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

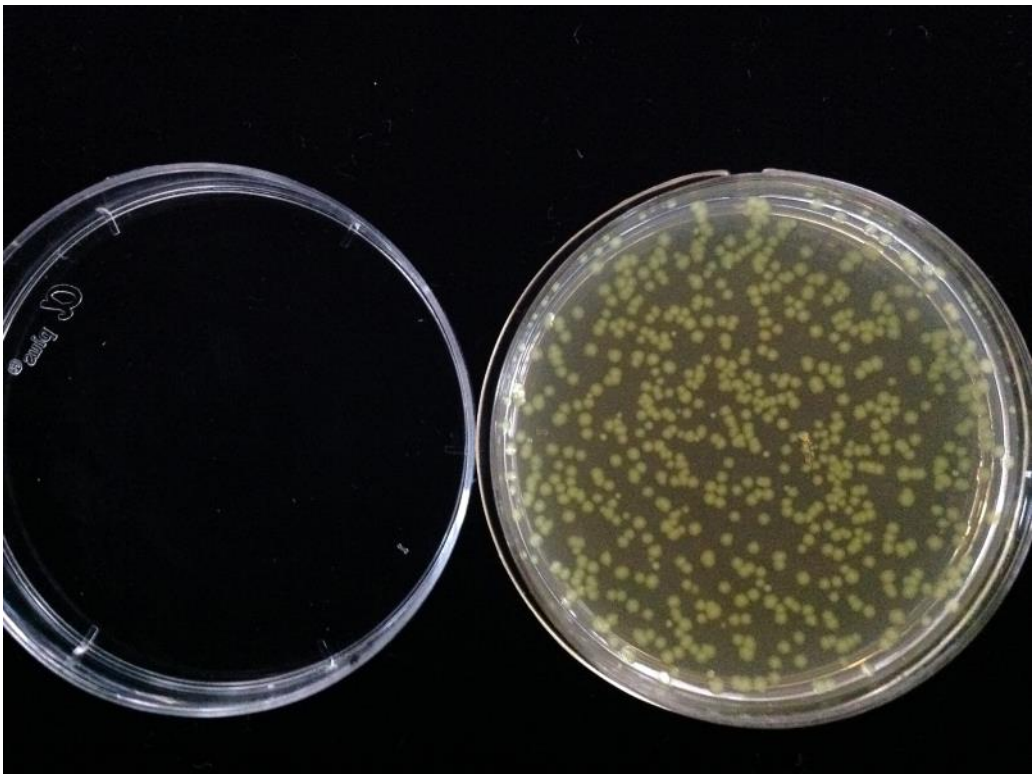


Figure 1: Plate with 0.001µl Liquid Culture

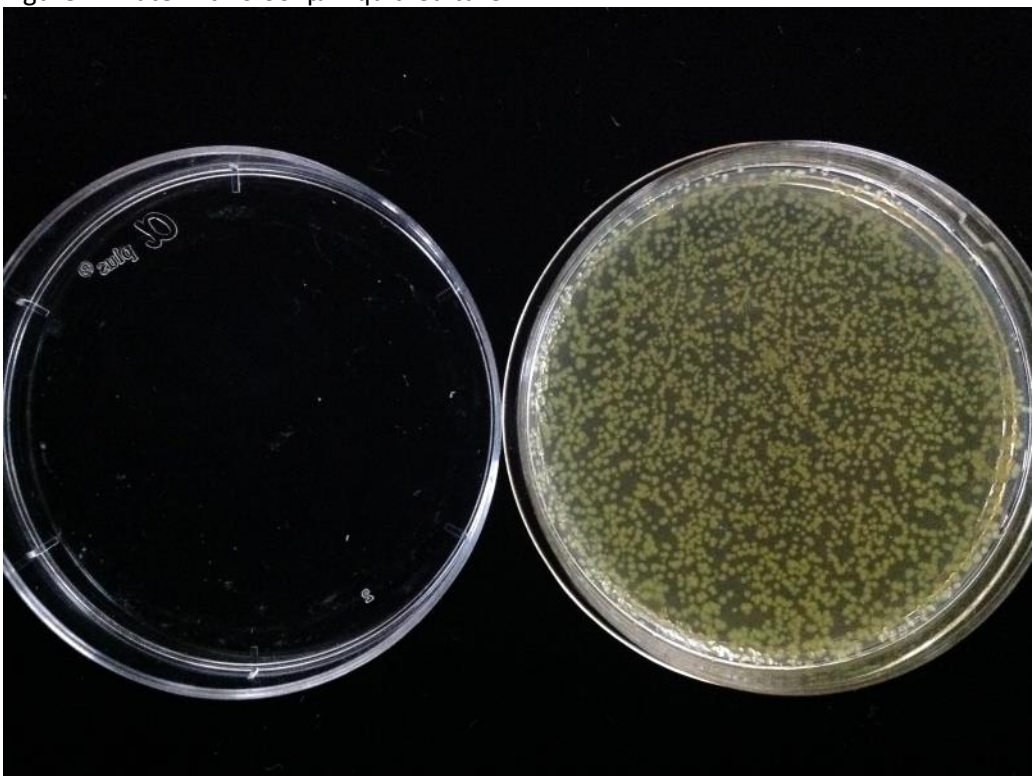


Figure 2: Plate with 0.01µl Liquid Culture

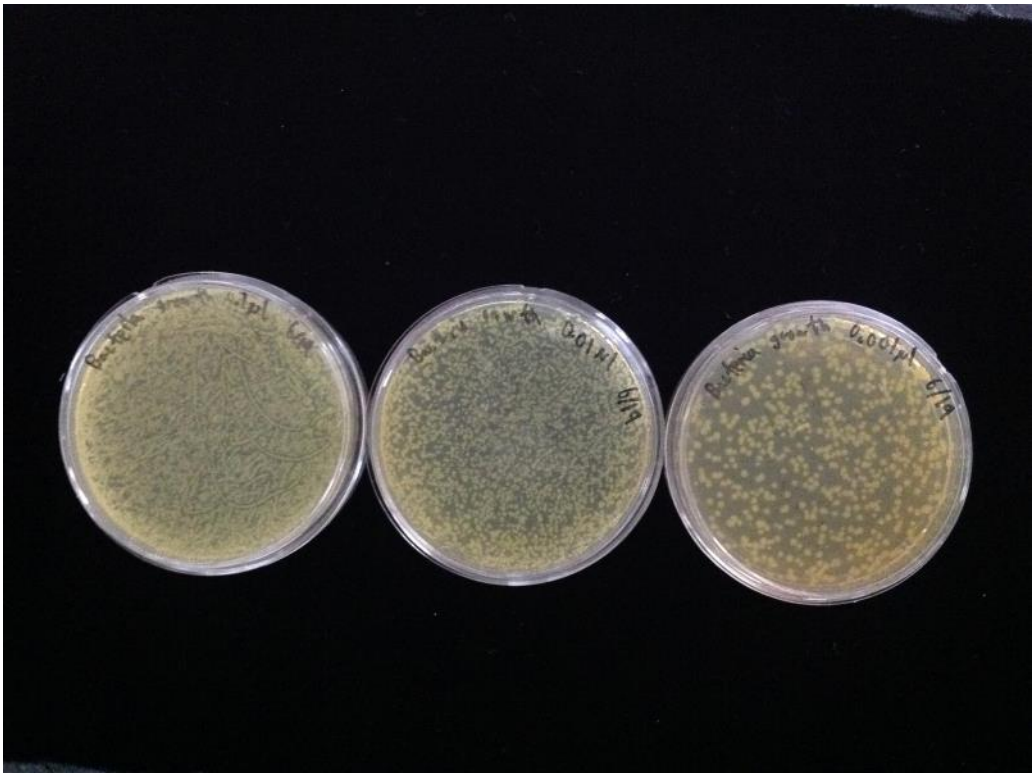


Figure 2: Comparison for Bacteria growth

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

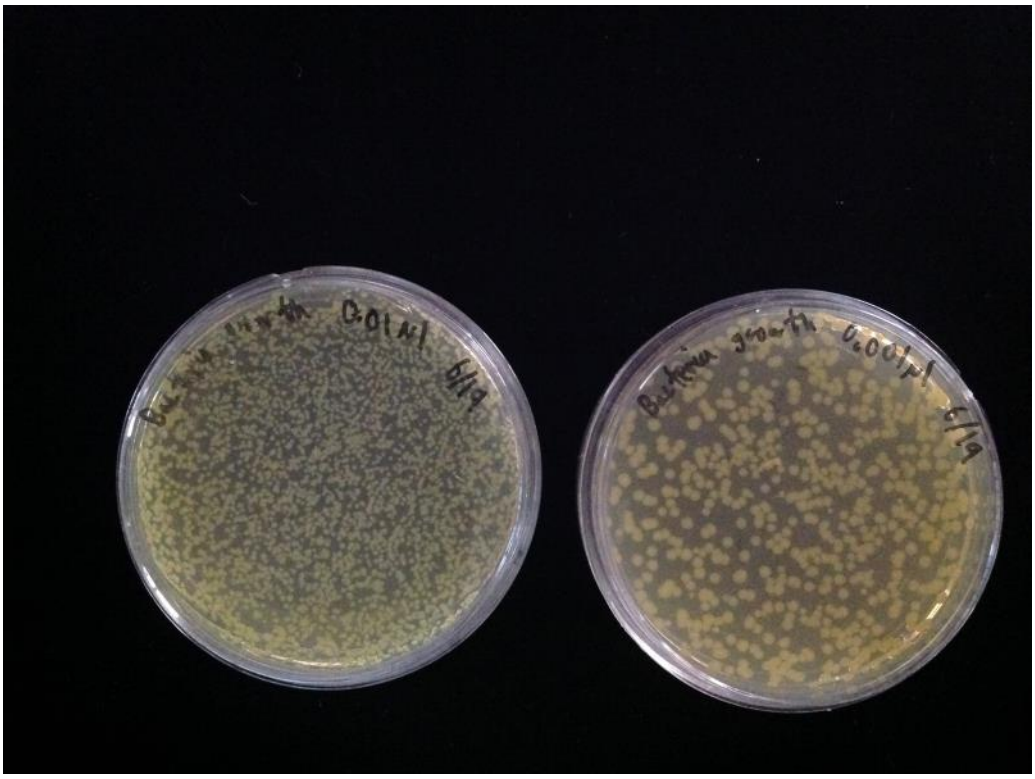


Figure 1: Comparison

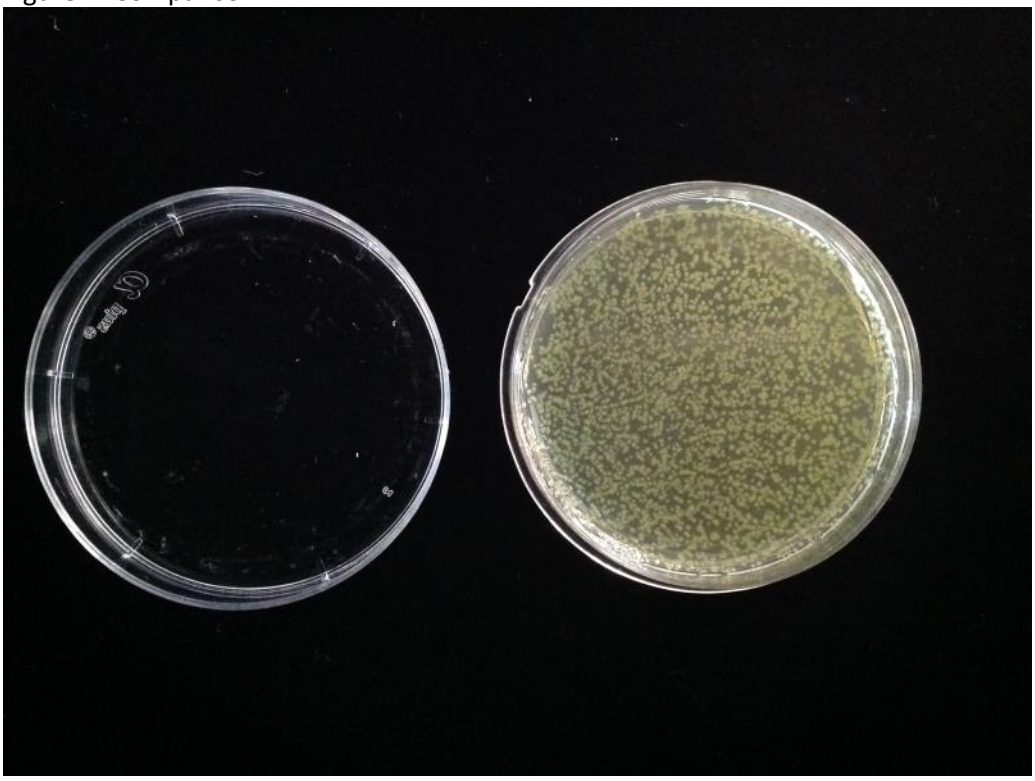


Figure 2: Plate with 0.01µl Liquid Culture

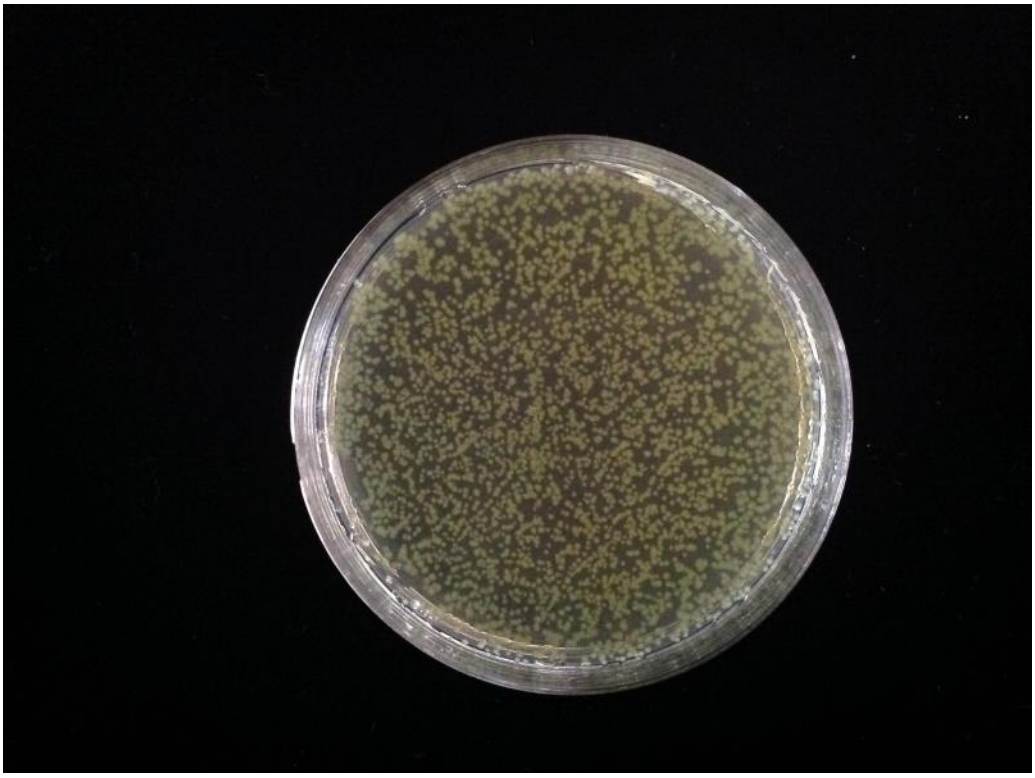


Figure 3: Plate with 0.01µl Liquid Culture (Detailed)

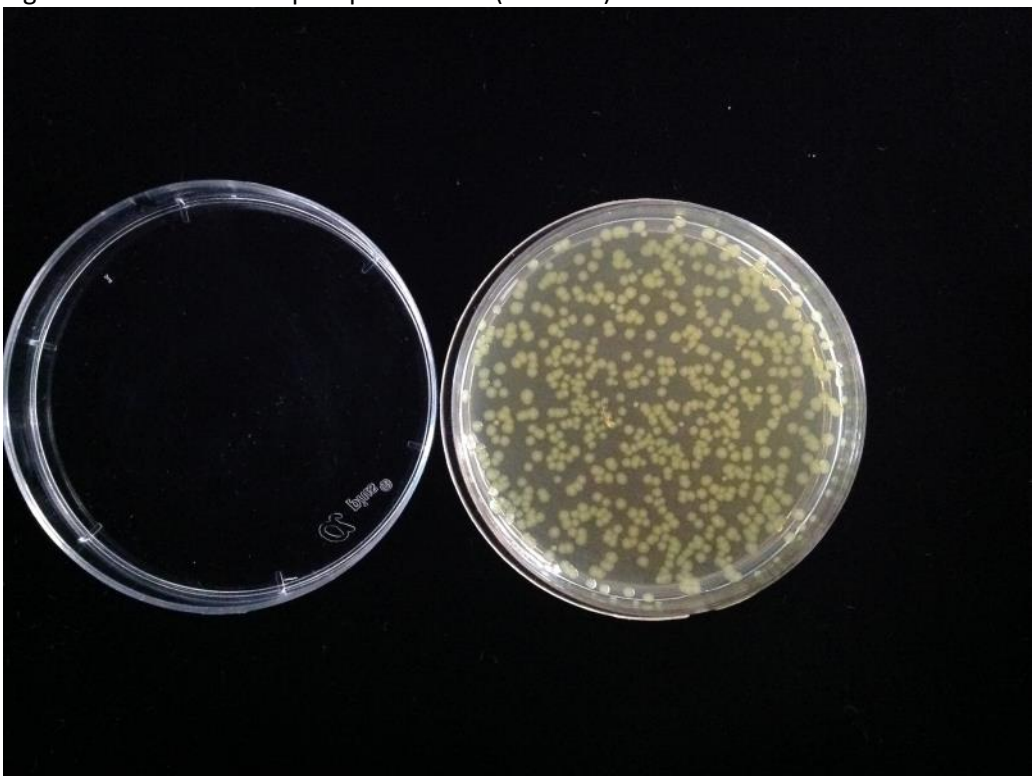


Figure 4: Plate with 0.001µl Liquid Culture

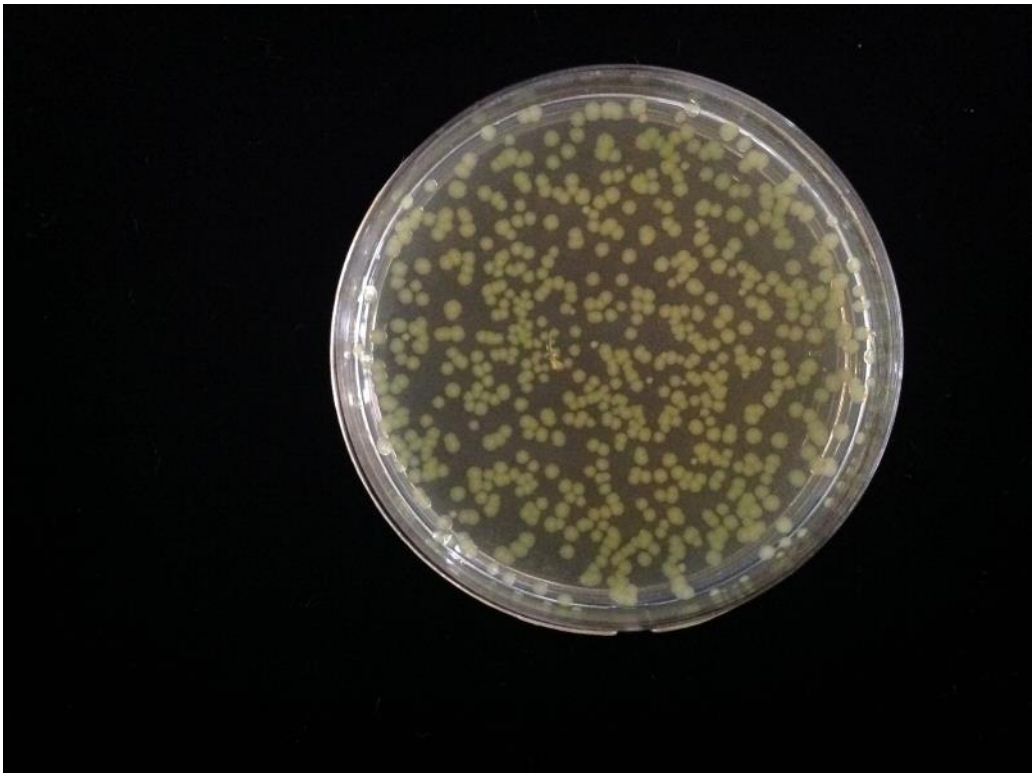


Figure 5: Plate with 0.001µl Liquid Culture (Detailed)

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

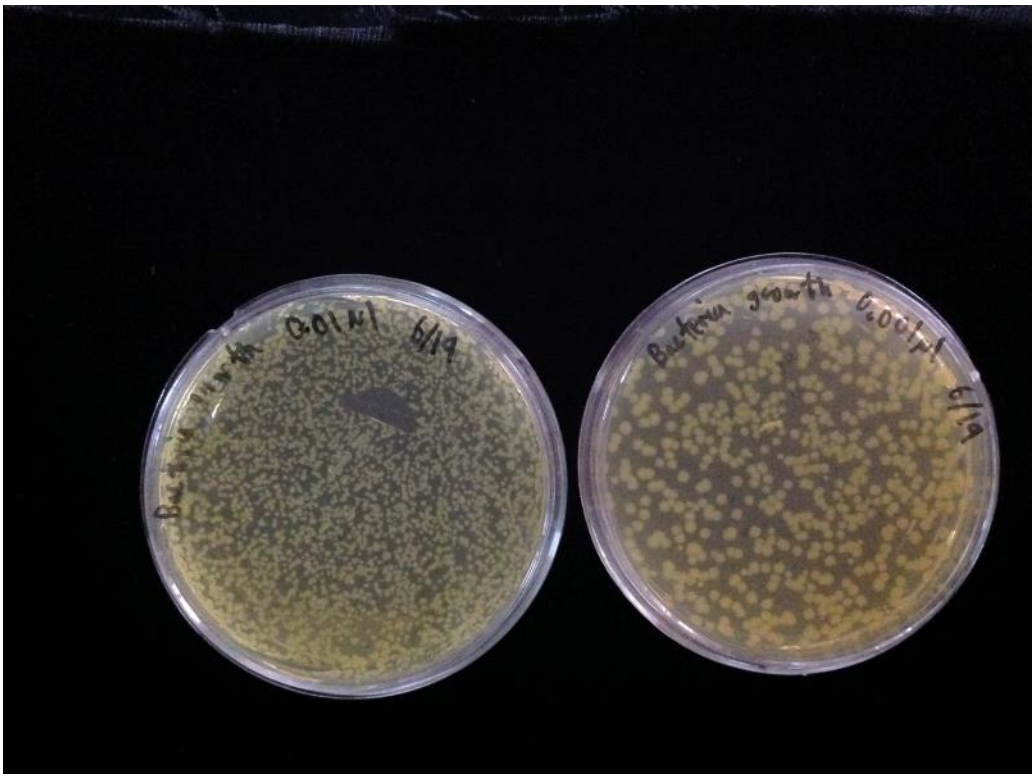


Figure 1: Comparison

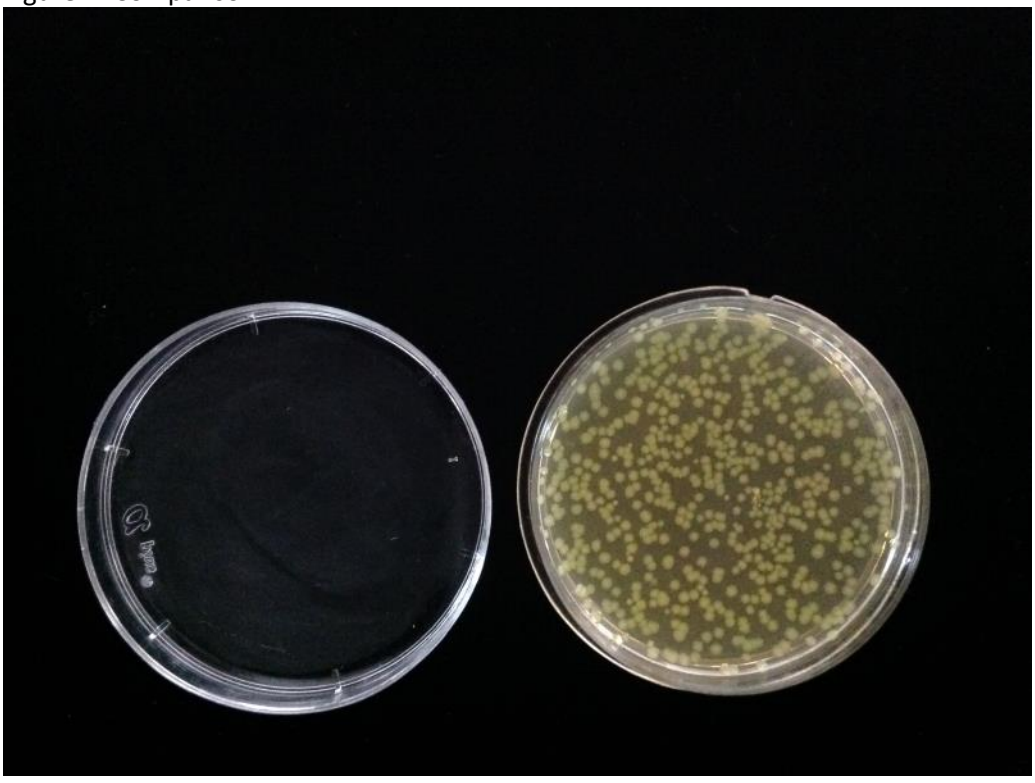


Figure 2: Plate with 0.01µl Liquid Culture

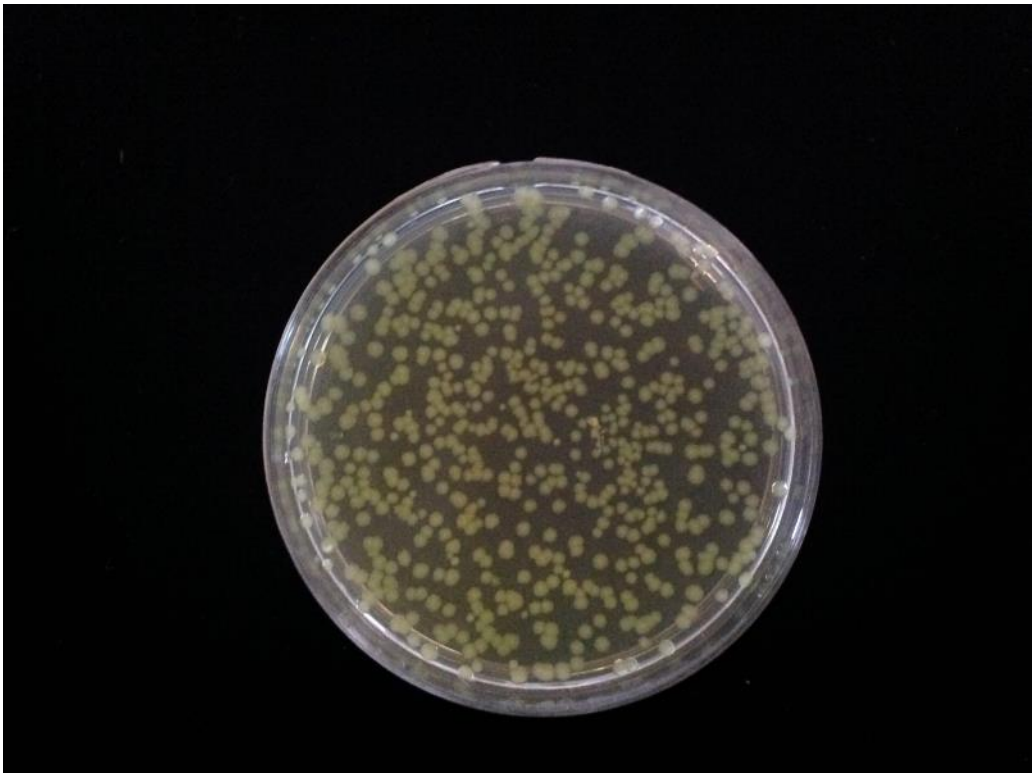


Figure 3: Plate with 0.01µl Liquid Culture (Detailed)

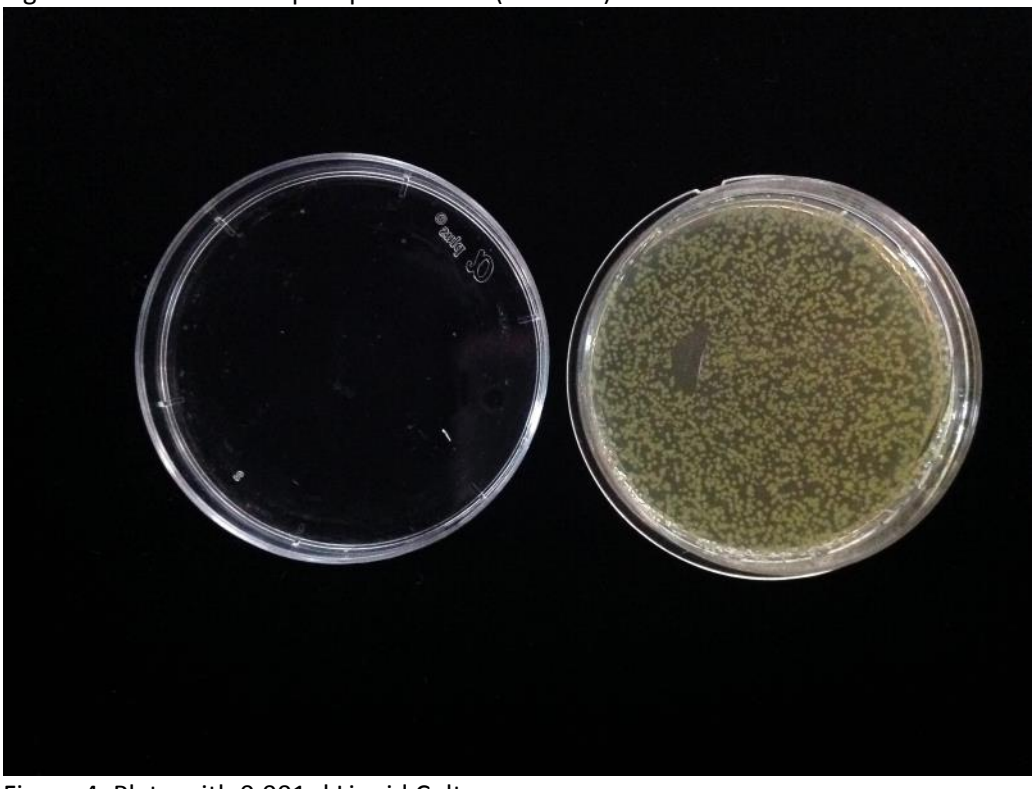


Figure 4: Plate with 0.001µl Liquid Culture

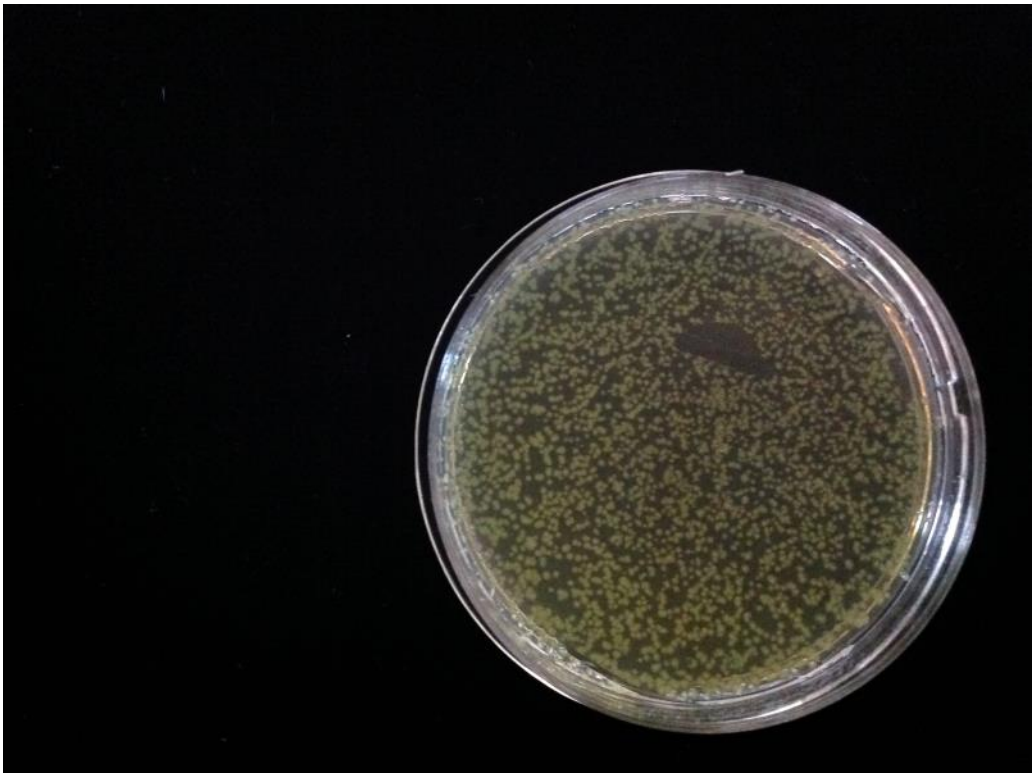


Figure 5: Plate with 0.001µl Liquid Culture (Detailed)

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

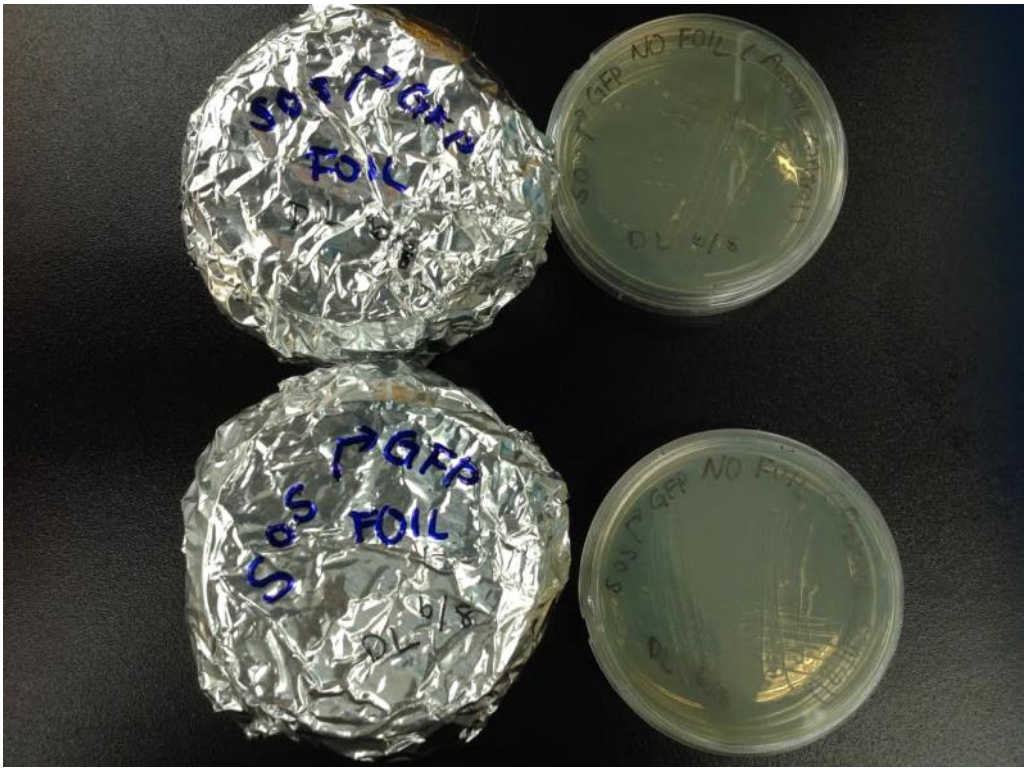


Image 1. SOS testing on/off experiment setup, 2 plates on left are covered with cloth and 3 layers of foil. 2 plates on the right are not covered.

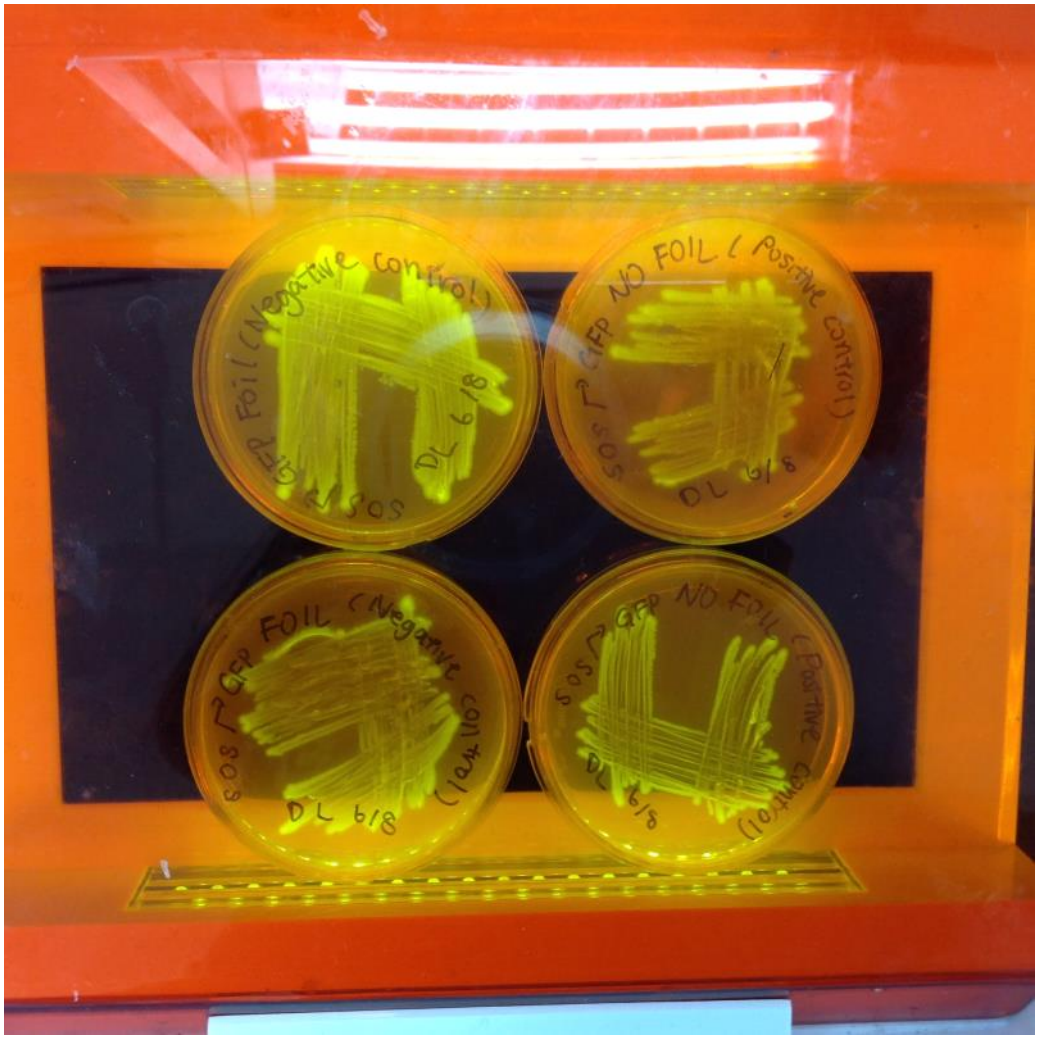
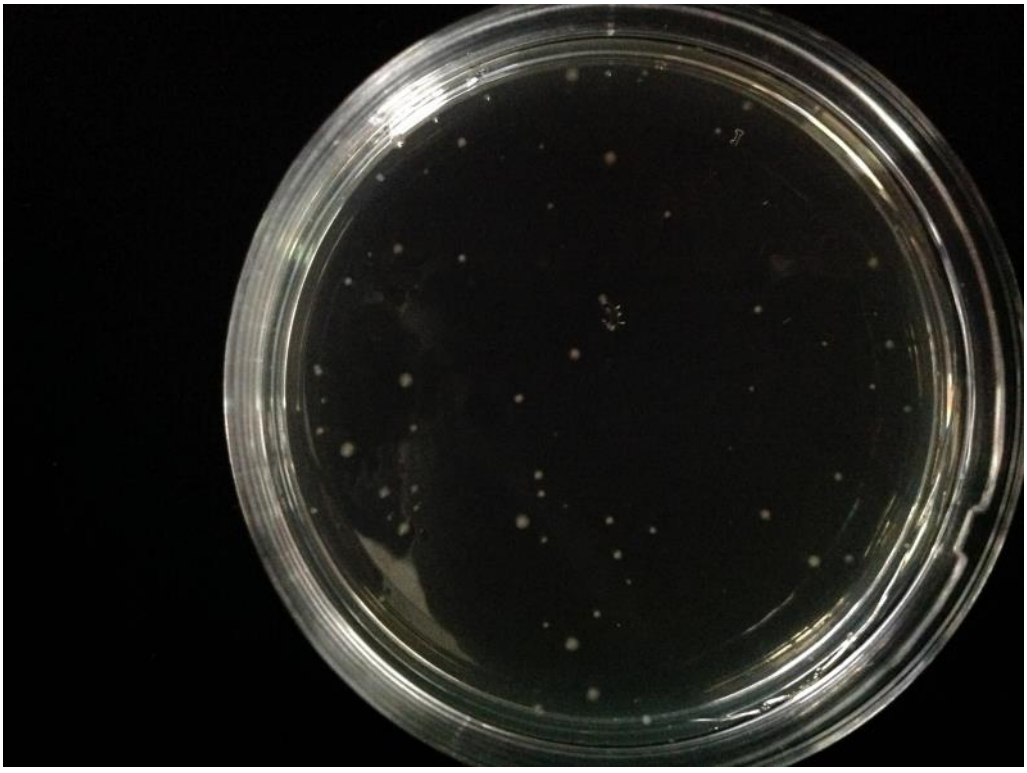


Image 2. SOS testing on/off Result, all 4 of them glowed.



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

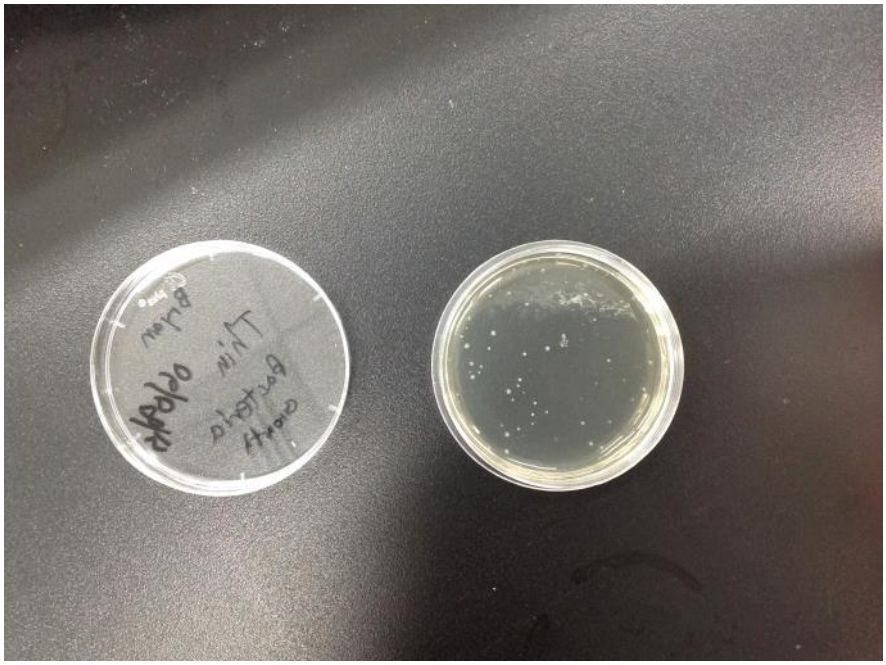


Image 1. Bacteria growth result after 20 hours (Day 1)

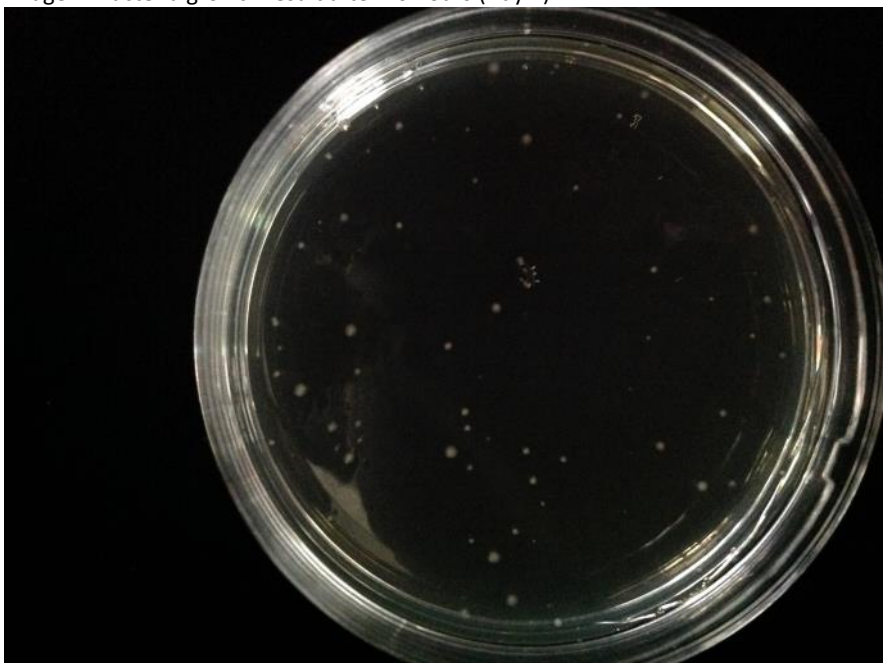
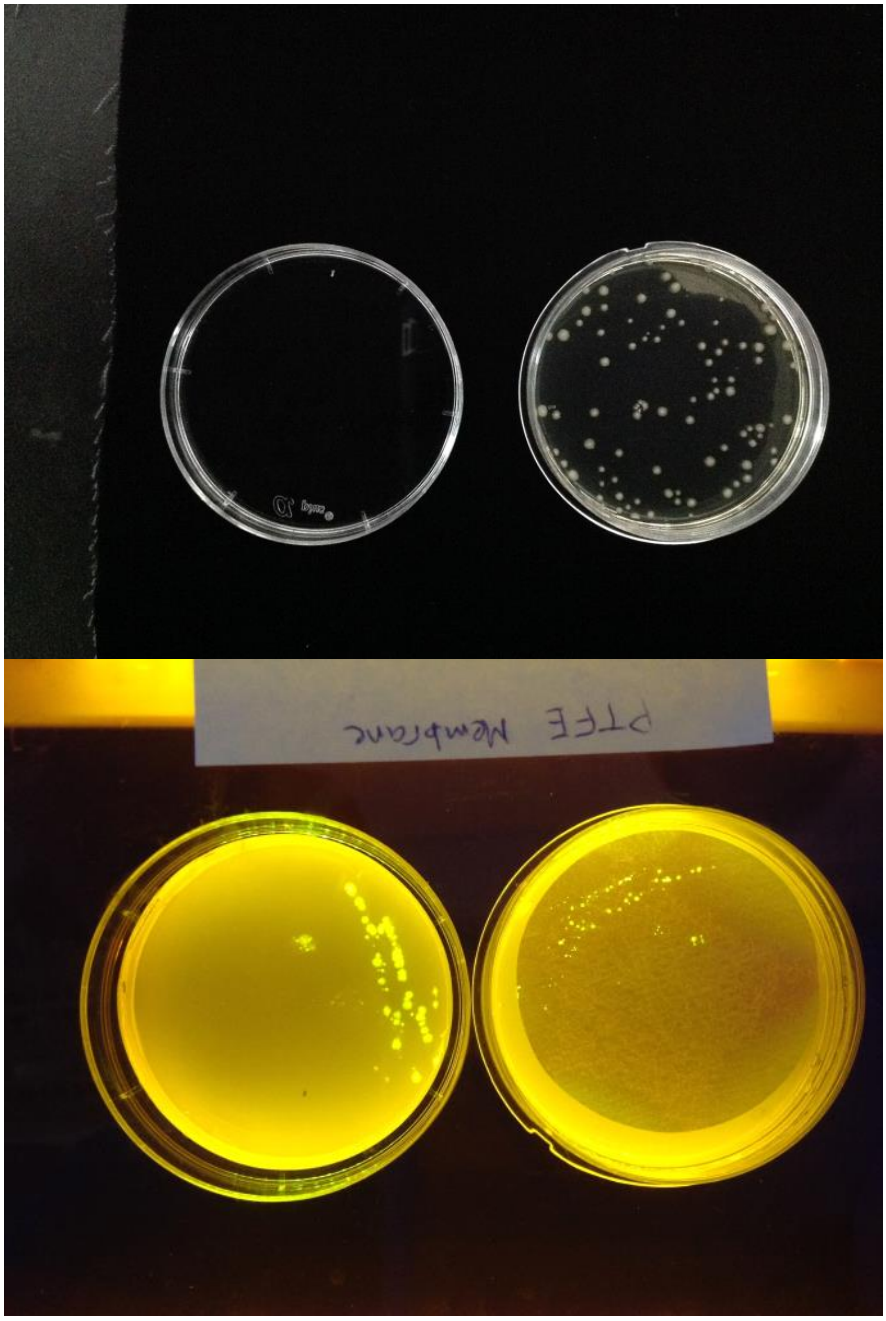
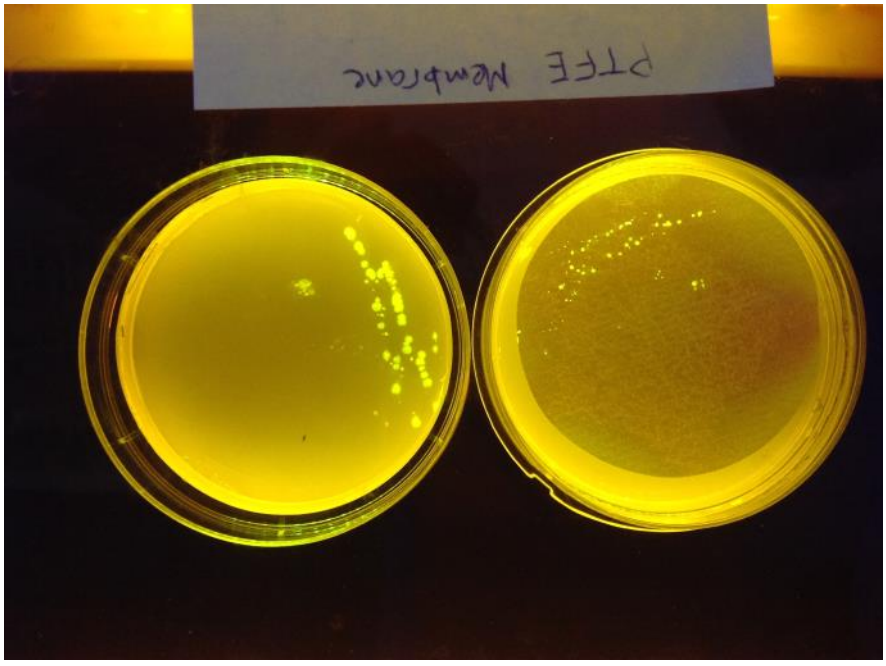
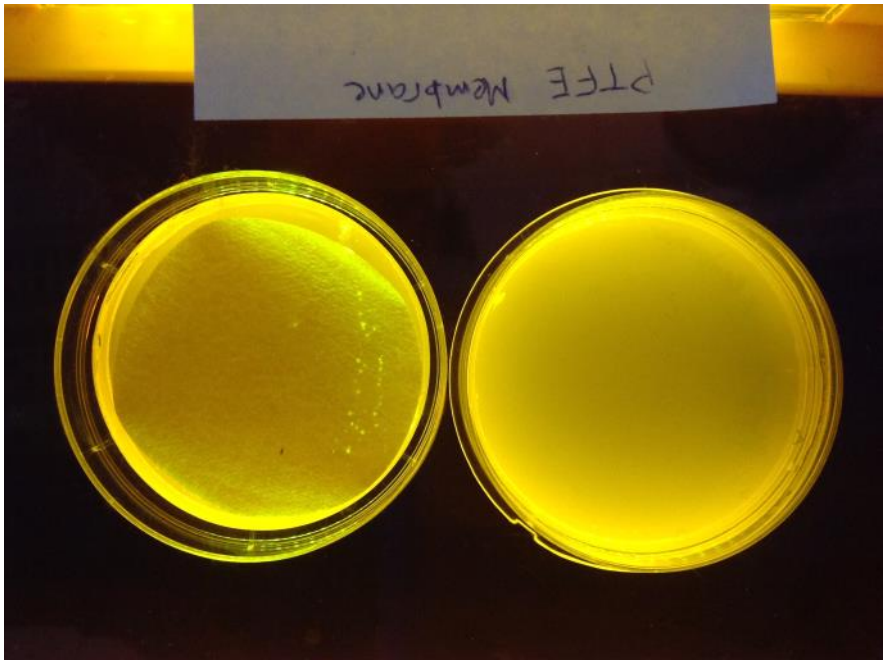
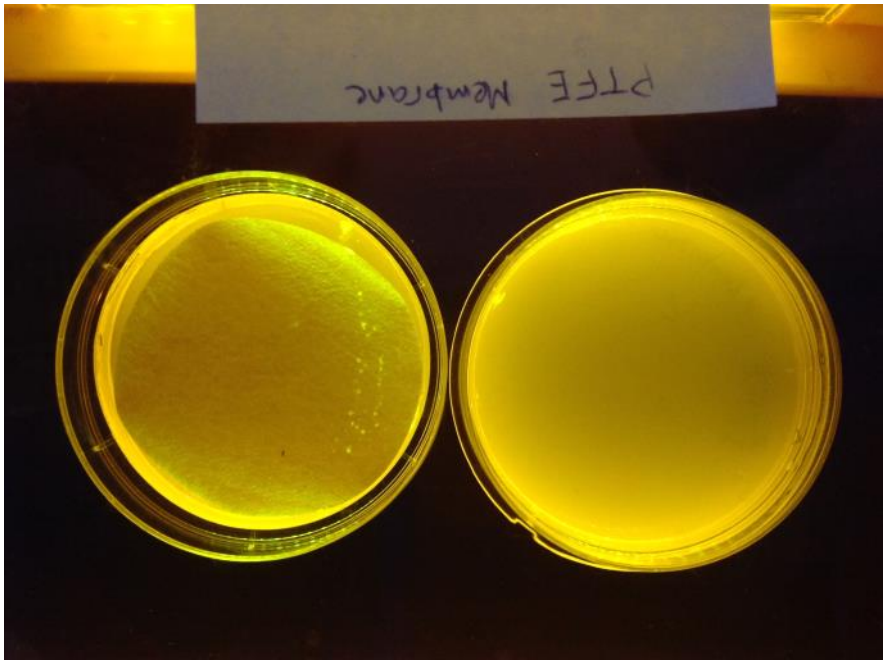


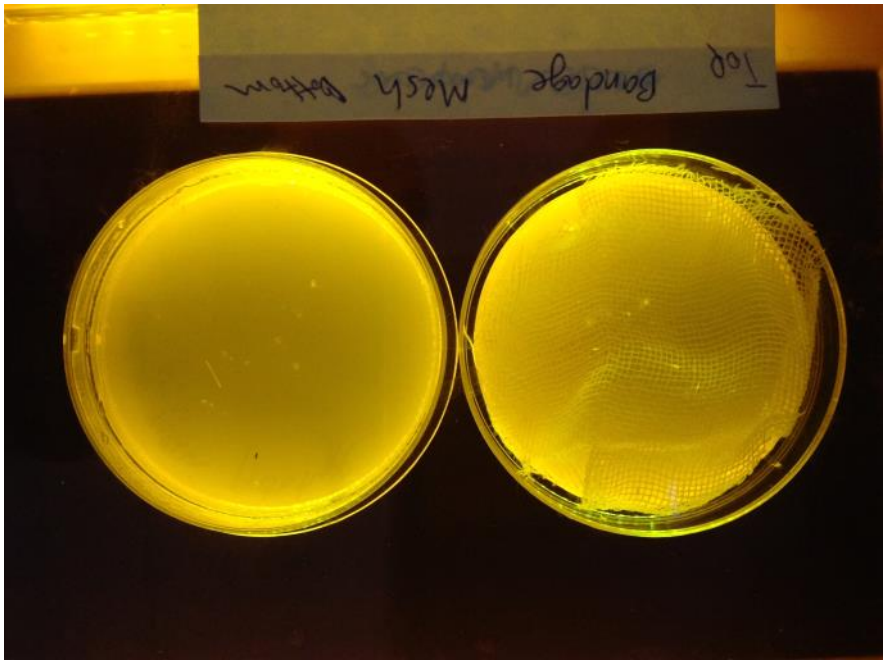
Image 2. Bacteria growth result after 20 hours detailed (Day 1)

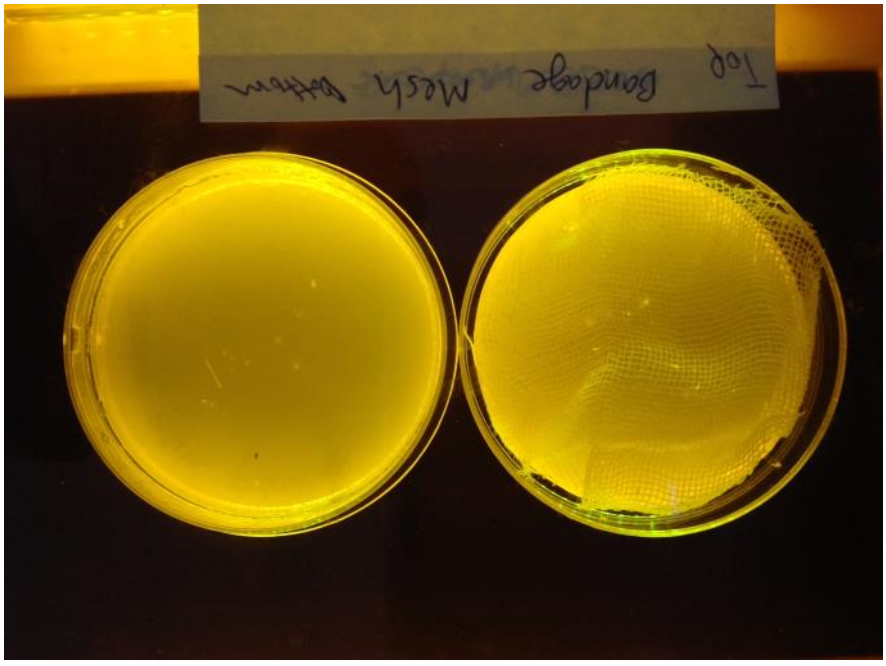

PTFE Membrane

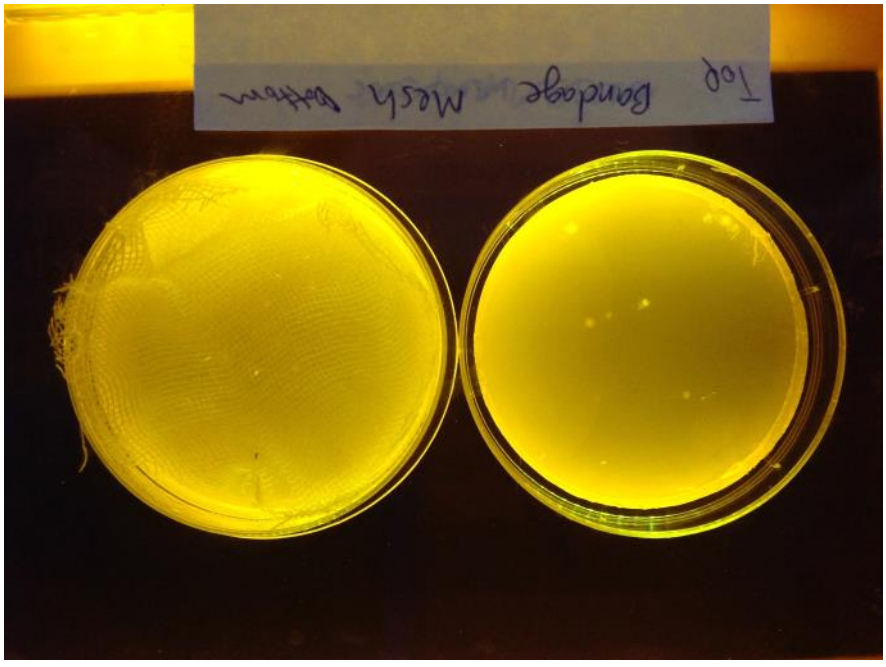

PTFE Membrane

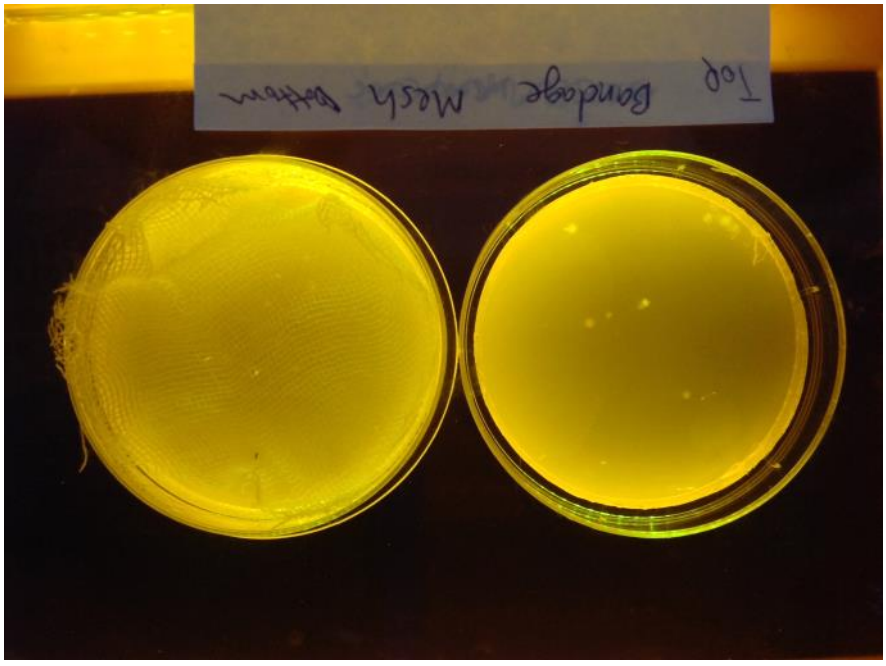

PTFE Membrane


PTFE Membrane

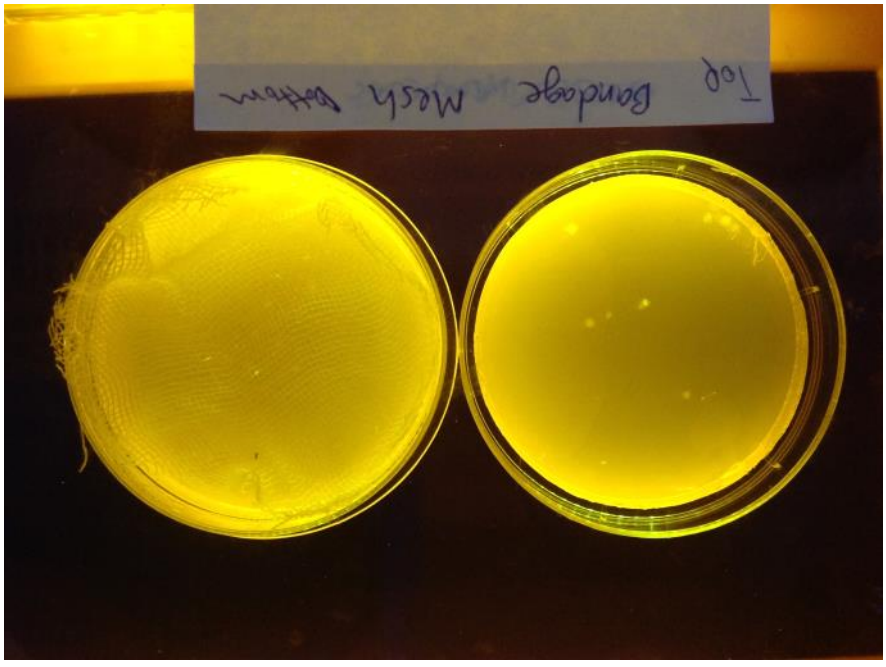

Top Bandage Mesh Pattern


Top Bandage Mesh Pattern

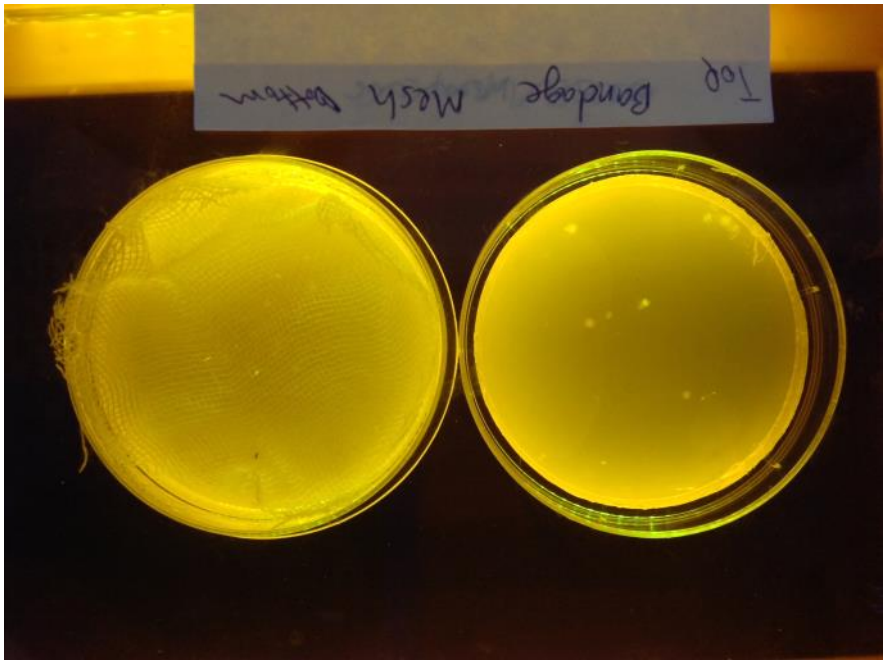

Top Bandage Mesh Pattern



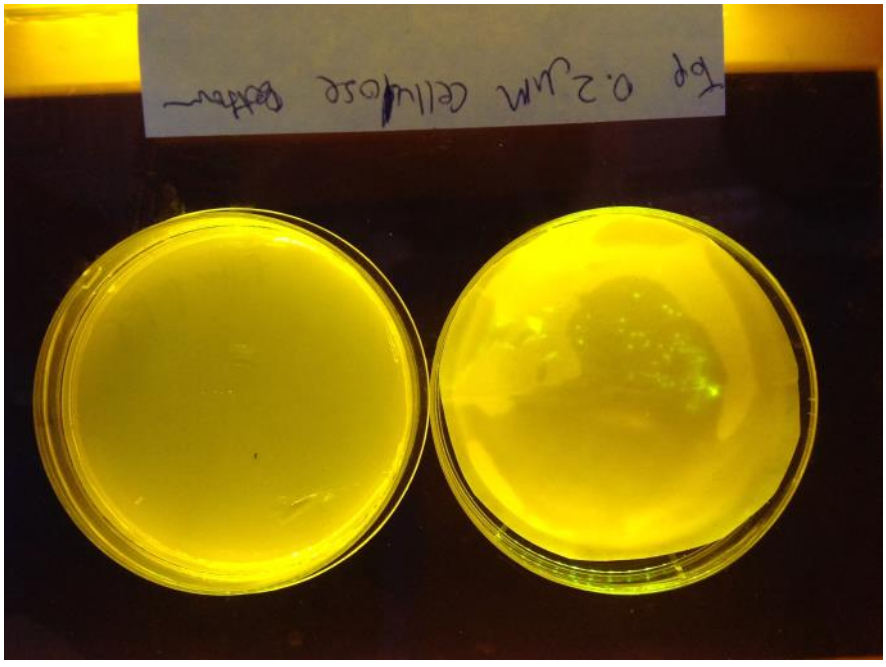
NOT CELLULOSE BUT .45 PTFE



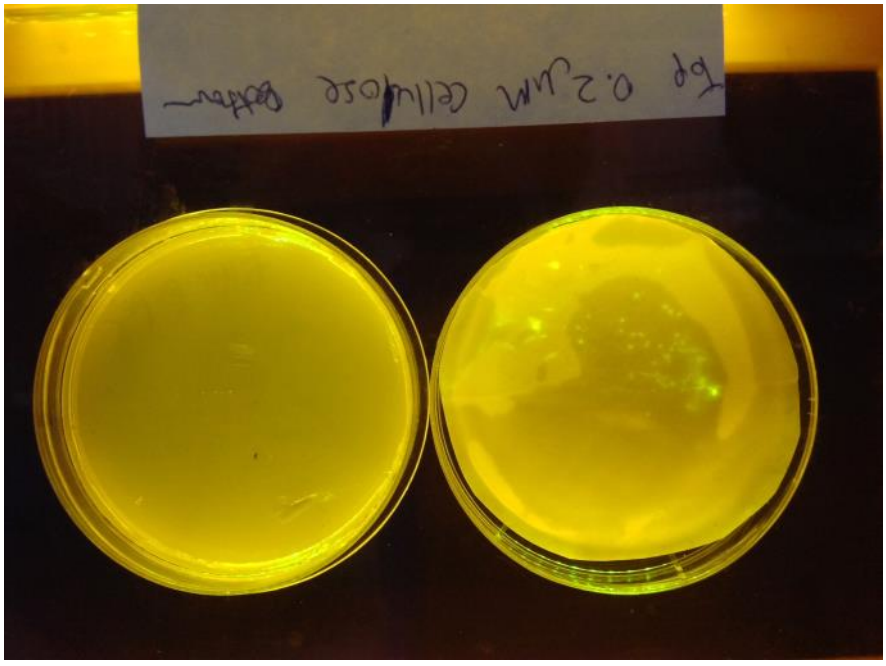
NOT CELLULOSE BUT .45 PTFE

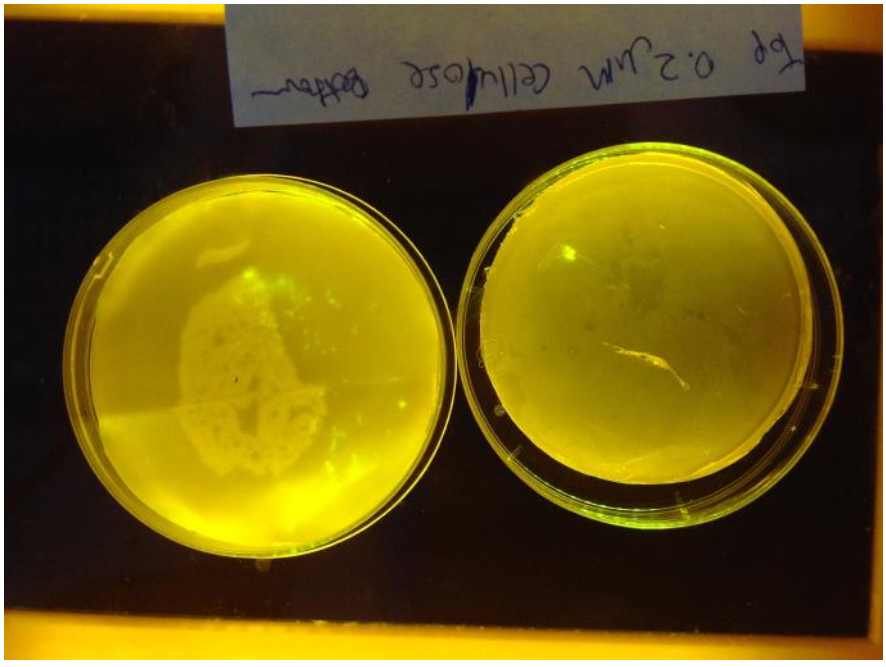


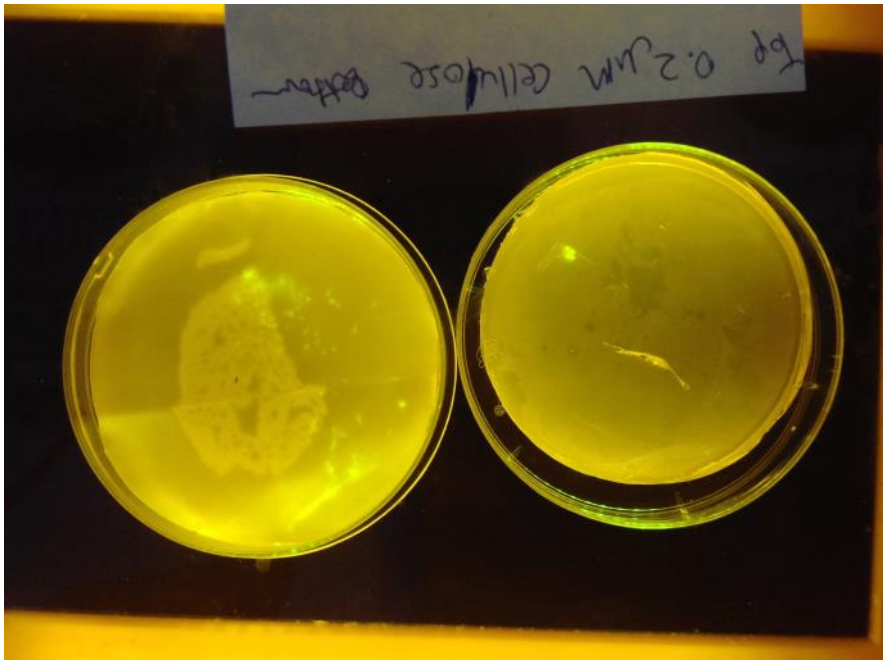
NOT CELLULOSE BUT .45 PTFE

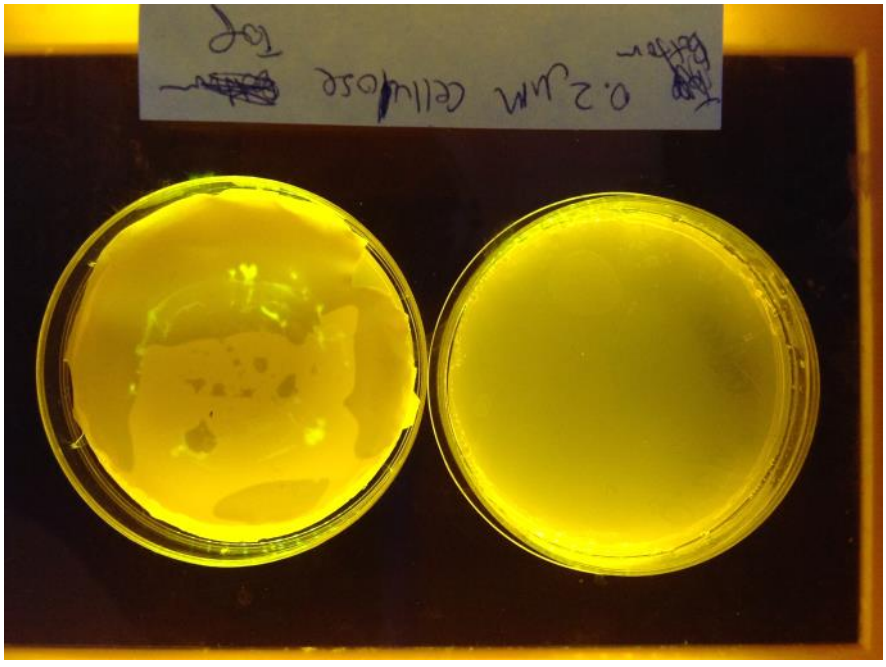


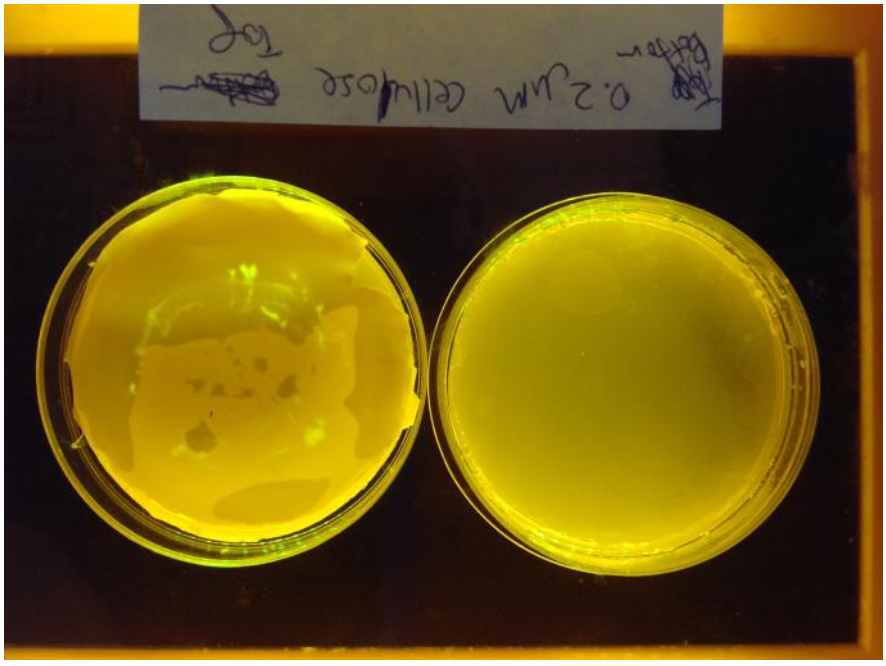
NOT CELLULOSE BUT .45 PTFE

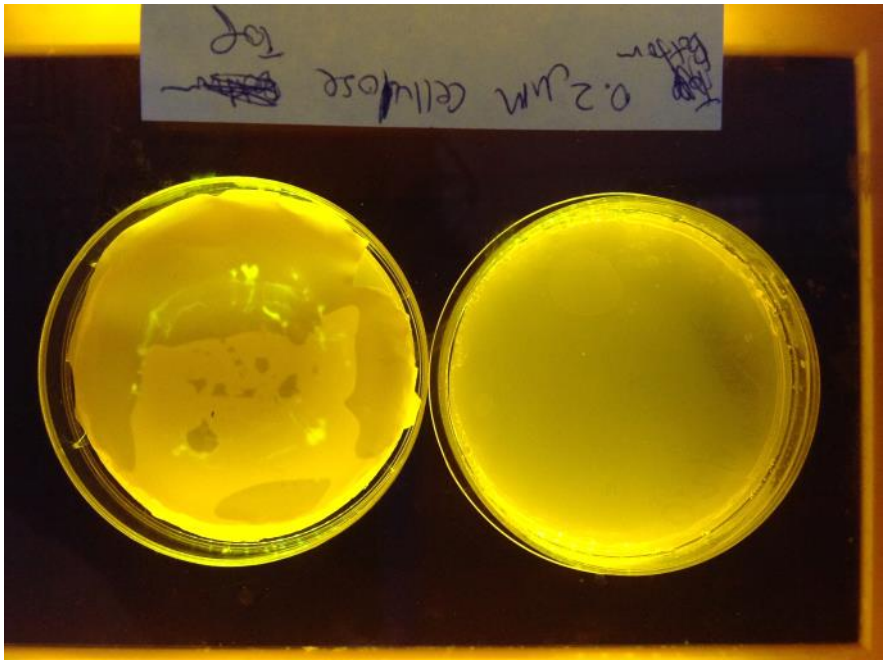

Bp 0.2µm cellulose

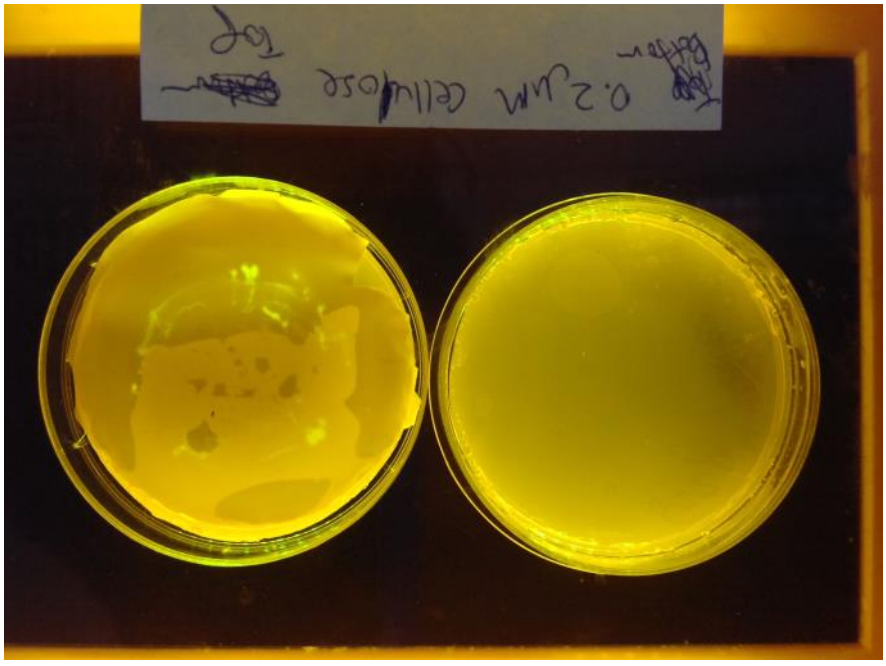

Bp 0.2µm cellulose

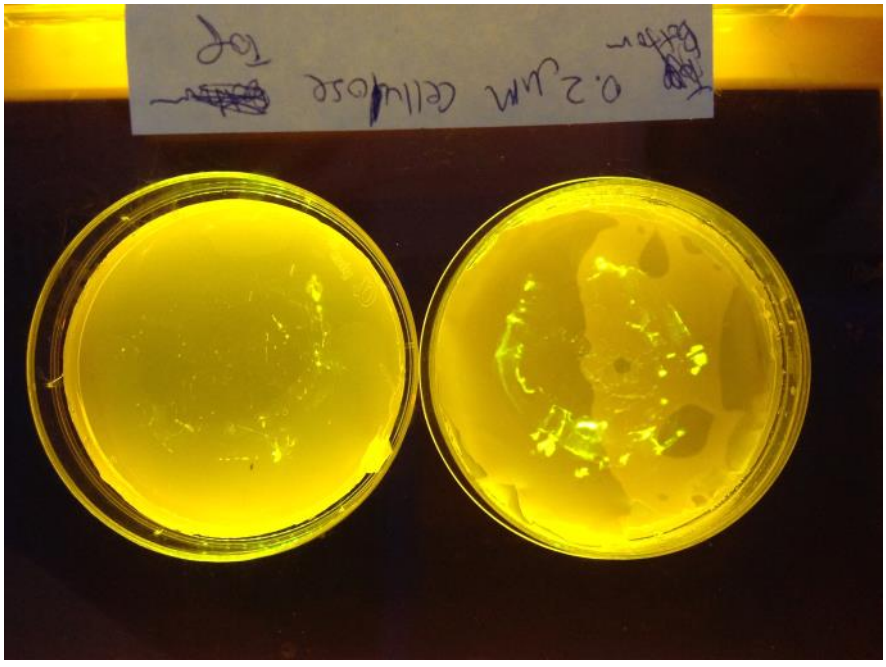

0.2 µm cellulose

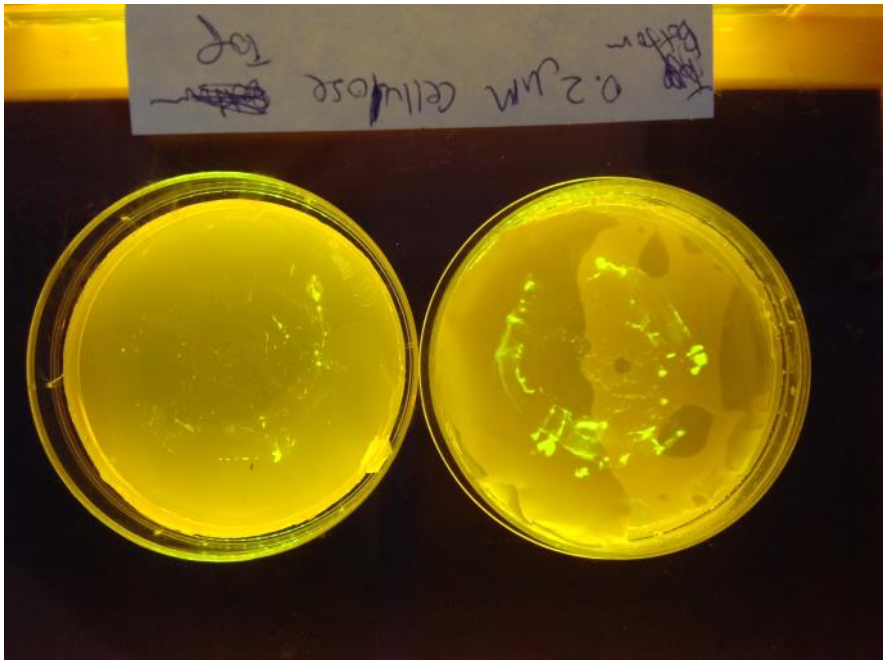

0.2 µm cellulose

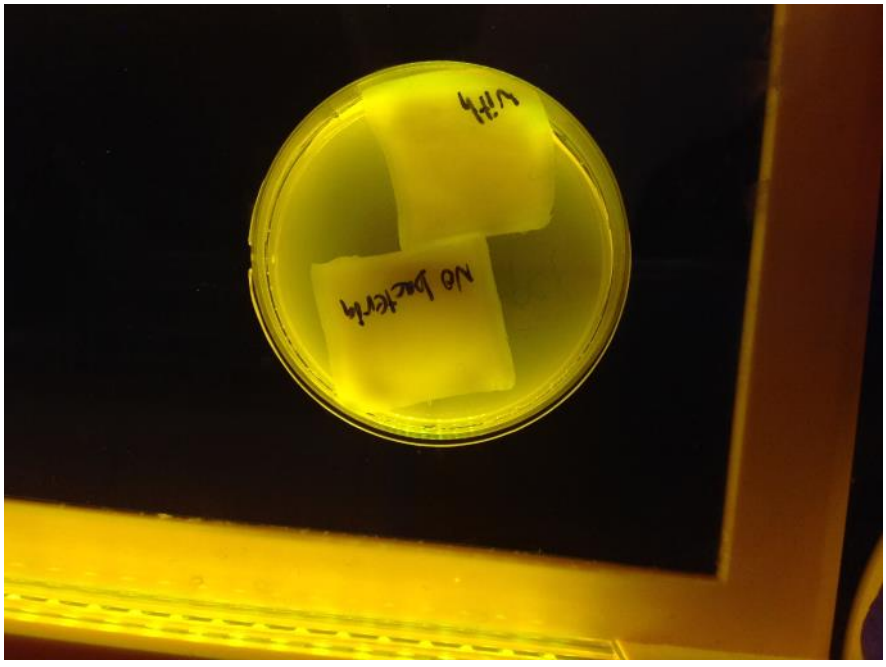

0.2µM cellulose

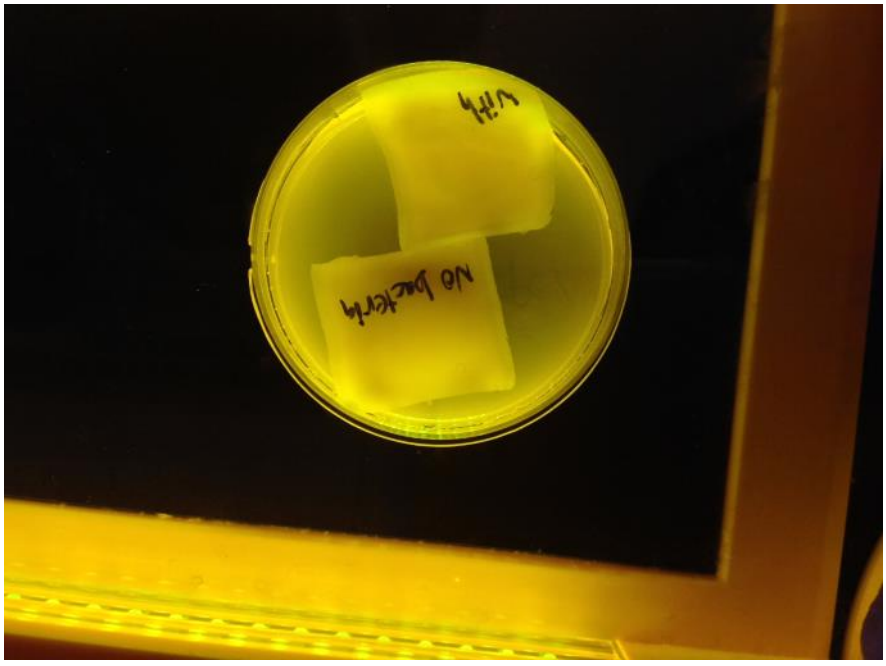

0.2µM cellulose

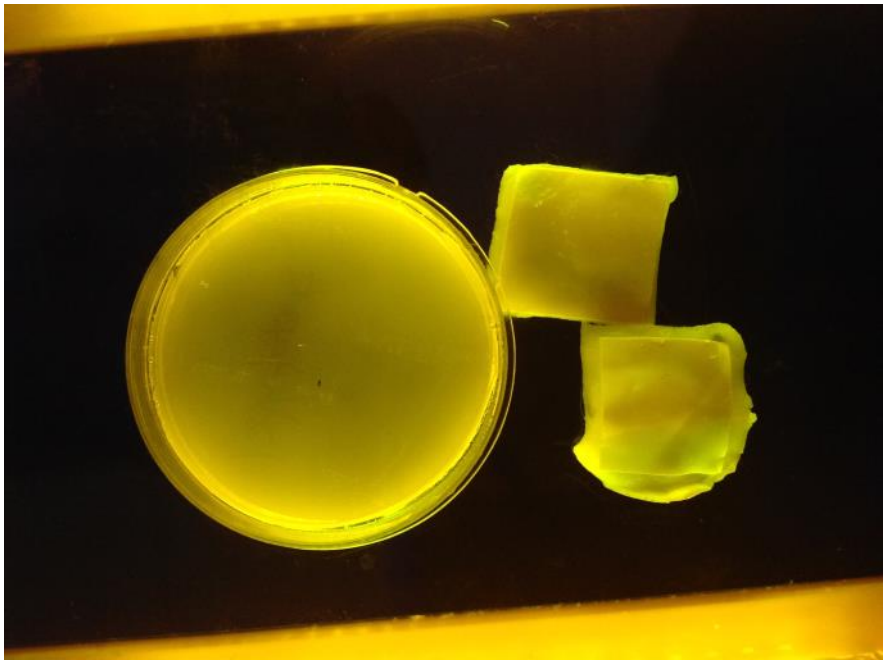

0.2µM cellulose

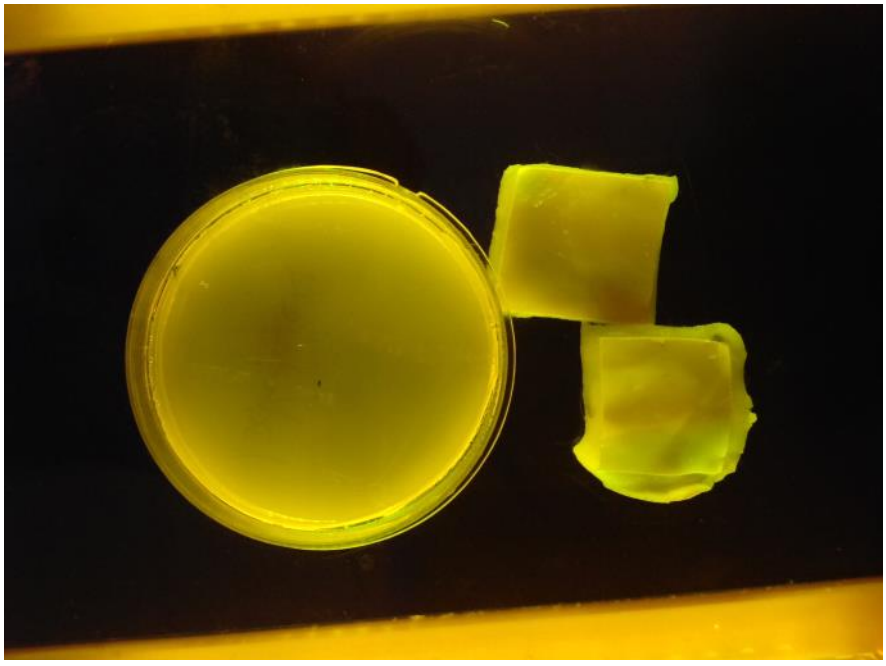

0.2µM cellulose

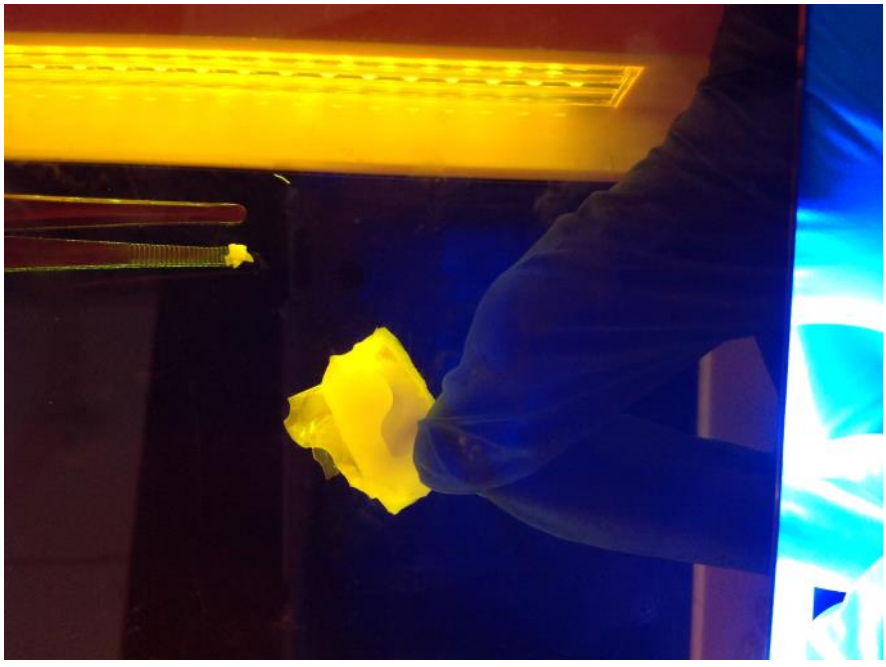

0.2 µM cellulose

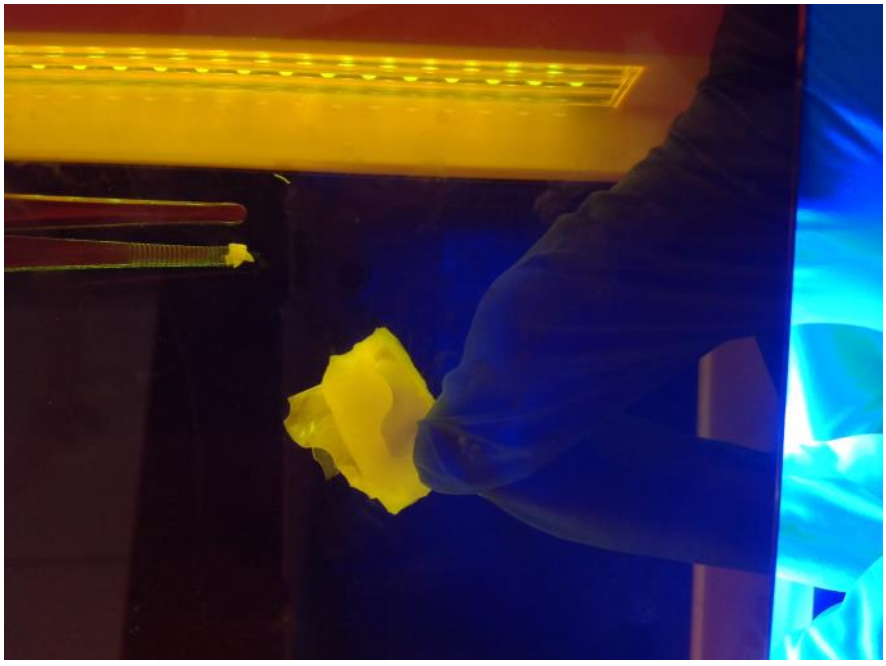

No bacteria
With

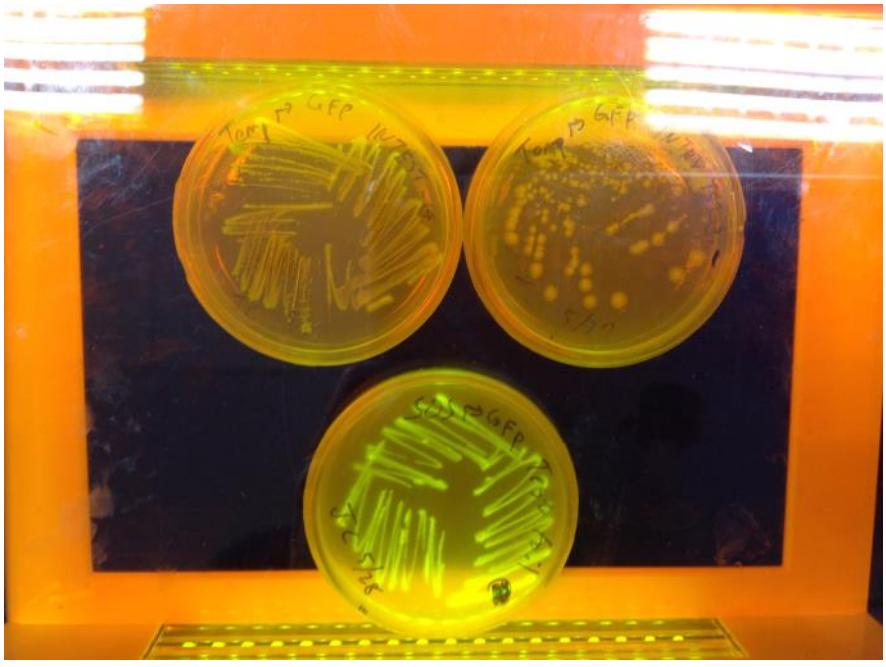

UV
No bacteria

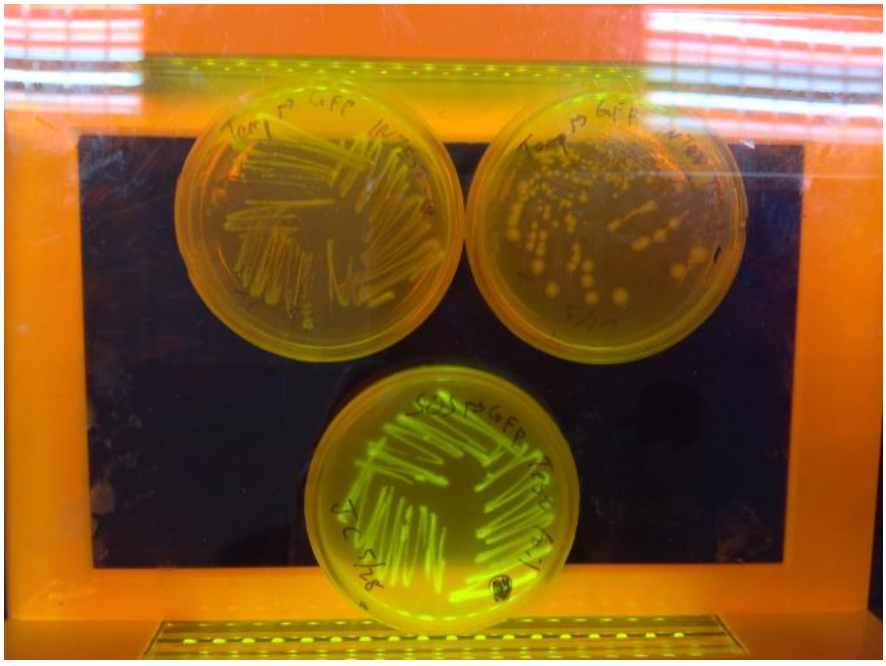












Experiments

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

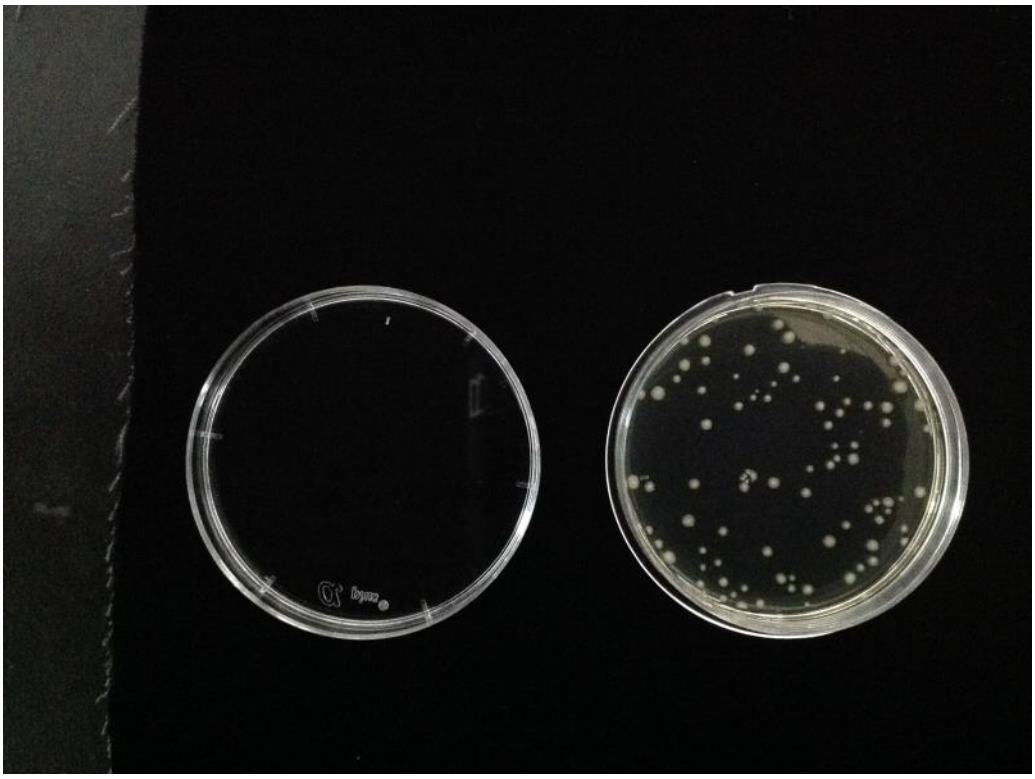


Image 1. Bacteria growth result after 44 hours (Day 2)

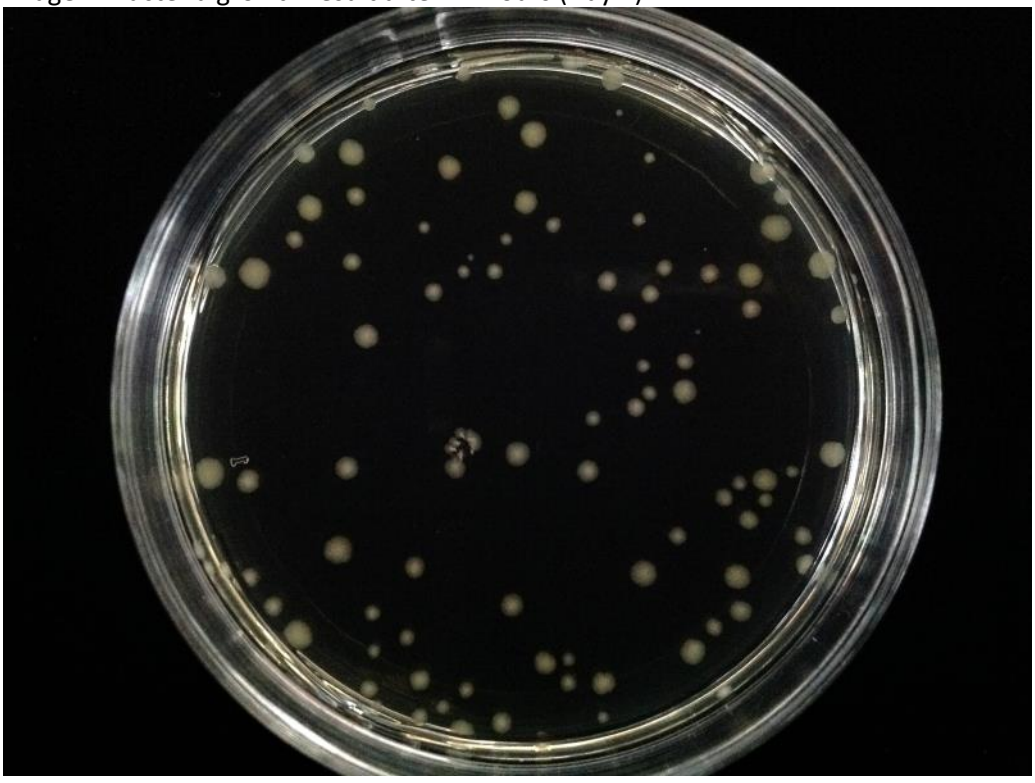


Image 2. Bacteria growth result after 44 hours detailed (Day 2)

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

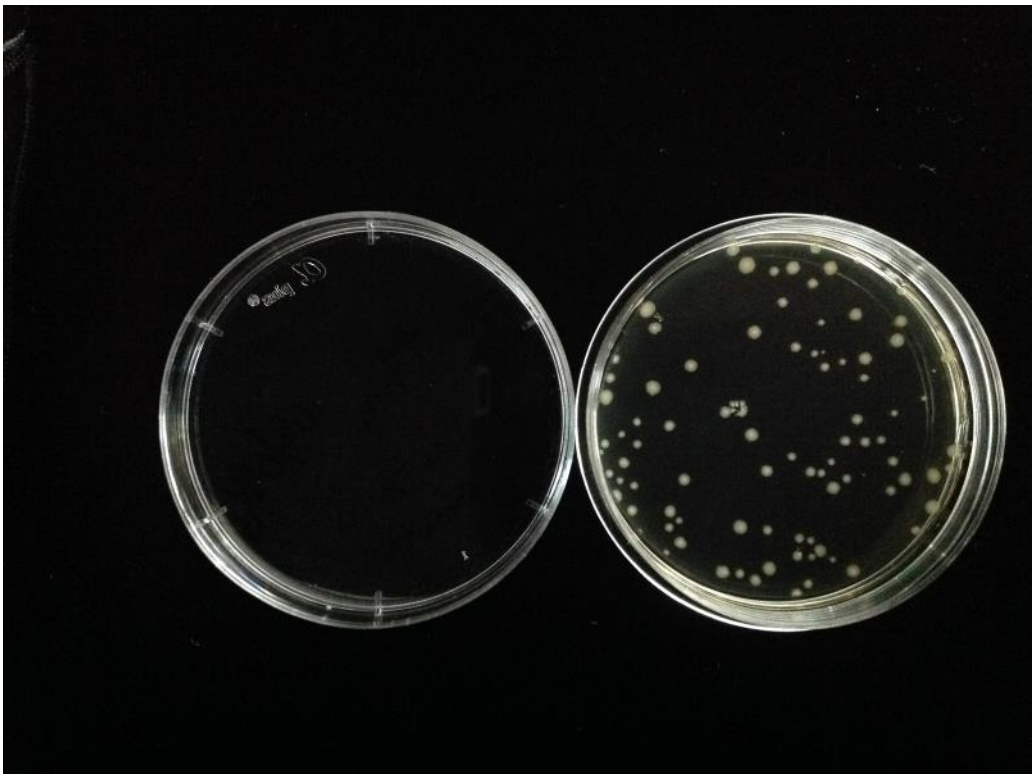


Image 1. Bacteria growth result after 68 hours (Day 3)

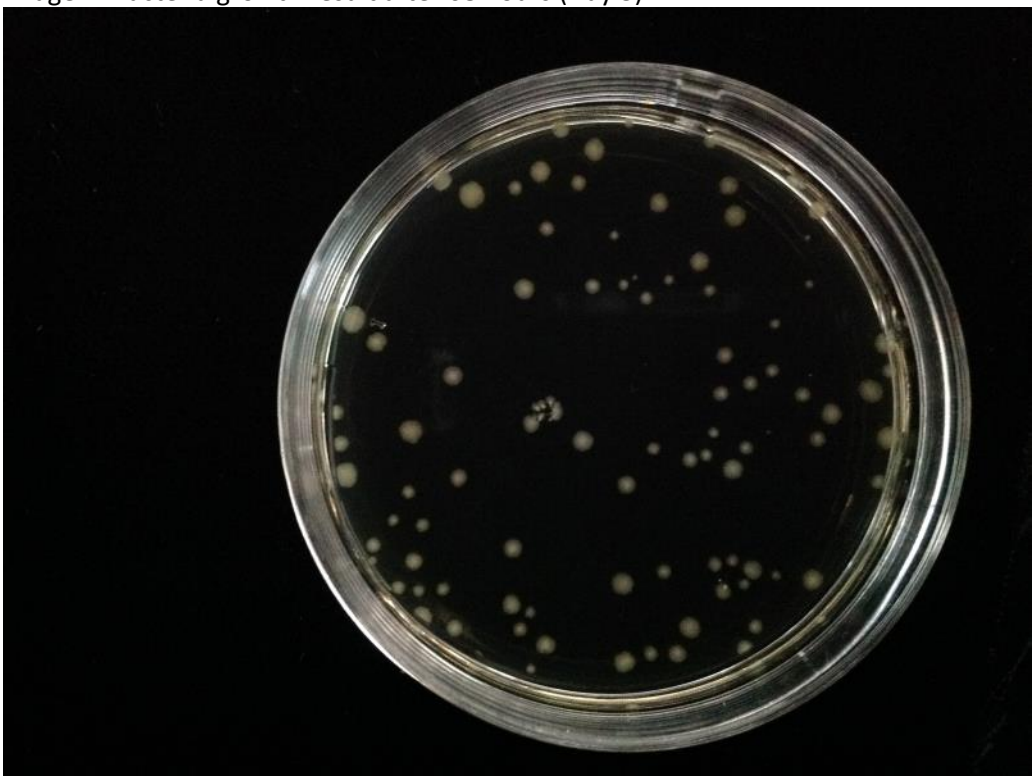
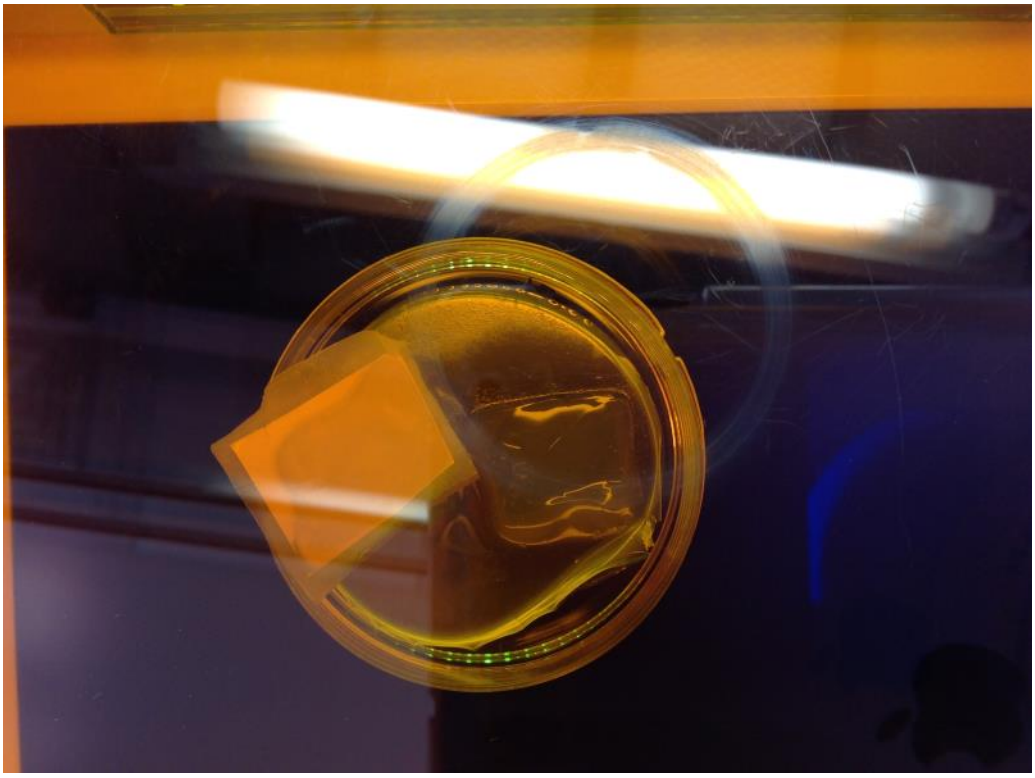
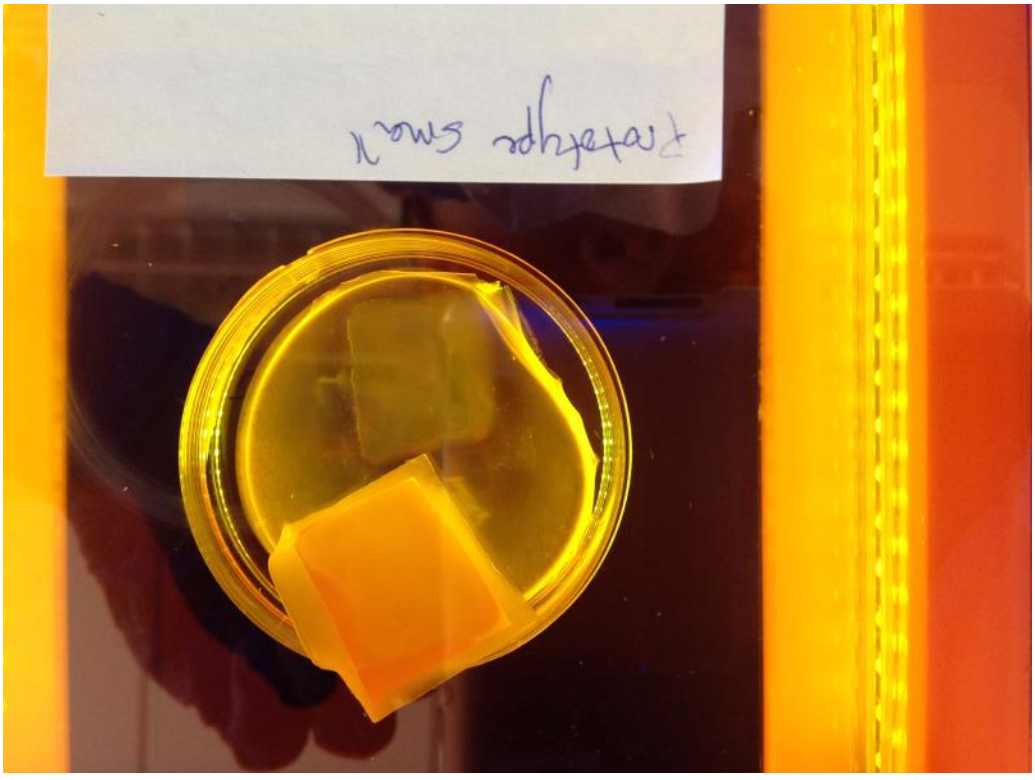
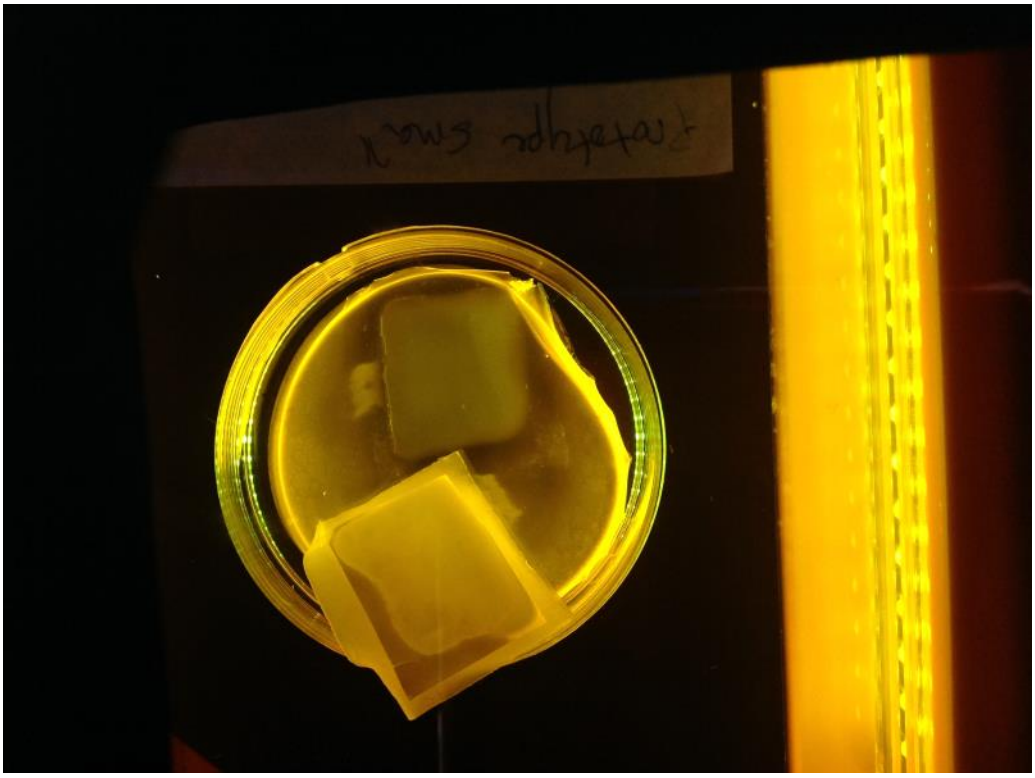
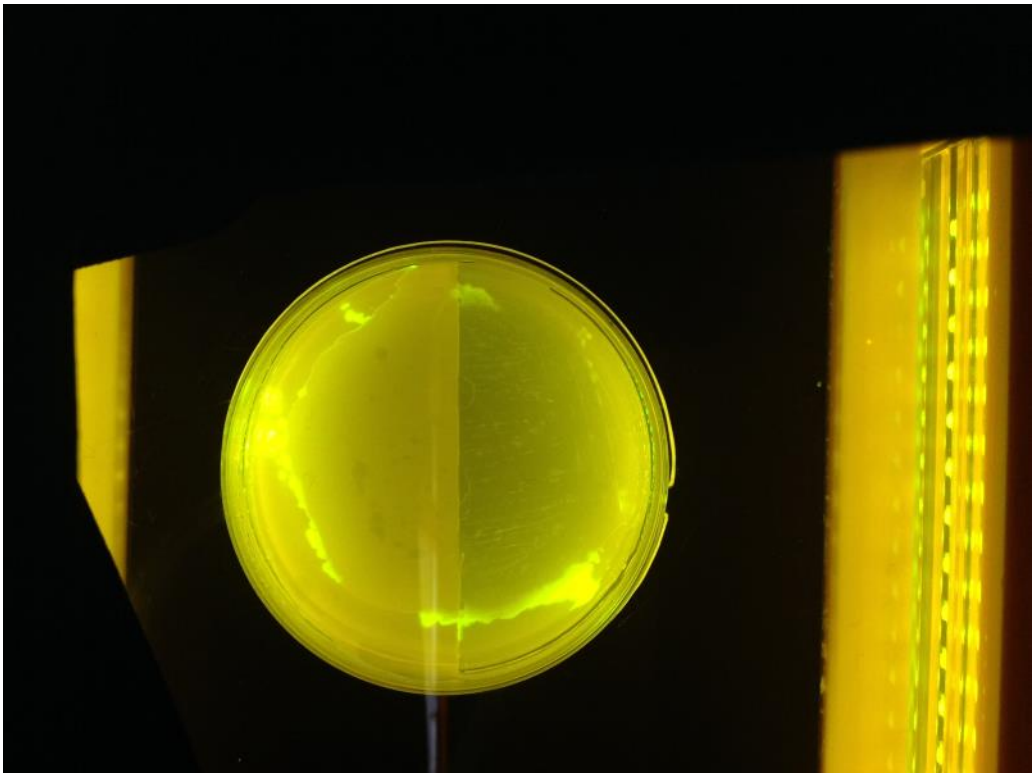


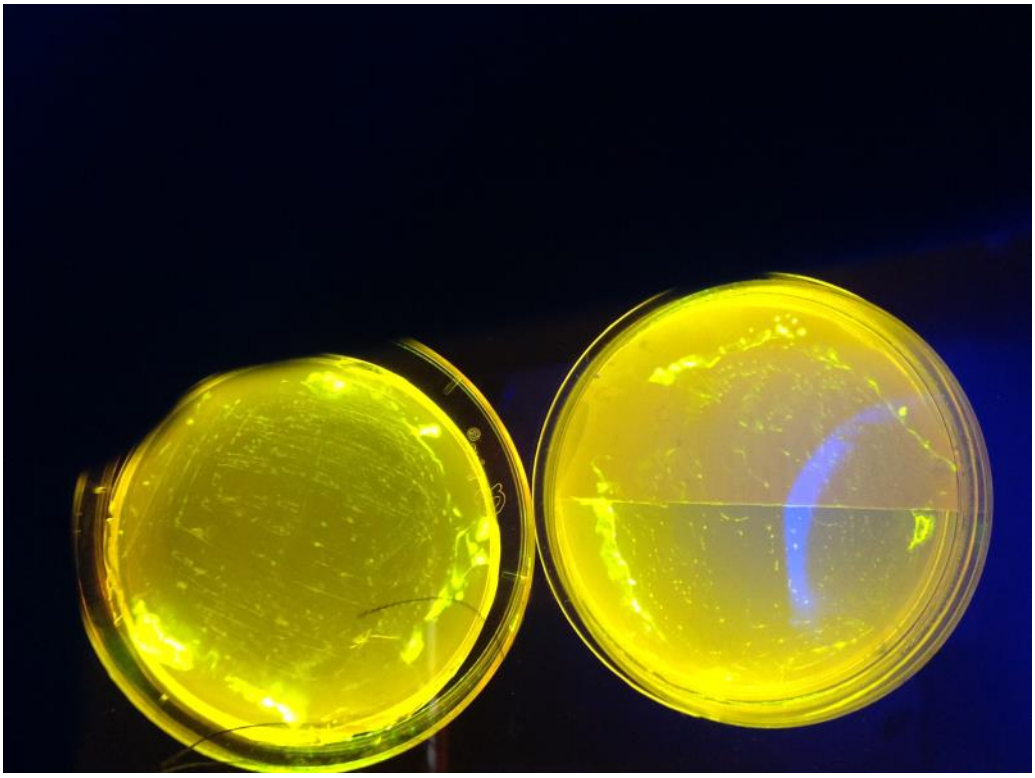
Image 2. Bacteria growth result after 68 hours detailed (Day 3)

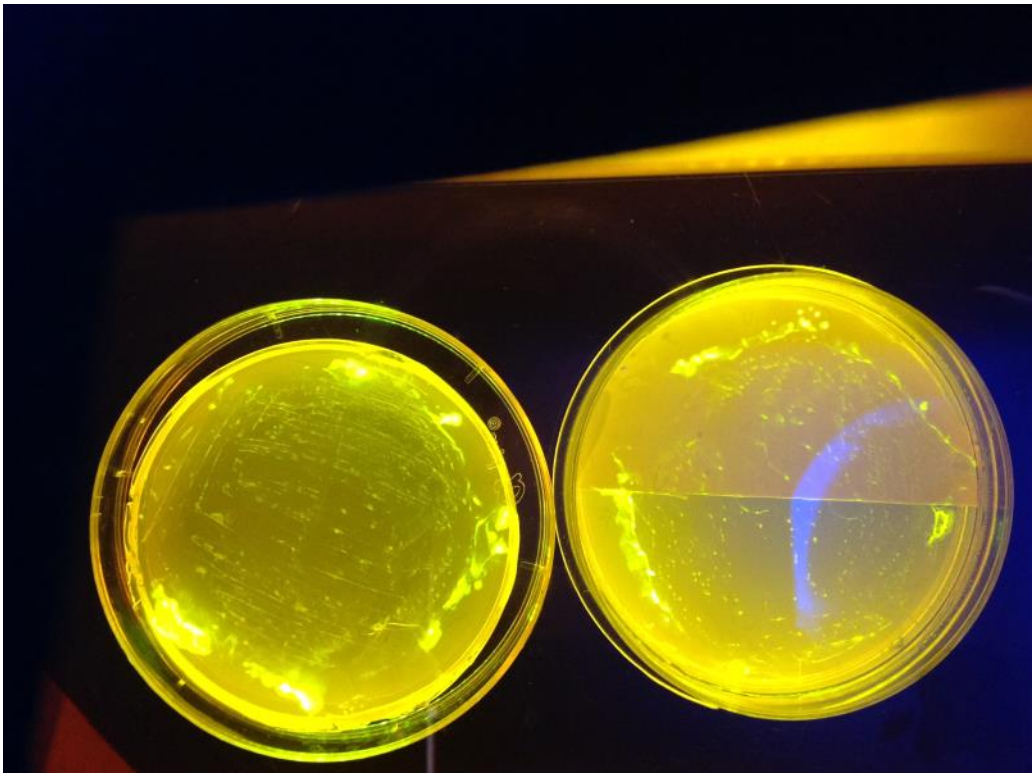


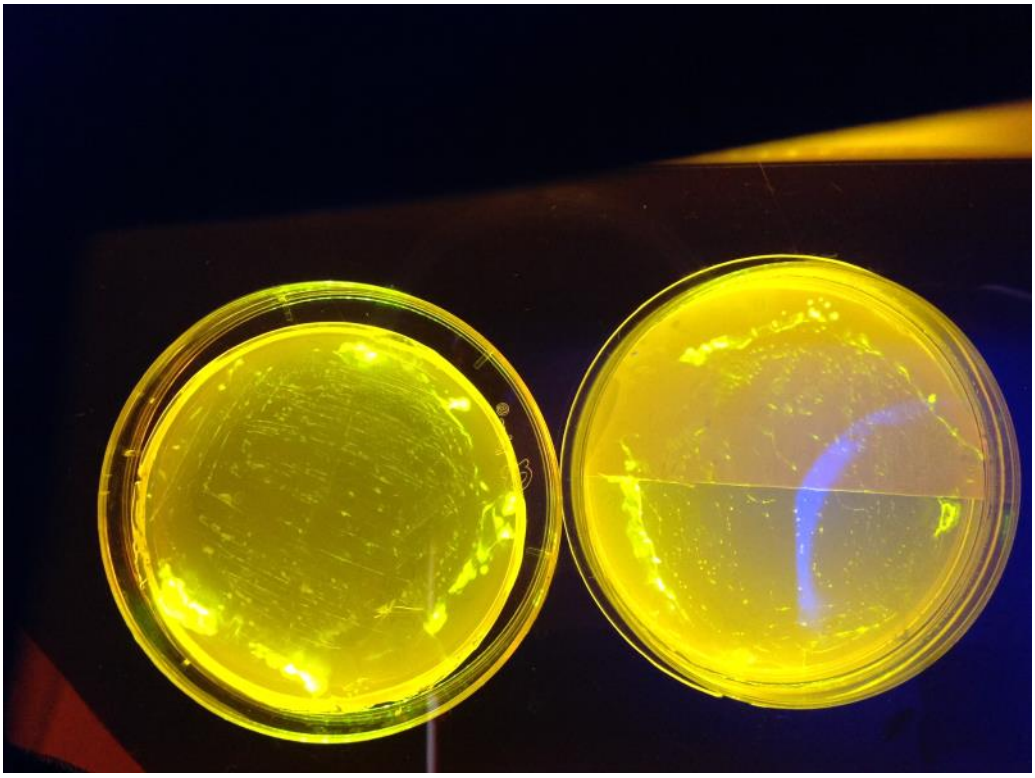


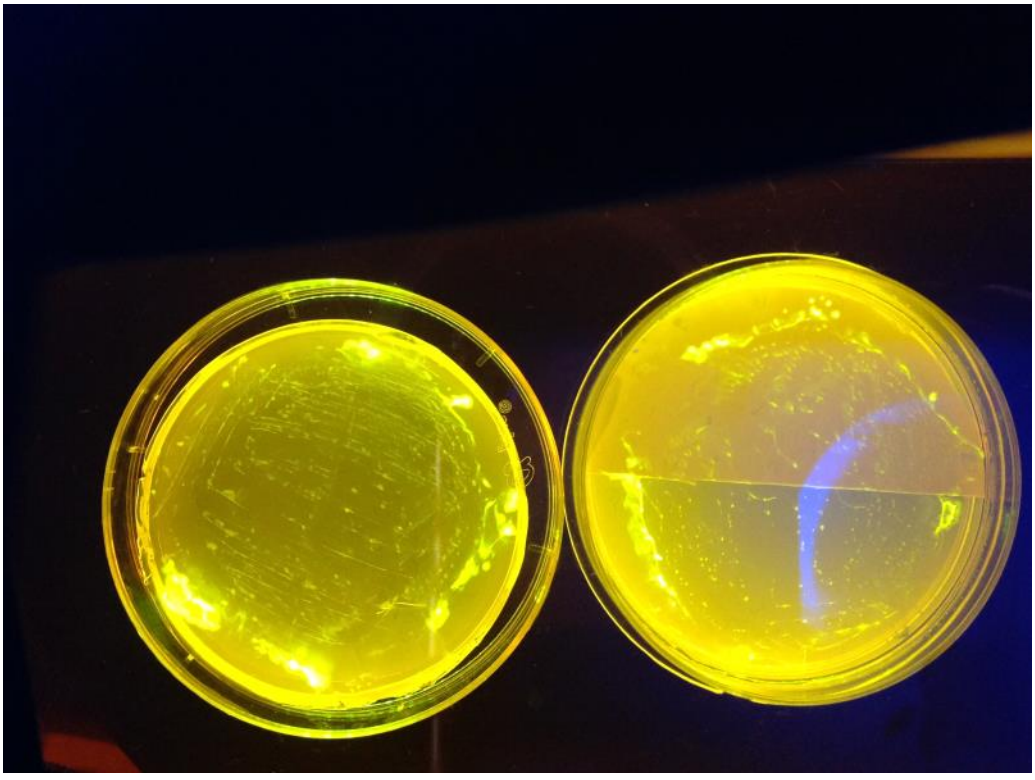


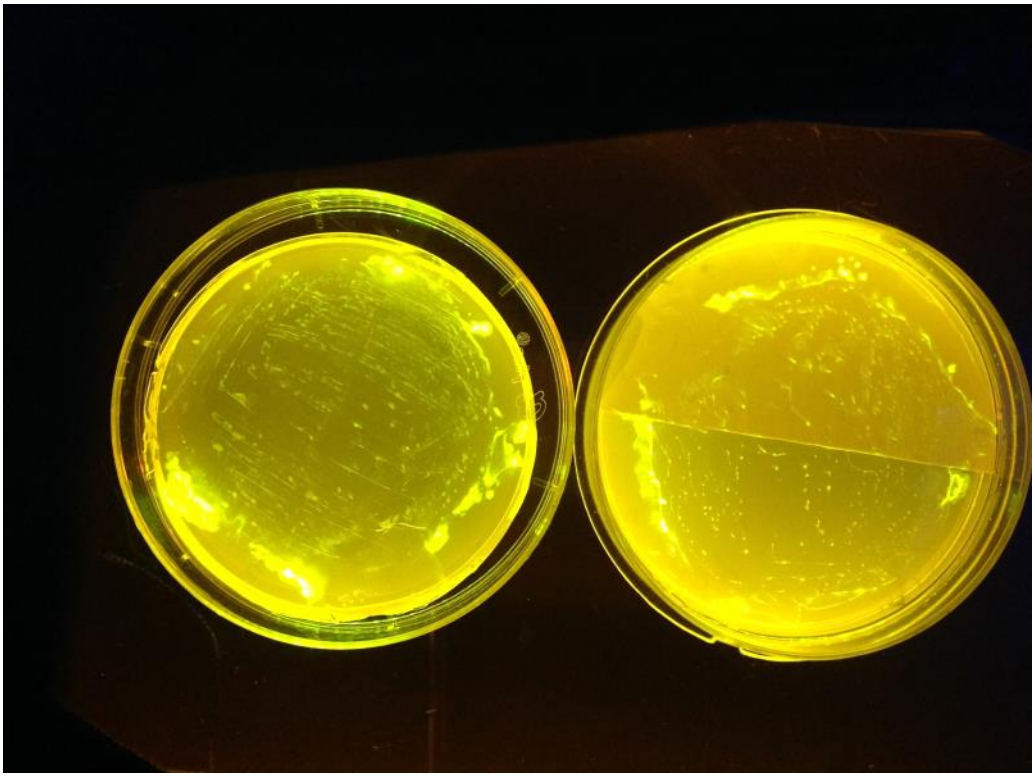


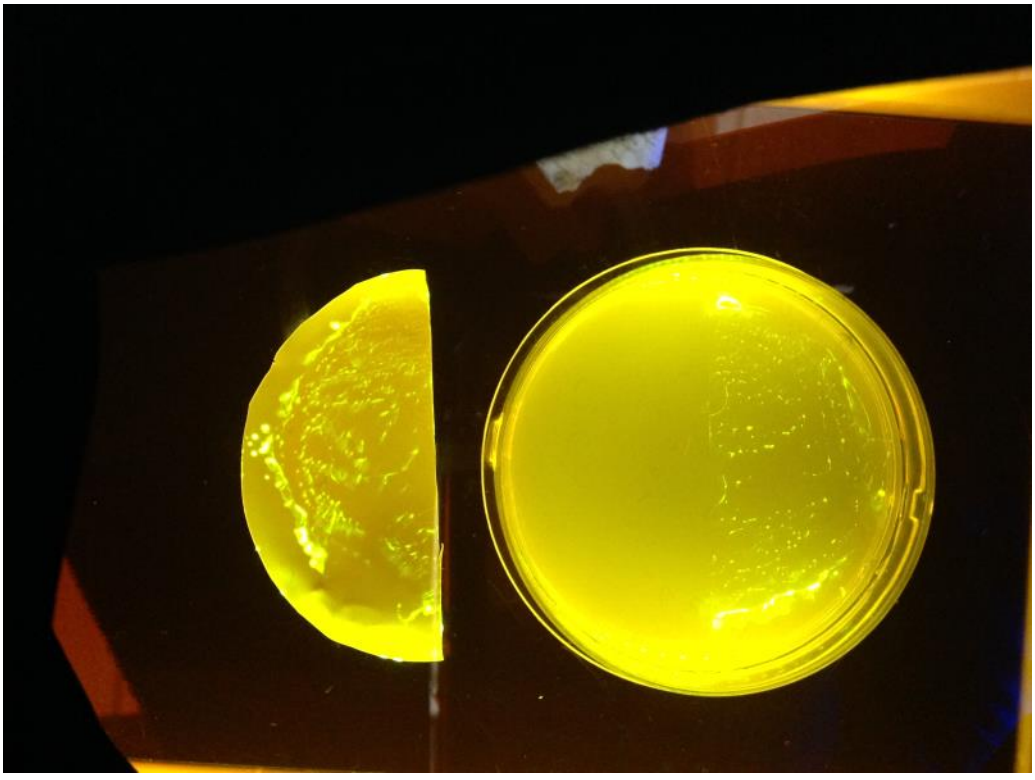


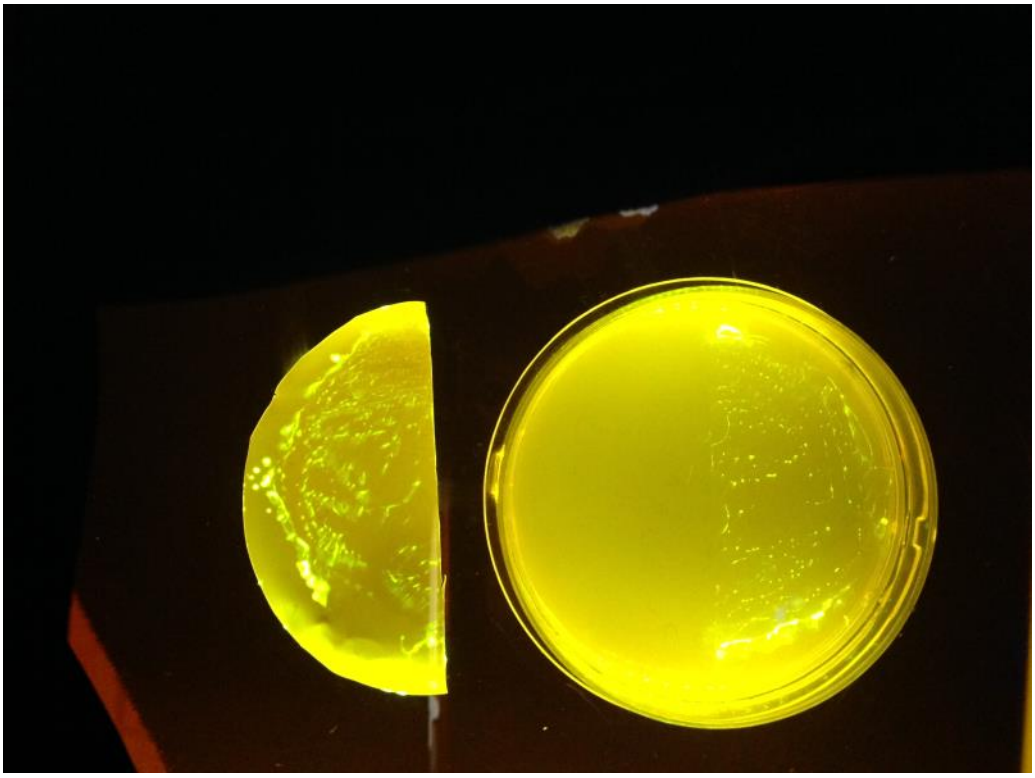


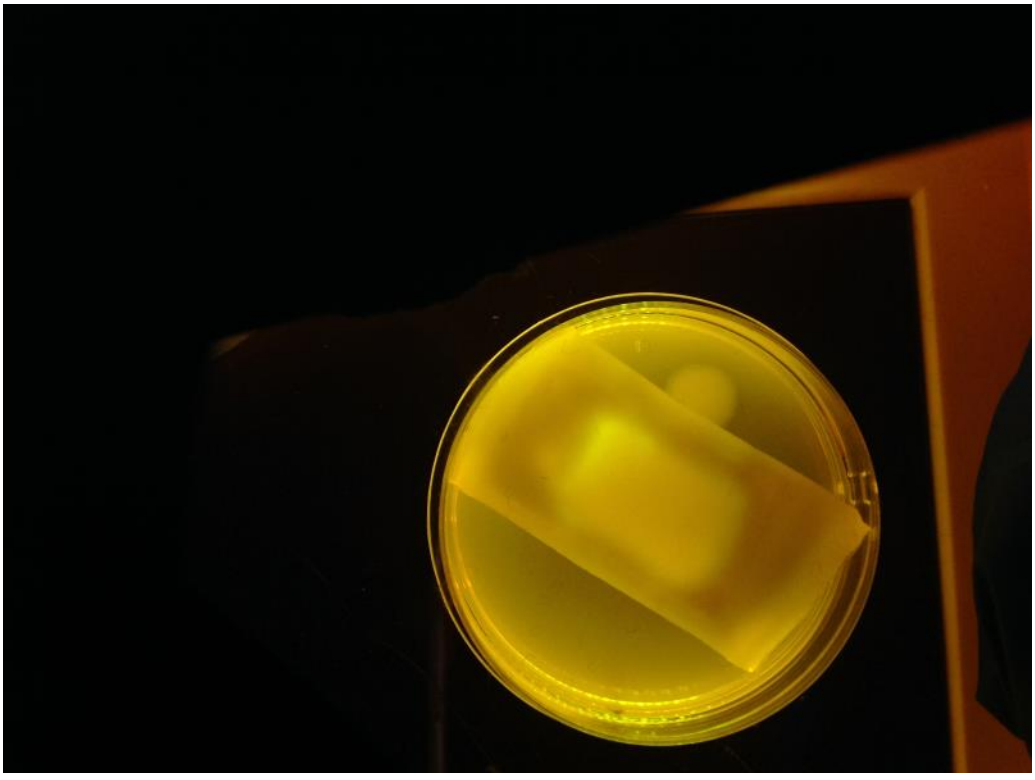


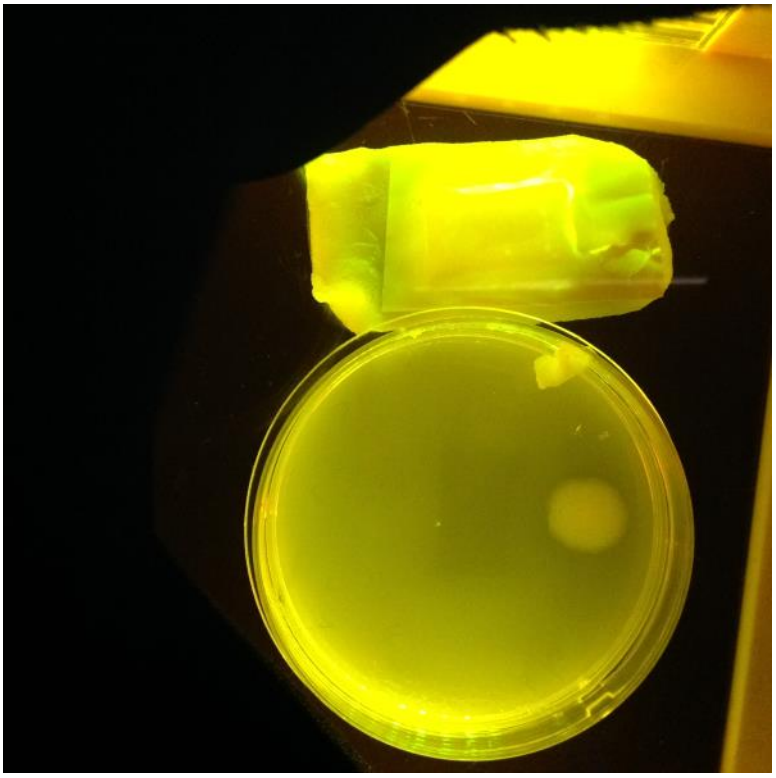


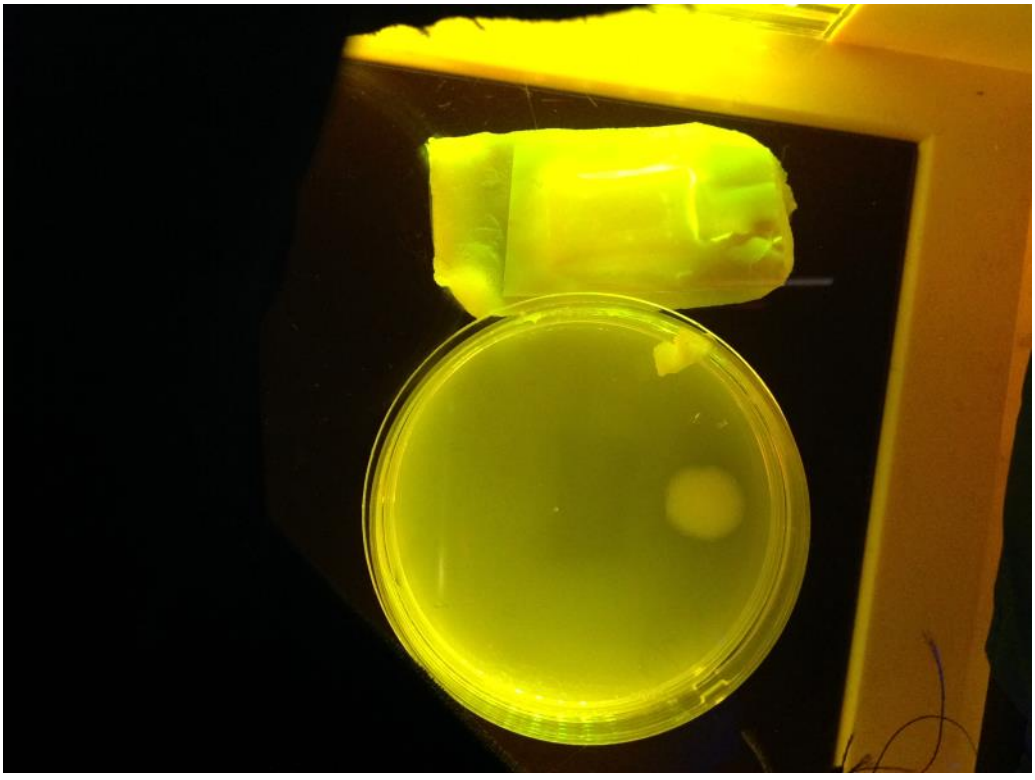


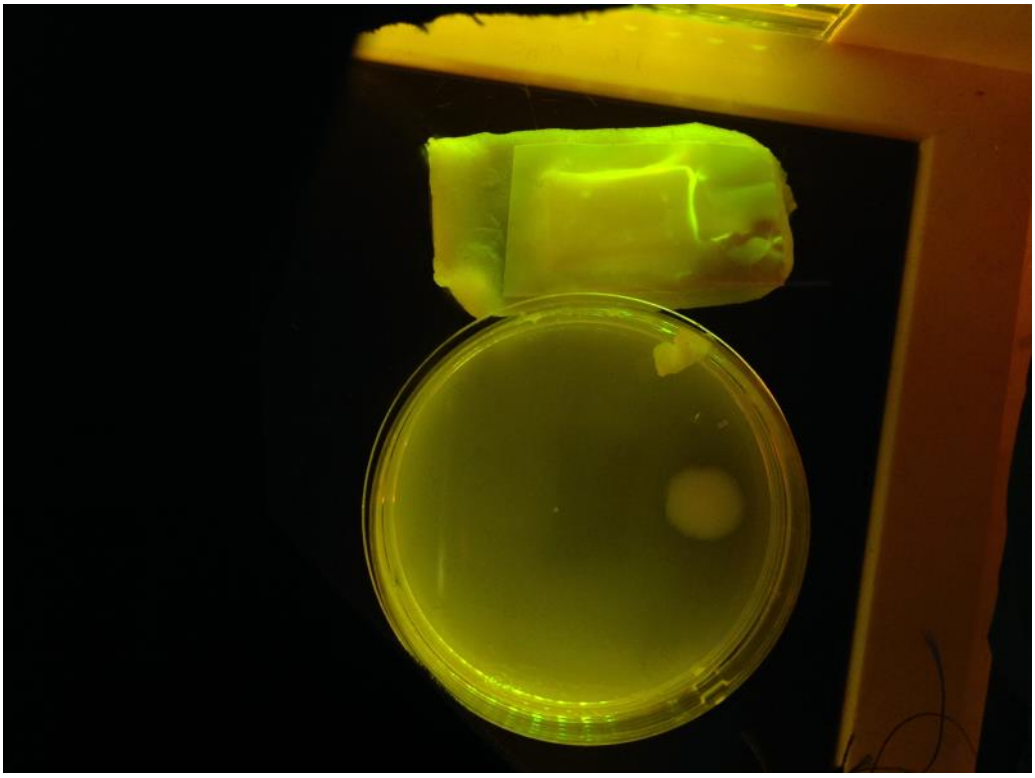


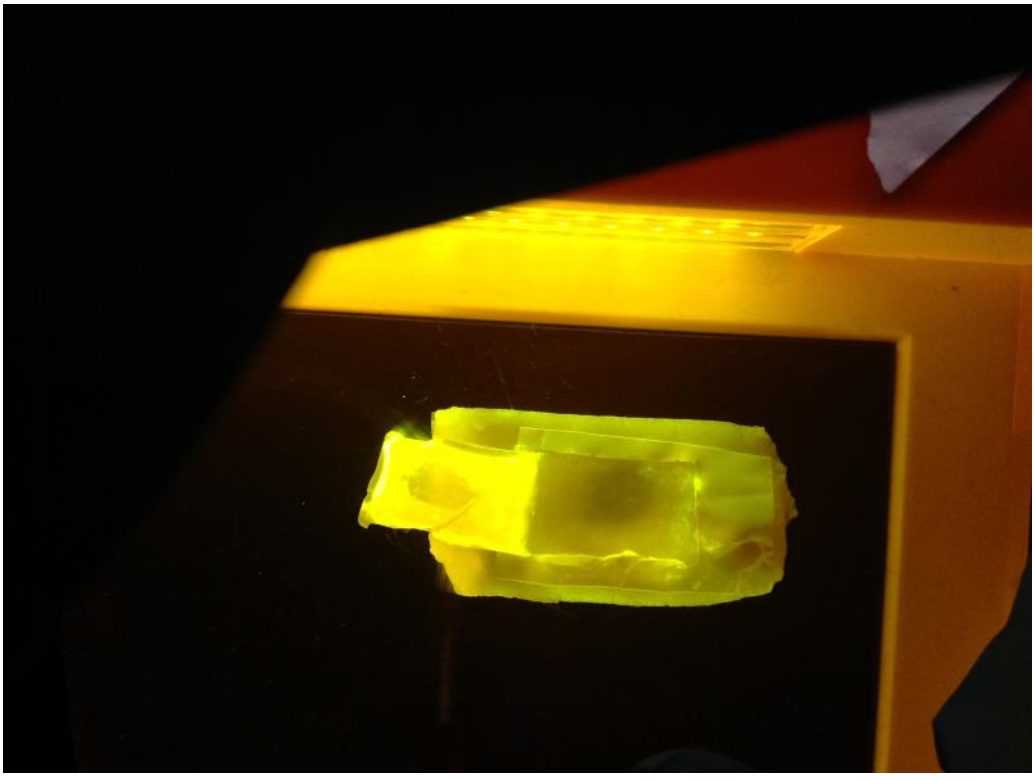


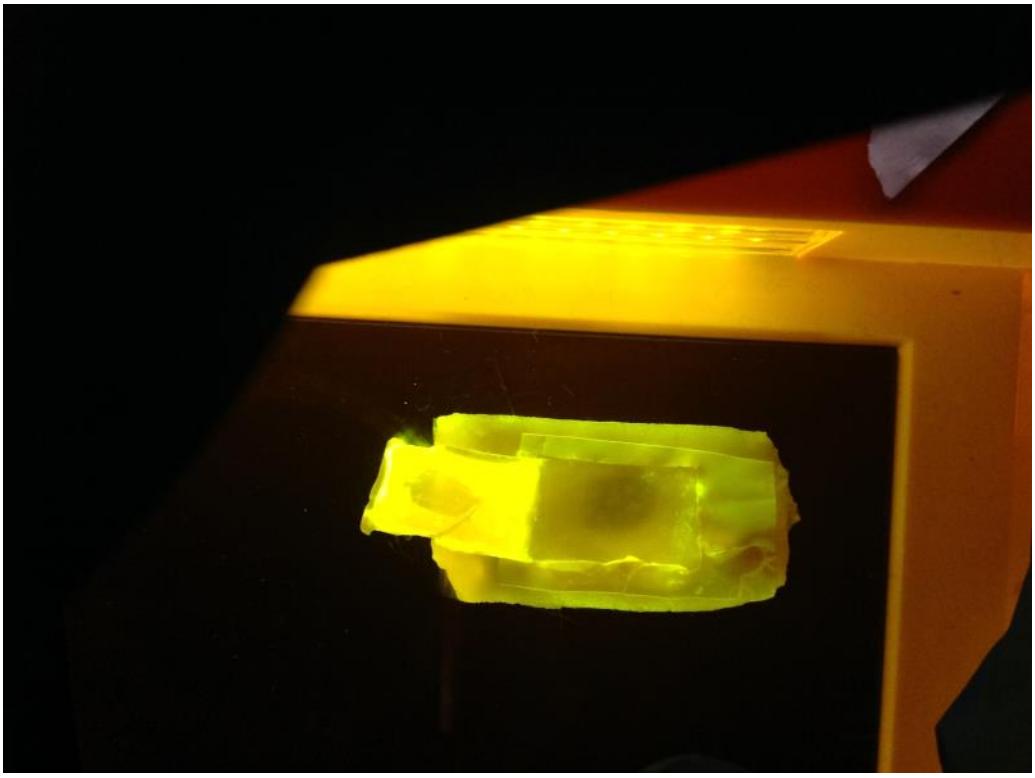












Experiments

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

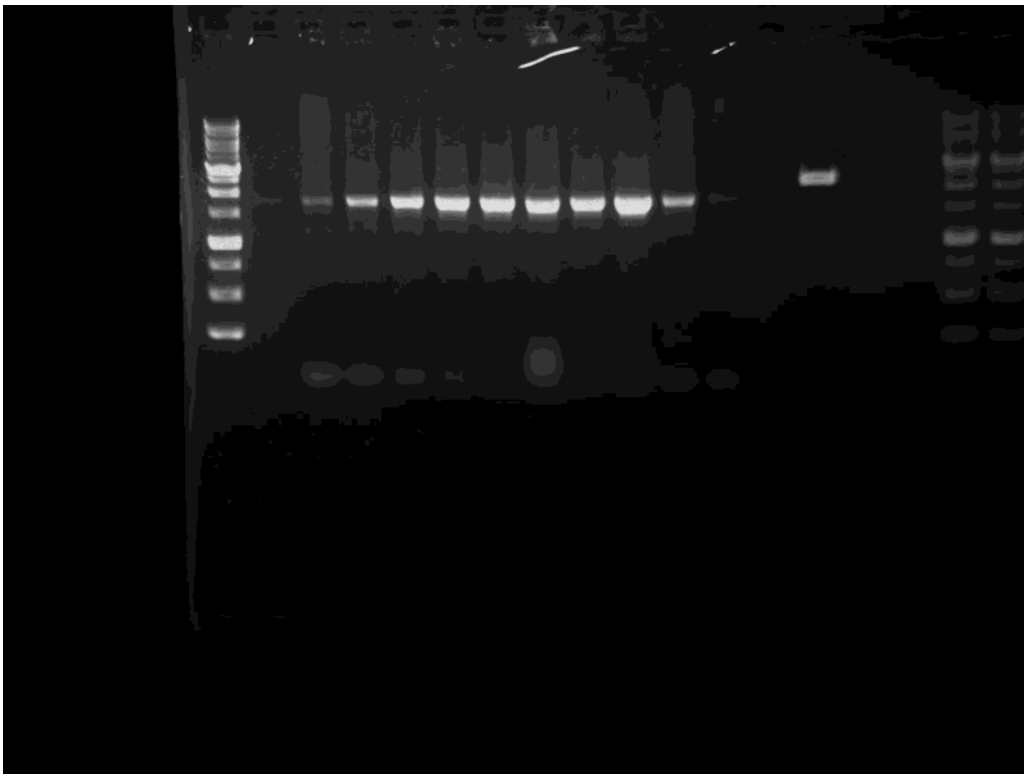
Experiments

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

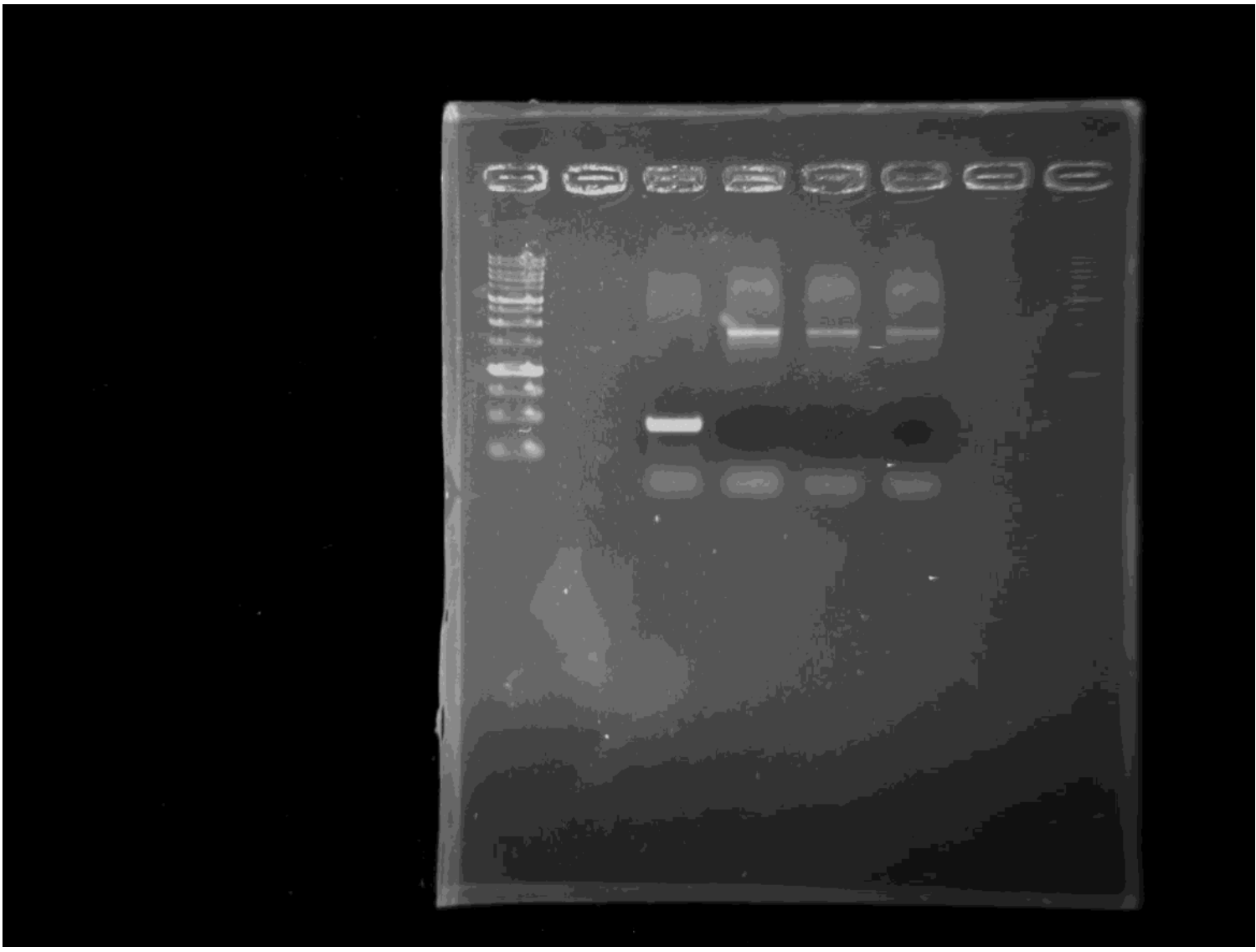
The lonely lane on the right is a terminator digested at E
    This linearized segment should be around 2kb which is also confirmed by the ladder on the left and right

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

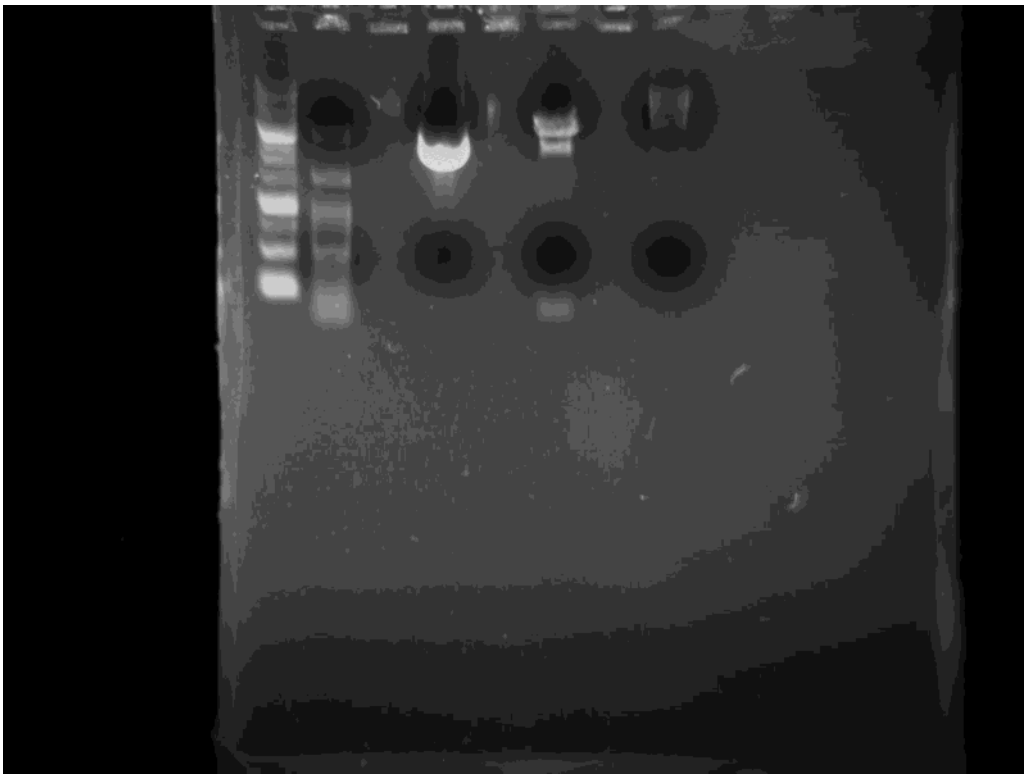


Tuesday, June 16, 2015
6:23 PM

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

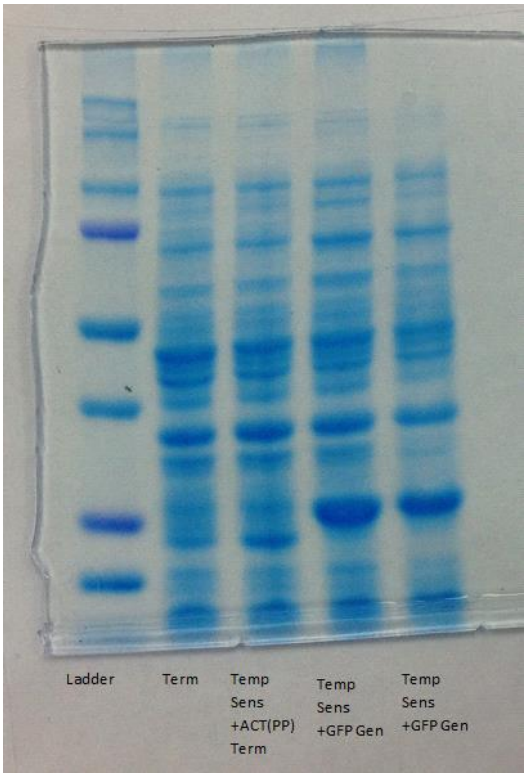
Experiments

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