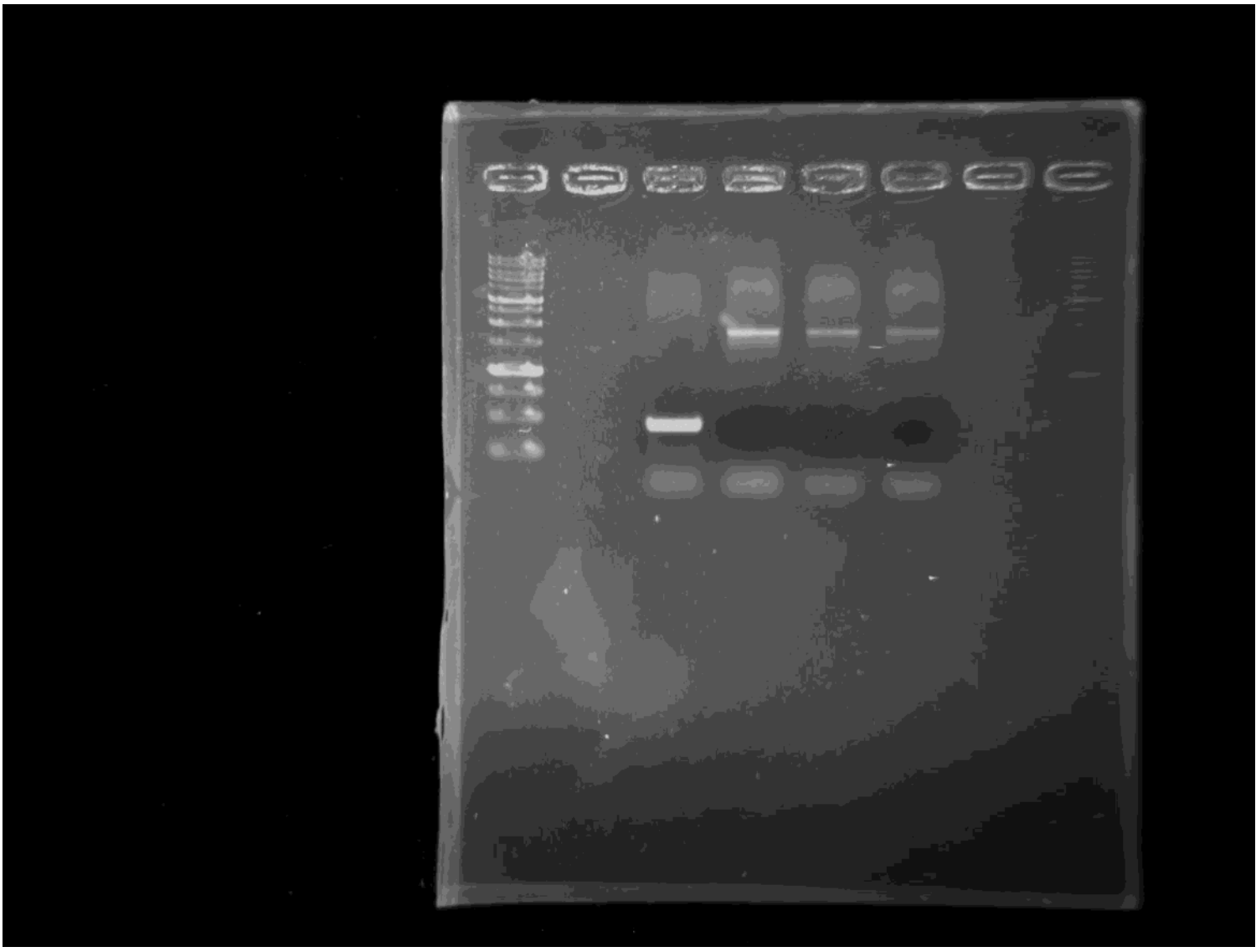
The lonely lane on the right is a terminator digested at E
This linearized segment should be around 2kb which is also confirmed by the ladder on the left and right

Experiments

Wednesday, June 17, 2015
7:21 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
Act exp	Leon	Miniprep Elution buffer	Good high concentration	Ready for next step	Digestion
ACT exp	Leon	Digestion Temp + RBS at ES ACT + Term at EX	Good gel bands	Gel looks ready for ligation	Gel run
ACT exp	Leon	Gel electrophoresis Temp + RBS at ES ACT + Term at EX	Seems to be good	Can't confirm experiment worked but the bands are in the right places	Gel extraction
ACT exp	Leon	Gel extraction Elution buffer	Concentrations are low but ok?	Can't tell if the DNA is nice and purified but moving on	ligation
ACT exp	Leon	Ligation Temp rbs ES + ACT term EX Low concentration parts.	Find out tomorrow!	Hopefully it worked?	transformation
ACT exp	Leon	Transformation Control with no DNA	Find out if they grew	They were…..	Find out tomorrow and 3 in 1
RBS and term	Leon	Miniprep CFP + term Term Elution buffer	Good high concentration for digestion	Stored in Leon's secondary box	Digestion
YebF	Leon	Gel check PCR samples	Bad, gel smudges and ladder didn't show up	We need to rerun the PCR	Rerun PCR
ACT exp, temp RBS	Leon	2 in 1 Culture Restreak	Check tomorrow	Grew culture and restreaks of temp sens prom + RBS for higher conc dna for digestion	Miniprep + digestion
ACT muta	Leon	Ran gel of Ecor1 and Pst1 cuts of ACT + term	Good found consistent gel bans at expected cuts	Can now perform mutagenesis and check success by disappearance of bands	Execute the holy experiment of SDM

Wednesday, June 17, 2015
7:53 PM

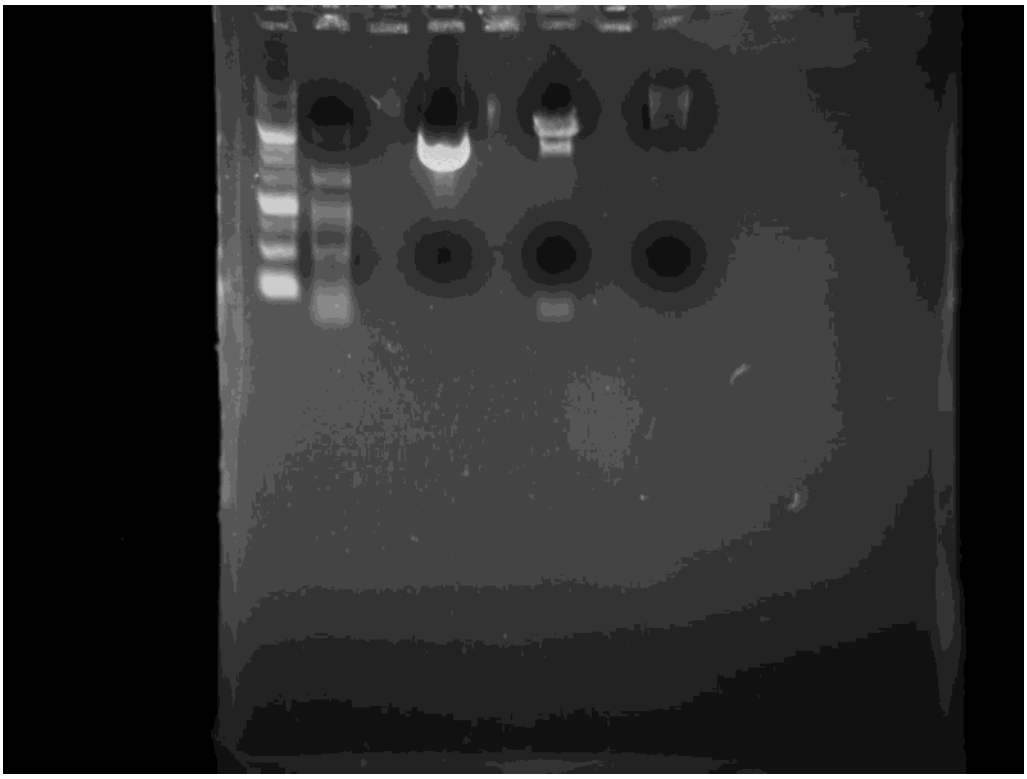


Tuesday, June 16, 2015
6:23 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
ACT digestion	JC	Digested original and mutated ACT, then ran gels	Undigested ACT and mutated ACT had one band at about 3kbp, digested bp had two - one at a little less than 3kbp and another at about 100bp	Mutation of p-sites was successful, since mutated ACT was not digested	
ACT cloning	Leon	Completed full cycle Transformed a ligated temp sens + rbs + ACT + term	Not sure	None found	Pcr to check
Plac rbs + CFP term	Andrew leon Interns	Ligation + transformation of plac rbs + cfp tern	Not successful	None grew	redo
Bacteria Growth	Bryan	LB Prep for streaking	Check after growth	Ready for streaking the cells	Streak cells with difference concentrations

Pics

Friday, June 19, 2015
10:43 AM



1kb ladder, 100bp ladder, ACT undigested, ACT digested at P, Mutated ACT digested at P

ACT-digested shows a band at around 100bp, corresponding to length of DNA between p sites

Friday, June 19, 2015
4:24 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
ACT cloning	Leon	Digestion of ACT mutated Digestion of terminator Front insert style	The bands are correct	Move on forwards	Gel run gel extract
ACT cloning	Leon	Gel ran and extracted digested ACT and digested terminator	Good concentrations of 100+	High concentrations after digestion and gel run	Ligation
ACT cloning	Leon	Ligation using ligation calculator	Good?	Good	Transformation
ACT cloning	Leon	Transformed using long protocol	Good	Good	Gel check pcr
Bacteria Growth	Bryan	Streaked with a total volume of 30µl 5µl, 1µl, 0.1µl, 0.01µl, and 0.001 µl of Liquid cultures were added Placed in 33 degrees Celsius	Check after first day	Continue after Dr. Chiang move the plates into the fridge on Saturday	Keep on growing

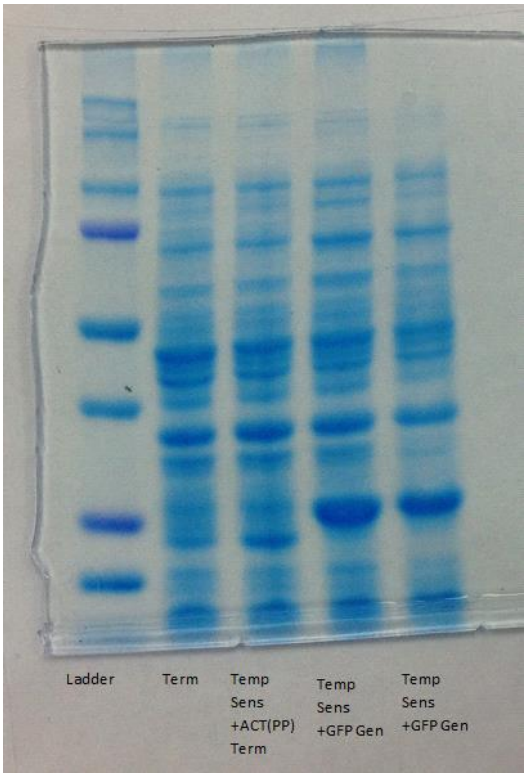
Experiments

Tuesday, June 30, 2015
3:22 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
ACT cloning	Leon	Grow cultures of yebF	Grew	High concentration with miniprep	Cloning stuff together

Pictures

Monday, June 29, 2015
5:29 PM



Preparing Protein Gel

1. Use 1ml of overnight liquid culture
2. Spin it down at max speed for 1 min
3. Decant supernatant
4. Add 50 uL of 1x SB
5. Heat at 95 degrees for 10 minutes
6. Spin down at max speed for 5 minutes
7. Transfer supernatant

Experiments

Wednesday, July 01, 2015
4:21 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
ACT cloning	Leon	Grow cultures of yebF	Grew	High concentration with miniprep	Cloning stuff together

Experiments

Wednesday, July 01, 2015
4:21 PM

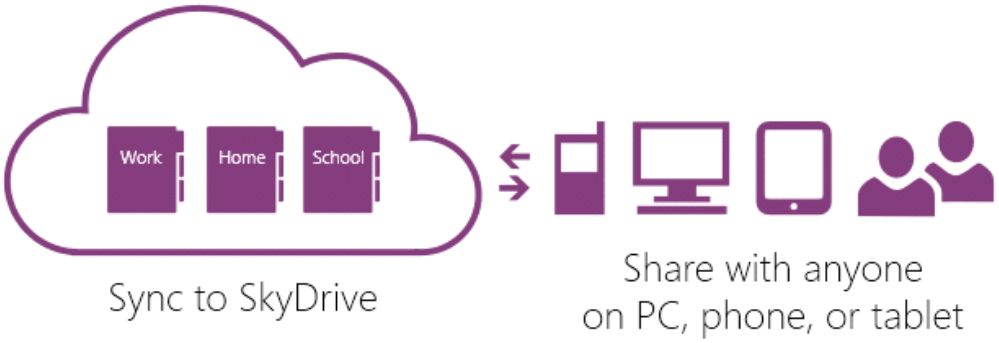
Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
ACT cloning	Leon	Grow cultures of yebF	Grew	High concentration with miniprep	Cloning stuff together

Experiments

Wednesday, July 01, 2015
4:21 PM

Purpose	Group	Experiments	Results	Conclusions/Notes	Next steps
YebF promotion	Leon	Grow cultures of yebF	Grew	High concentration with miniprep	Cloning stuff together

OneNote: one place for all of your notes



Watch the 2 minute video

1. Take notes anywhere on the page

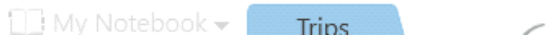
Write your name here

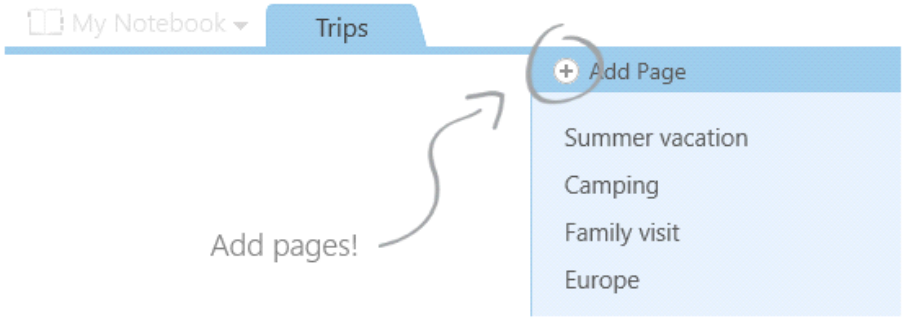
2. Get organized

You start with "My Notebook" - everything lives in here

Add sections for activities like:

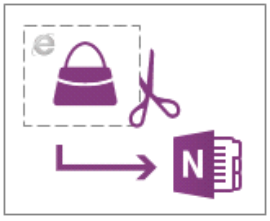
Add pages inside of each section:



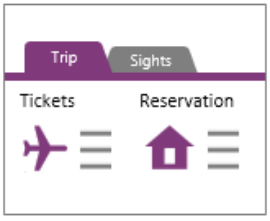


(Pages are over there)

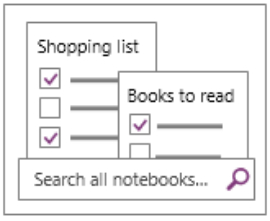
3. For more tips, check out 30 second videos



Clip from the web



Plan a trip with others



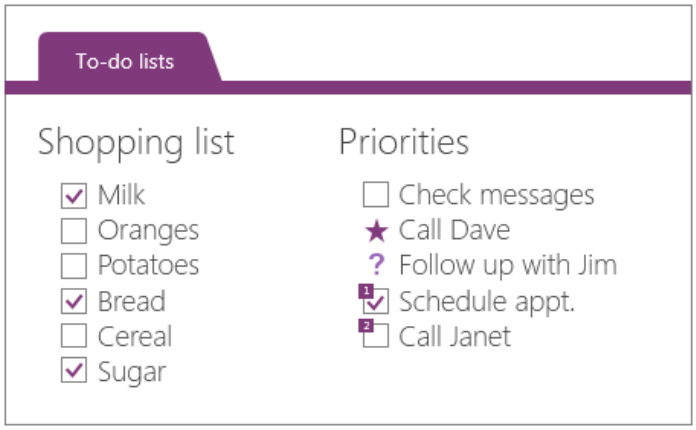
Search notes instantly

Write notes on slides

4. Create your first page

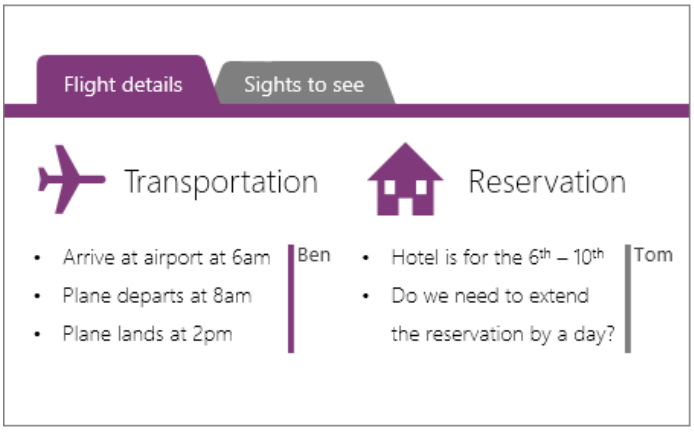
You're in the Quick Notes section - use it for random notes

OneNote Basics



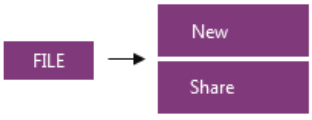
Remember everything

- Add Tags to any notes
- Make checklists and to-do lists
- Create your own custom tags



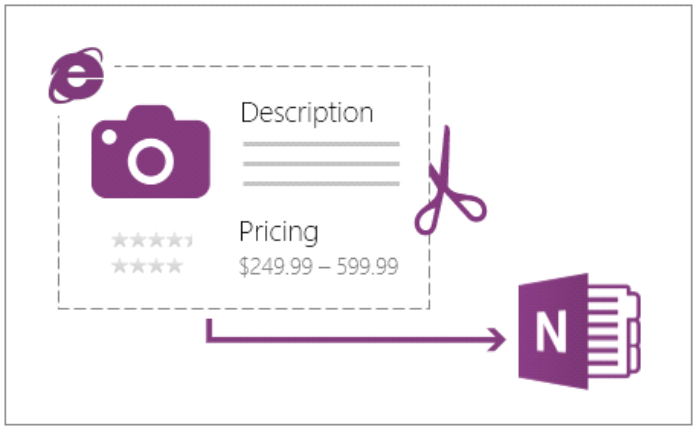
Collaborate with others

- Keep your notebooks on SkyDrive
- Share with friends and family
- Anyone can edit in a browser



Keep everything in sync

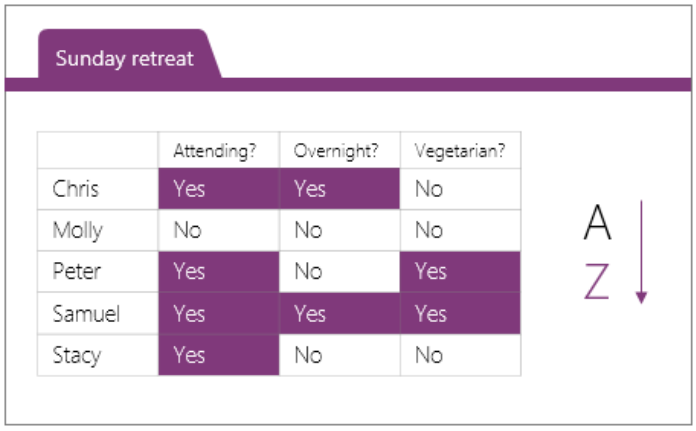
- People can edit pages at the same time
- Real-Time Sync on the same page
- Everything stored in the cloud
- Accessible from any device



Clip from the web

- Quickly clip anything on your screen
- Take screenshots of products online
- Save important news articles

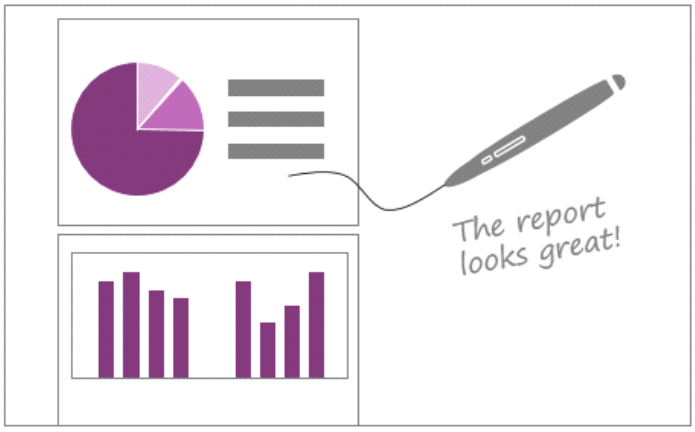
in your taskbar
OR
+ S on your keyboard



Organize with tables

- Type, then press TAB to create a table
- Quickly sort and shade tables
- Convert tables to Excel spreadsheets

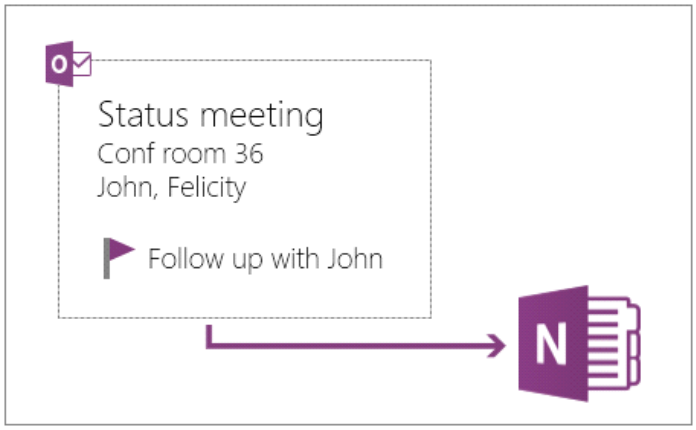
TABLE TOOLS
LAYOUT
Shading
Sort
Convert to Excel Spreadsheet



Write notes on slides

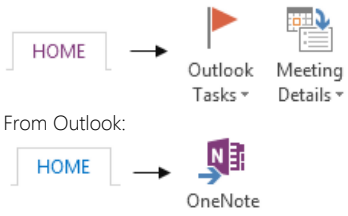
- Send PowerPoint or Word docs to OneNote
- Annotate with a stylus on your tablet
- Highlight and finger-paint

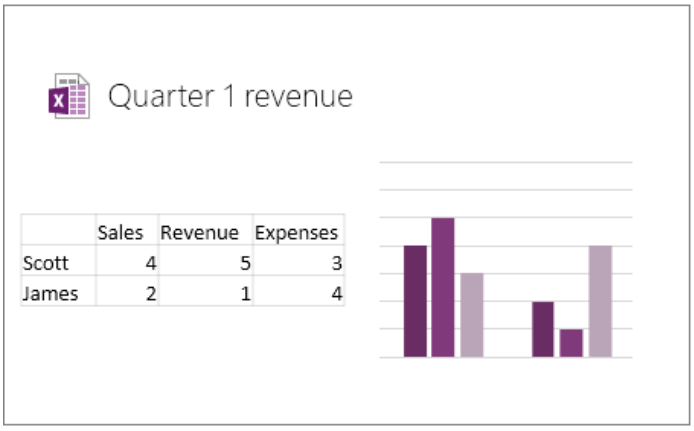
in your taskbar
OR
+ N on your keyboard



Integrate with Outlook

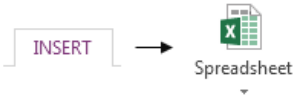
- Take notes on Outlook or Lync meetings
- Insert meeting details
- Add Outlook tasks from OneNote

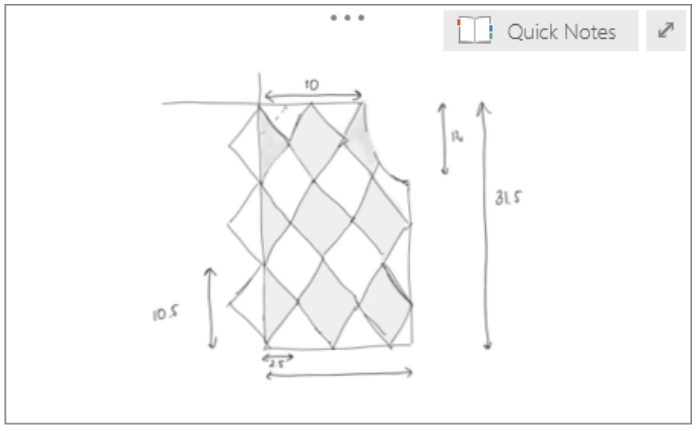




Add Excel spreadsheets

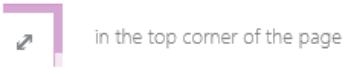
- Track finances, budgets, & more
- Preview updates on the page

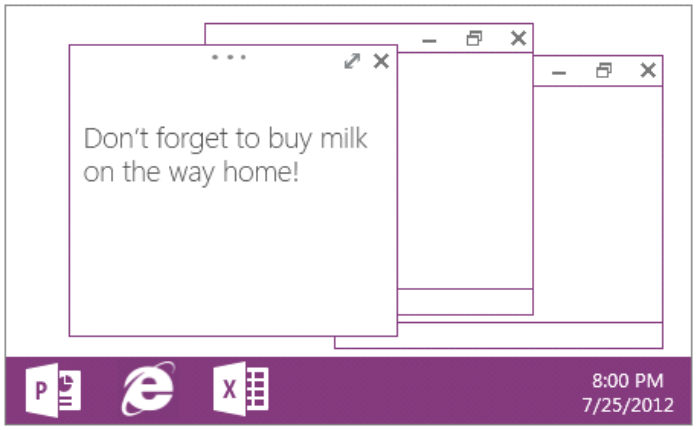




Brainstorm without clutter

- Hide everything but the essentials
- Extra space to focus on your notes





Take quick notes

- Quickly jot down thoughts and ideas
- They go into your Quick Notes section

in your taskbar

OR

+ N on your keyboard

Experiments

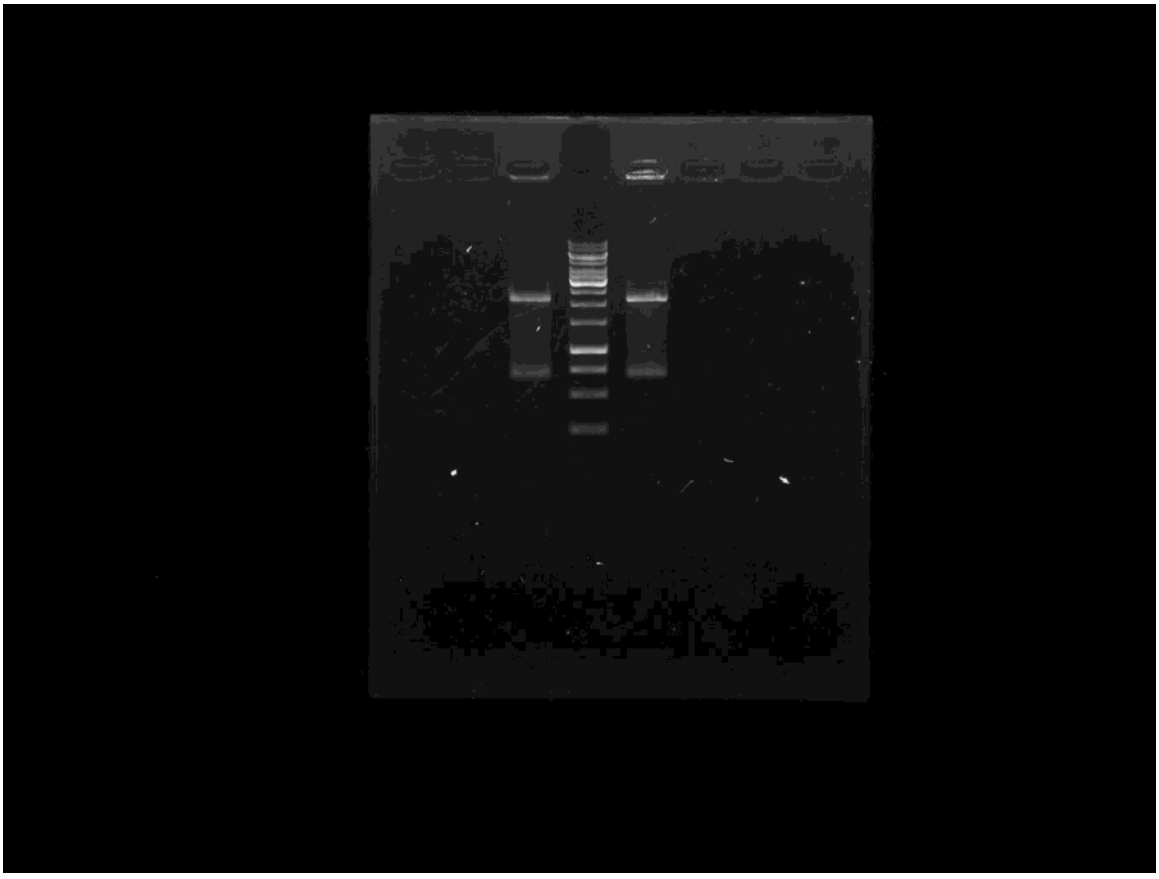
2015年7月17日
上午 09:30

Group	Experiments	Results	Conclusions/Notes	Next steps
Phillip	T4 Testing	You gotta be patient and wait for it man! Wait for the base drop! I mean the buffers.	Continued the steps towards ligation. Days seem dim, that's because the weather is actually cloudy today. Finished digestion and ran gels for the products for the pTempSens +RBS and also T4+Term.	The real deal. Ligation.
Phillip	Transferred stuff from TAS NYMU.			

Pictures

2015年7月17日
下午 12:25

Gel Pictures



T4

Experiments

2015年7月24日
下午 04:43

Group	Experiments	Results	Conclusions/Notes	Next steps
Phillip	Measured and rechecked the concentrations of a lot of the purified plasmid samples that we had. Please refer to the page "Checking with NYMU Nanodrop"	The results of this nanodrop concluded with the current NYMU Nanodrop.		
Phillip				

Checking with NYMU Nanodrop

Friday, July 24, 2015
5:54 PM

	Original Concentration as stated on the side of the centrifuge tube	Remeasured Concentration as stated on the side of the centifuge	Difference	Percent Difference
T4 Term		22.4		
pTempSens +RBS		13.9		
Temp RBS	95.2	68.6	26.6	0.279411765
T4 Term 1	415.3	201.2	214.1	0.515530941
T4 Term 2	461.8	197.1	264.7	0.573191858
T4 Term 3	346.7	325.7	21	0.060571099
ACT EXSP	191	-19	210	1.09947644
GFP	104.4	94.5	9.9	0.094827586

Research

Friday, July 24, 2015
6:56 PM

Part:BBa_K1510010

http://parts.igem.org/Part:BBa_K1510010:Experience

This page uses red fluorescent protein to test if YebF can successful transfer the protein outside of the cell by actually fusing the RFP at the C-terminal of the protein and testing if the signal protein works by measuring culture supernatant.

Experiments

Friday, July 24, 2015
5:54 PM

Group	Experiments	Results	Conclusions/Notes	Next steps
Phillip	Going to chill off on experiments because I'll have to redo the past week's experiments again. Another failure is just a step towards success.			

Additional Research

2015年7月27日
上午 10:34

http://www.diabetesforecast.org/2013/jul/making-insulin.html?referrer=https://www.google.com.tw/
The following article talks about how scientists engineered genetically to change pig insulin that differed by one amino acid to become the human version of insulin. Our model takes a similar approach, but instead we mutate it with 3 amino acids.

https://en.wikipedia.org/wiki/High-performance_liquid_chromatography for purification

There are several problems with insulin as a clinical treatment for diabetes:

- Mode of administration.
- Selecting the 'right' dose and timing. Usually one unit of insulin is ~15grams of CHO.
- Selecting an appropriate insulin preparation (typically on 'speed of onset and duration of action' grounds).
- Adjusting dosage and timing to fit food intake timing, amounts, and types.
- Adjusting dosage and timing to fit exercise undertaken.
- Adjusting dosage, type, and timing to fit other conditions, for instance the increased stress of illness.
- Variability in absorption into the bloodstream via subcutaneous delivery
- The dosage is non-physiological in that a subcutaneous bolus dose of insulin alone is administered instead of combination of insulin and C-peptide being released gradually and directly into the portal vein.
- It is simply a nuisance for patients to inject whenever they eat carbohydrate or have a high blood glucose reading.
- It is dangerous in case of mistake (most especially 'too much' insulin).

0

SPRING BREAK

Tuesday, April 21, 2015
2:51 PM