

Table of Contents

September 2022	3
Wednesday, September 7, 2022	3
Notes for aseptic technique	3
Thursday, September 8, 2022	3
Cystine heart agar with hemoglobin plates (CHAH) protocol	5
Analyzing colony size using ImageJ	6
Friday, September 9, 2022	7
Making competent cells	7
Monday, September 12, 2022	7
Electroporate cells	10
Tuesday, September 13, 2022	10
Making Glycerol Stocks (Single Use Aliquots)	10
Wednesday, September 14, 2022	11
Thursday, September 15, 2022	11
Friday, September 16, 2022	18
Monday, September 19, 2022	19
Freezing permanent bacterial strain stocks	22
Add strain to strain list	22
Freeze down strain	23
Tuesday, September 20, 2022	23
To make lysate	23
Buffers to Prep	24
Wednesday, September 21, 2022	25
Mueller-Hinton broth protocol	25
Inoculating test tube cultures of <i>Francisella tularensis</i> protocol	25
Thursday, September 22, 2022	26
Cell harvesting protocol	26
Cell lysis protocol	27
Friday, September 23, 2022	27
Monday, September 26, 2022	29
Sucrose gradient protocol	31

Fractionating Protocol	32
Tuesday, September 27, 2022	33
Colony PCR Protocol	34
Wednesday, September 28, 2022	35
gDNA extraction protocol	36
Thursday, September 29, 2022	36
Friday, September 30, 2022	37
Monday, October 3, 2022	37
Bibliography	40

September 2022

Wednesday, September 7, 2022

To Do:

1. Streak out $\Delta rpsU1$ and $\Delta rpsU2$

Results and Data:

With the help of Hannah Trautmann, I streaked $\Delta rpsU1$ $\Delta rpsU2$ onto 2 CHAH (cystine heart agar with hemoglobin in it) plates to be used for making competent cells.

Notes for aseptic technique

These are some things that I have been told will help reduce contamination in the lab

1. Make sure to always wear gloves when dealing with anything that may have been in contact with cells
2. Make sure to wipe lab bench down with ethanol wipes before starting any experiment
3. Make sure to spray hands with ethanol when starting something new
4. Make sure to spray micropipettes, tube holders, etc. before using them
5. Make sure to always do sterile activities within 6 inches of the flame

Thursday, September 8, 2022

To Do:

1. ~~Streak out LVS~~
2. ~~Image plates Kathryn struck out and learn to use ImageJ~~
3. ~~Make Kan plates~~

Results and Data:

Hannah and I made CHAH-Kan plates to use for imaging colony sizes. Along with that, she showed me how to electroporate cells and I plan to practice sterile technique over a flame. We also imaged the plates that Dr. Kathryn Ramsey had previously struck out and I used ImageJ to quantify the colonies. For the area, I used the number that was given to me via ImageJ but I'm not sure if I should be calculating that myself. I then streaked out LVS from a single aliquot to be used for making competent cells.

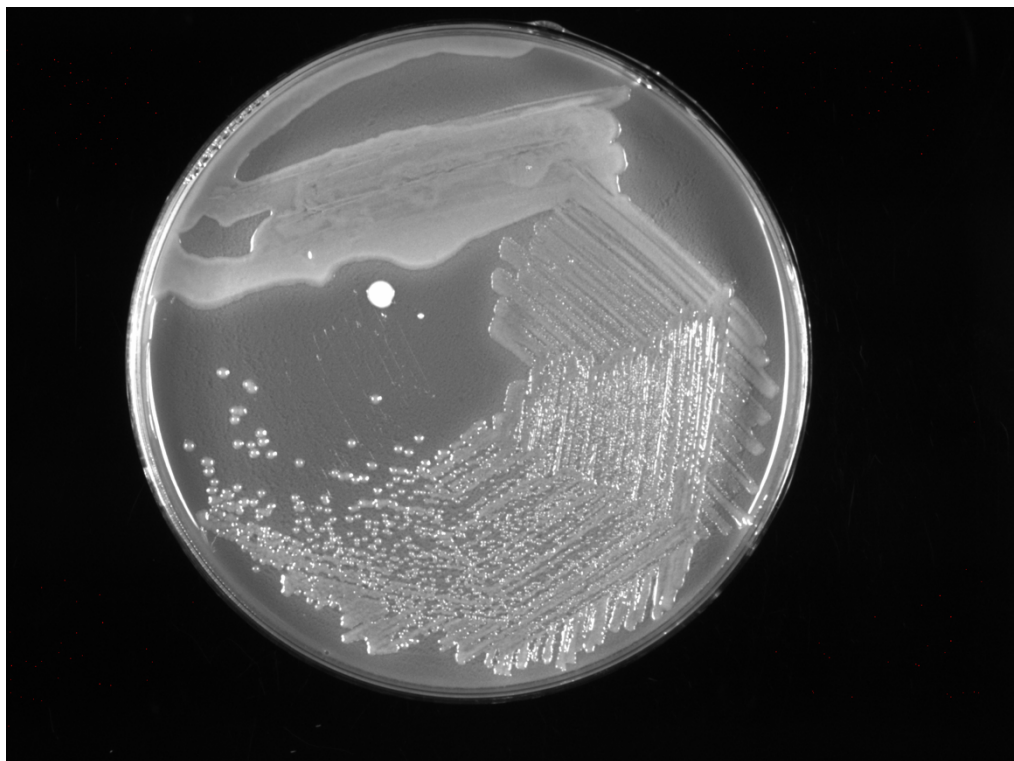


Figure 1. LVS colonies

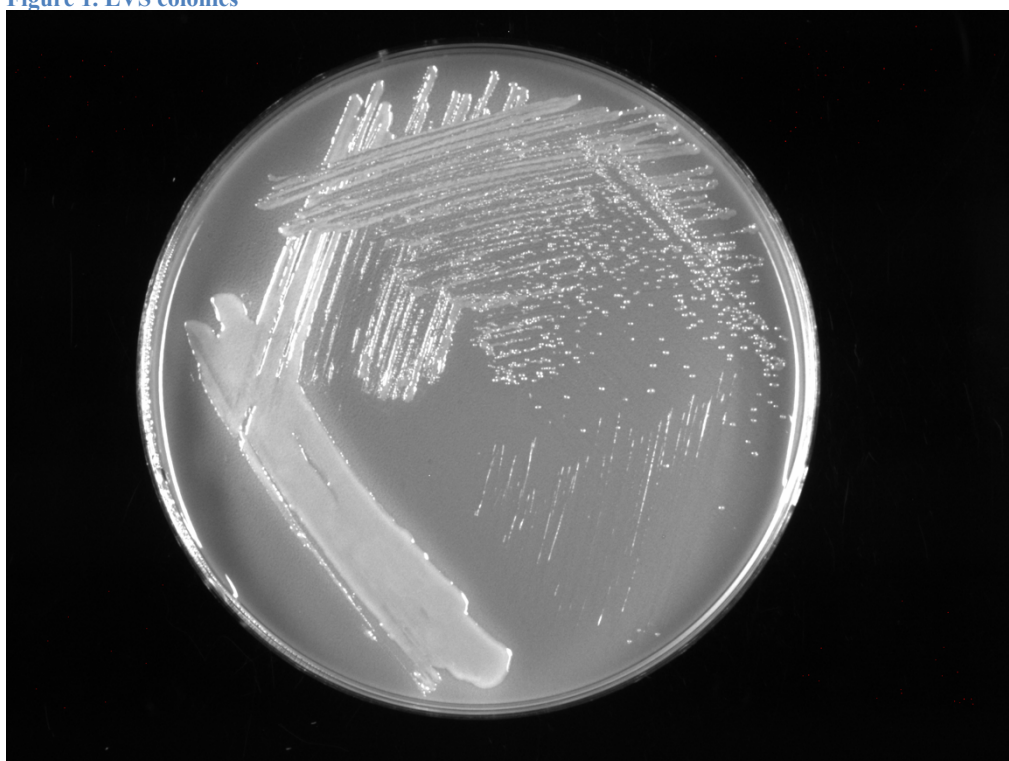


Figure 2. $\Delta rpsU1 \Delta rpsU2$ colonies

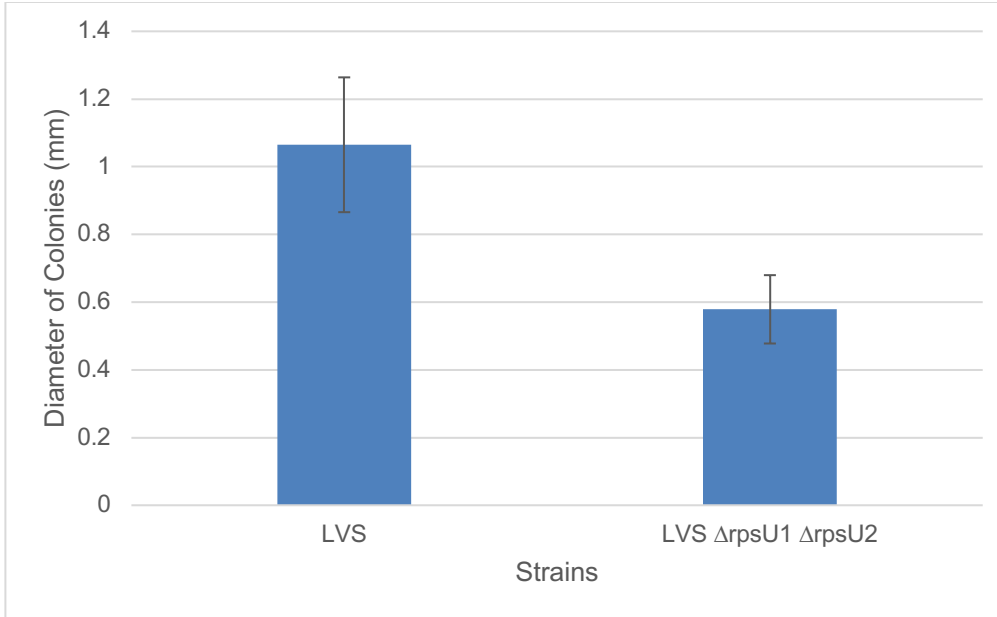


Figure 3. This graph compares the average diameter of colonies on the LVS plate and the $\Delta rpsU1 \Delta rpsU2$ plate. It shows that the LVS colonies are bigger than the $\Delta rpsU1 \Delta rpsU2$ colonies, as hypothesized.

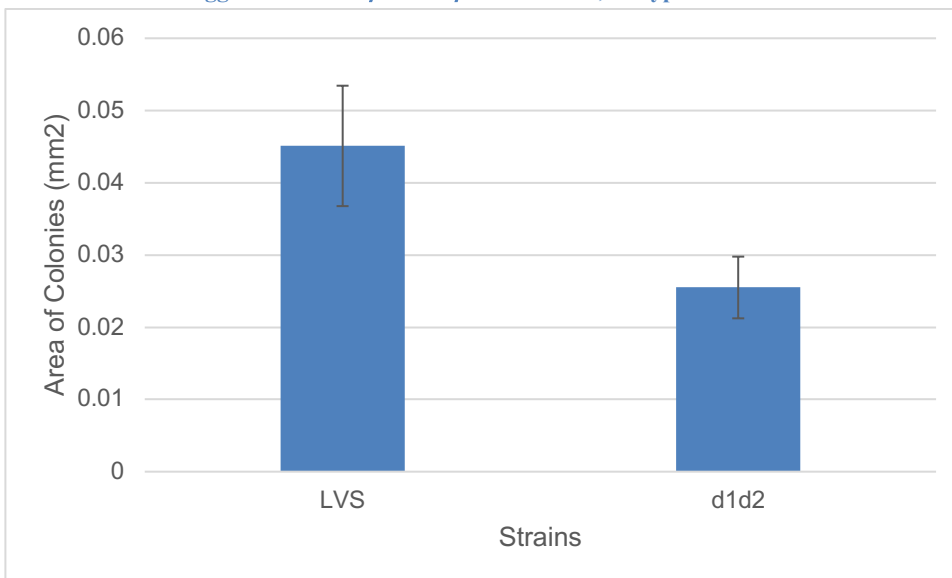


Figure 4. This graph compares the average area of colonies on the LVS plate and the $\Delta rpsU1 \Delta rpsU2$ plate. It shows that the LVS colonies are larger than the $\Delta rpsU1 \Delta rpsU2$ colonies, as hypothesized.

Next time, try to get more isolated cells to try to count 50 instead of 10. Since the second set will be coming from frozen stock with glycerol there will probably be less cells in general so it may work out that they will be more spread out. Hannah and Kathryn talked about maybe doing a serial dilution to ensure that the colonies are more spread out. We want the colonies more spread out so that they are not limited by the other colonies around them – I should not count ones that are clumped together because they may be smaller due to limited area rather than the missing genes.

Cystine heart agar with hemoglobin plates (CHAH) protocol

For 600 mL of CHA

1. Measure out 15.3 g of cystine agar into 2 500 mL flask

2. Add 150 mL of ddiH₂O (type 1) to each flask
3. Add stir bar to flasks
4. Heat on low, stirring, for about 10 minutes (media should be totally dissolved)
5. Autoclave on 30' liquid cycle, filling the water bin up to the height of the media
 - a. When using the instapot:
 - i. Do pressure cook for 30 minutes, then let it keep warm for 20 minutes
6. Cool down (ideally to ~55C)
7. Separately (before), prepare hemoglobin 2% solution
8. Add 3g freeze-dried hemoglobin 2% solution
9. Autoclave on 20' liquid cycle with water in the bin
 - a. If you use one that's already been made, make sure to heat it up in the water bath
10. Cool down (ideally to ~55C)
11. Using sterile technique, pour hemoglobin into CHA
12. For making CHAH Kan plates, need a concentration of 5 ug/mL but the stock is 50mg/mL
 - a. To do the math, use the formula $C_1V_1=C_2V_2$
 - b. For this scenario, $C_1=50\text{mg/mL}$ (this is the stock concentration of Kan), $C_2=5\text{ ug/mL}$ (concentration of Kan you want in the plates), $V_2=600\text{mL}$
 - c. $V_1=60\text{uL}$ (how much Kan you need to add to the CHAH mixture)
13. Using 50mL pipet, aliquot 24mL of CHAH Kan mixture into each 100mm plate

Analyzing colony size using ImageJ

1. For imaging plates, open image lab
2. Under custom, pick 'zone of inhibition assay'
3. Put gel in the bio-rad gel imager
4. Press 'position gel' and make sure the image on the screen looks good
 - a. If not, open middle drawer of imager and fix it
5. Run protocol
6. After, hit export and export for publication
7. Make sure it is 600 dpi and then save the image
8. After imaging plates, open ImageJ
9. File > Open the ruler image through ImageJ
 - a. I saved a copy of the ruler image to my folder
10. Set the scale by using the ruler/measure.tif. Use the straight-line tool to measure out 10mm.
11. Analyze > Set Scale
12. Distance in pixels should be ~237, known distance should be changed to 10, unit of length should be changed to mm, select global option
13. File > Open plate image. DO NOT disable global scale
14. Count the number of single colonies per strain (left to right) excluding the colonies that seem to be touching or merging
15. The colonies are reflective and appear to have a halo around the sides. Measure the diameter of each colony from left to right, including the halo
16. Put in document the measurements for each colony and find the average diameter for each strain, then find the area ($2\pi*r$)
 - a. The area was given in the measurement
 - b. If by math, $r = \text{diameter}/2$, so $\text{area} = 2*\pi*(\text{diameter}/2)$

Friday, September 9, 2022

To Do:

1. Make competent LVS and $\Delta rpsU1/\Delta rpsU2$
2. Streak LVS and $\Delta rpsU1/\Delta rpsU2$ to single colony

Results and Data:

I streaked LVS and $\Delta rpsU1/\Delta rpsU2$ to single colony from frozen stock on to CHAH plates. For $\Delta rpsU1/\Delta rpsU2$ I had ended up doing the quadrant technique but for LVS I did the 5 streaking that Kathryn had done for the previous set. I am unsure how much of a difference this will make. I also made EC cells for LVS and $\Delta rpsU1/\Delta rpsU2$. I was able to get three 110uL tubes of $\Delta rpsU1/\Delta rpsU2$ EC cells and two of LVS that I have put in my box in the -80 C freezer.

Making competent cells

1. Scrape up entire plate of cells into 400 uL of sterile 10% sucrose and resuspend (alternatively, cells can be grown to mid-log in MHB)
2. Add 1.1 mL of 10% sucrose to a final volume of 1.5mL
3. Spin for 3 minutes at 10,000 rpm
4. Remove supernatant, throw out, and resuspend in fresh 1 mL 10% sucrose
5. Repeat 3x-5x in 10% sucrose
6. After final spin, remove all supernatant
7. Resuspend cells in 10% sucrose at high density (corresponding to $\sim 1 \times 10^{11}$); these are EC cells by slowly adding 110 uL at a time. It should be about equal amounts of cells as sucrose.
8. For any extra EC cells, aliquot $\sim 110 \mu\text{L}$ / sterile tube (enough for 2 electroporations) and freeze at -80°C

Monday, September 12, 2022

To Do:

1. Electroporate empty vector into LVS and $\Delta rpsU1/\Delta rpsU2$
2. ~~Streak LVS for single use aliquots~~
3. ~~Image plates~~
4. ~~Streak out from cloning library~~ 5 from cloning library and LVS pF (maybe)

Results and Data:

I electroporated the empty vector into LVS and $\Delta rpsU1/\Delta rpsU2$. With this, there should be colonies with the empty vector (EC1 and EC3) and no colonies without the empty vector (EC2 and EC4). Along with that, there should be bigger colonies.

Electroporation Labels

1	LVS with pF
2	LVS with no DNA
3	$\Delta rpsU1 \Delta rpsU2$ with pF
4	$\Delta rpsU1 \Delta rpsU2$ with no DNA

When streaking for single use aliquots, you do not want to streak to single colonies – rather you want to make sure that there is a lot of cells concentrated in the middle. For this, want to streak from glycerol stock and make sure you grab a good clump of cells.

I also streaked out from cloning library. The cloning library was made by Hannah. To do this, she cut *Francisella tularensis* DNA into ~1.5 kB pieces, big enough to fit a gene or two into. From this, she electroporated those plasmids into $\Delta rpsU1 \Delta rpsU2$ cells. She then streaked the strains onto big plates and froze down bigger colonies. The bigger colonies should be $\Delta rpsU1 \Delta rpsU2$ cells that have some gene(s) in their plasmid that helped restore growth. Right now, I am streaking out some of the frozen down strains to verify that they do have bigger growth compared to $\Delta rpsU1 \Delta rpsU2$. We hope to find some interesting strains that we can sequence and want to see if there are any patterns in the plasmids that helped restore growth to wildtype.

For this, I streaked to single colonies and these strains are $\Delta rpsU1/\Delta rpsU2$ but do not show the growth defect because of additional plasmid added to them (possibly) We want to image these plates and compare them to LVS pF and $\Delta rpsU1/\Delta rpsU2$ with no plasmid (or an empty vector once that is made) to confirm that the cloning library ones do not have that growth defect that is expected with the deletion of *rpsU1/2*.

Strains for Experiment

KRLVS/Clone Number	Genotype	Plate Type
120	LVSpf	Kan
47	$\Delta 1/\Delta 2$	No ab
E2-21	FTL_1860, FTL_1861	Kan
E2-27	FTL_1797	Kan
E2-29	trkA (FTL_1232)	Kan
E2-30	FTL_0917, FTL_0918	Kan
E2-38	FTL_0789	Kan

Streak all to single colonies (quad)

Cloning library is 7E, want to take it out 1 at a time

3A KRLVS strains box 2 to find 47/ $\Delta 1/\Delta 2$

Imaging Plates – Refer to **Thursday, September 8** protocol.

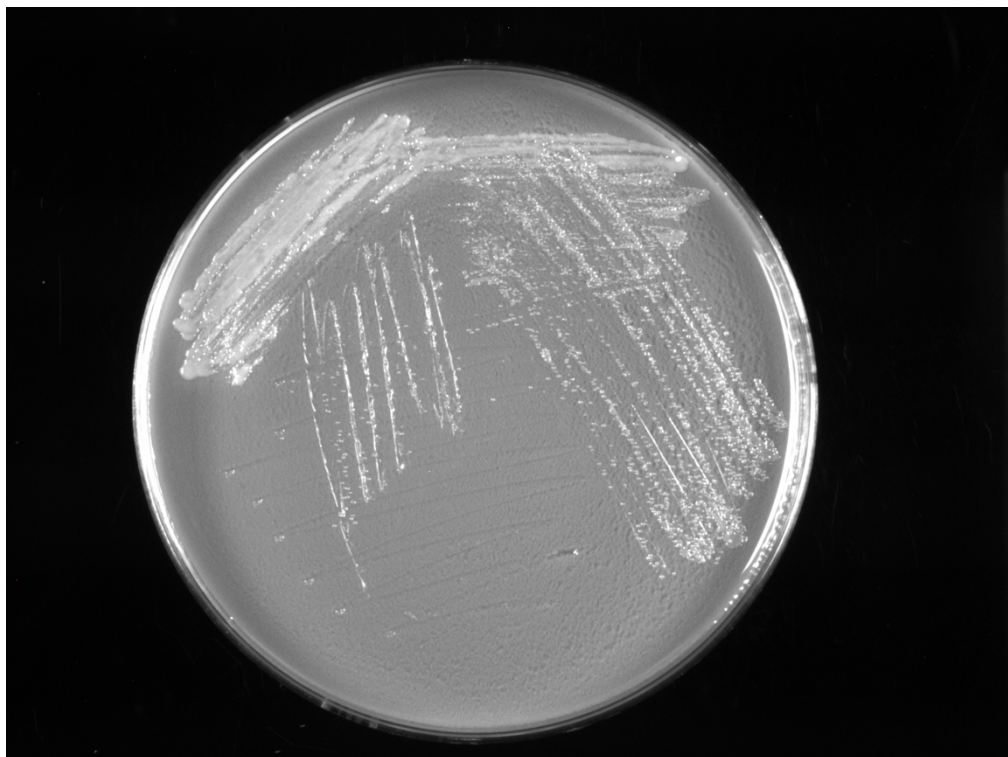


Figure 5. $\Delta rpsU1 \Delta rpsU2$ plate image

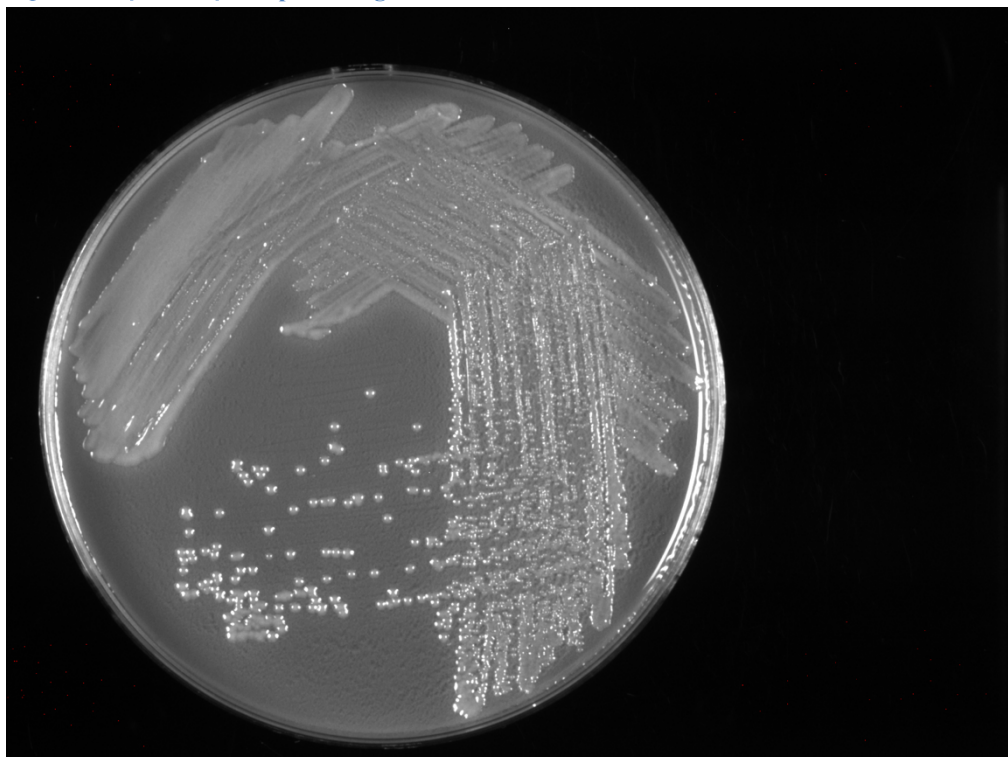


Figure 6. LVS plate image

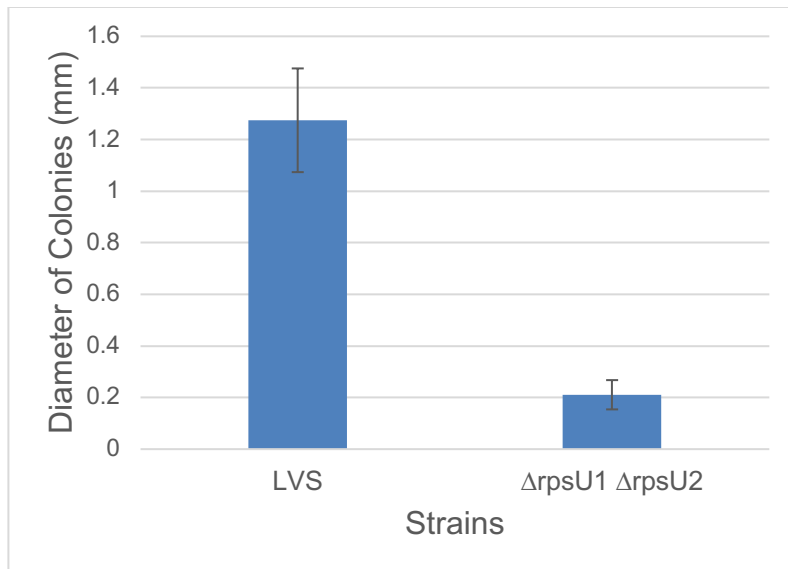


Figure 7. This graph compares the average diameter of colonies on the LVS plate and the $\Delta rpsU1 \Delta rpsU2$ plate. It shows that the LVS colonies are bigger than the $\Delta rpsU1 \Delta rpsU2$ colonies, as hypothesized.

Electroporate cells

1. For each electroporation, aliquot 4mL MHB into glass test tubes for recovery, warm in shaker at 37 C
2. For each electroporation, in a 2 mm sterile electroporation cuvette (green lid), combine:
 - a. 5 uL of pEX-based allelic exchange construct (mini-prep concentration, at least 100 ng/uL)
 - b. 50 uL electrocompetent cells
3. Have recovery media ready
4. Electroporate using the following settings: 2.5 kV, 25 uF, and 600 Ω (EC2)
5. Immediately after individual electroporations, use 1 mL warm recovery media from test tube to wash cells out of cuvette and transfer cells to recovery test tube
6. Recover cells for 4-8 2 hours, shaking at 37C
7. Pellet cells by centrifugation in sterile 2 mL tubes, 1' at 10,000 x g
8. Plate on CHAH-Kan plates, using 1 plate for each 2mL tube (2 plates per electroporation)
9. Incubate plates at 37 C for 3 days (or until single colonies appear)
10. Always include a control electroporation with no plasmid. Eventually single break-through colonies may start appearing; at that point, single colonies on the experimental plates are also likely to just be breakthrough growth

Tuesday, September 13, 2022

To Do:

1. ~~Make single use aliquots~~
2. ~~Quantify plate images~~

Results and Data:

Made single use aliquots of $\Delta rpsU1 \Delta rpsU2$ and LVS and put it in the -80 freezer in my box. I ended up making 2 cryovials each of LVS and $\Delta rpsU1 \Delta rpsU2$. For single aliquots, I made 10 of $\Delta rpsU1 \Delta rpsU2$ and 15 of LVS.

Making Glycerol Stocks (Single Use Aliquots)

1. Make 3 cryovials for each strain (permanent stocks), label!

- a. For this one, I only made 2 for each strain
2. Prepare 2.4mL of MHB in a 50mL conical (~~adjust if you are also making single use stocks~~)
3. Take at least half of a thickly spread plate and add cells to the MHB tube
4. Resuspend until there are no clumps in the MHB
5. Add 600ul of 75% glycerol to the 2.4mL mix by pipetting
6. Aliquot 1mL per cryovial, freeze at -80
7. With remaining 1 mL:
 - a. For $\Delta 1/\Delta 2$: make 100uL single use aliquots in flip top tubes (should make 9 or 10)
 - b. For LVS: make 50 uL single use aliquots in flip top tubes (can make 10)

Wednesday, September 14, 2022

To Do:

- ~~1. Make more Kan plates~~
- ~~2. Work on intro stuff (reread both background papers, take detailed notes and highlight to begin to put background slides together)~~
- ~~3. Start a spreadsheet of the library of ones I struck out, when I struck them out, when I imaged them, what the images say, any info about the plasmids~~

Results and Data:

Made CHAH-Kan plates.

Thursday, September 15, 2022

To Do:

- ~~1. Look at single colonies from electroporations~~
- ~~2. Image plates~~
- ~~3. Patch out electroporations (1 Kan plate)~~
- ~~4. Continue working on background research and ppt~~

Results and Data:

When looking at the single colonies from electroporations, we noticed that there was one colony of the $\Delta rpsU1 \Delta rpsU2$ pF was noticeably bigger than the other colonies. Hannah thinks that this could be a colony with a mutation that allows it to grow faster than the other colonies. Because of this, I made sure that I did not patch this colony out and she asked Kathryn if we should match out the possible mutant (we ended up patching the possible mutant out). We may want to sequence the mutant against another colony that does not grow as fast to see what the mutation is. Then I imaged plates from September 12. The first set I imaged were LVS pF and $\Delta rpsU1 \Delta rpsU2$ pF.

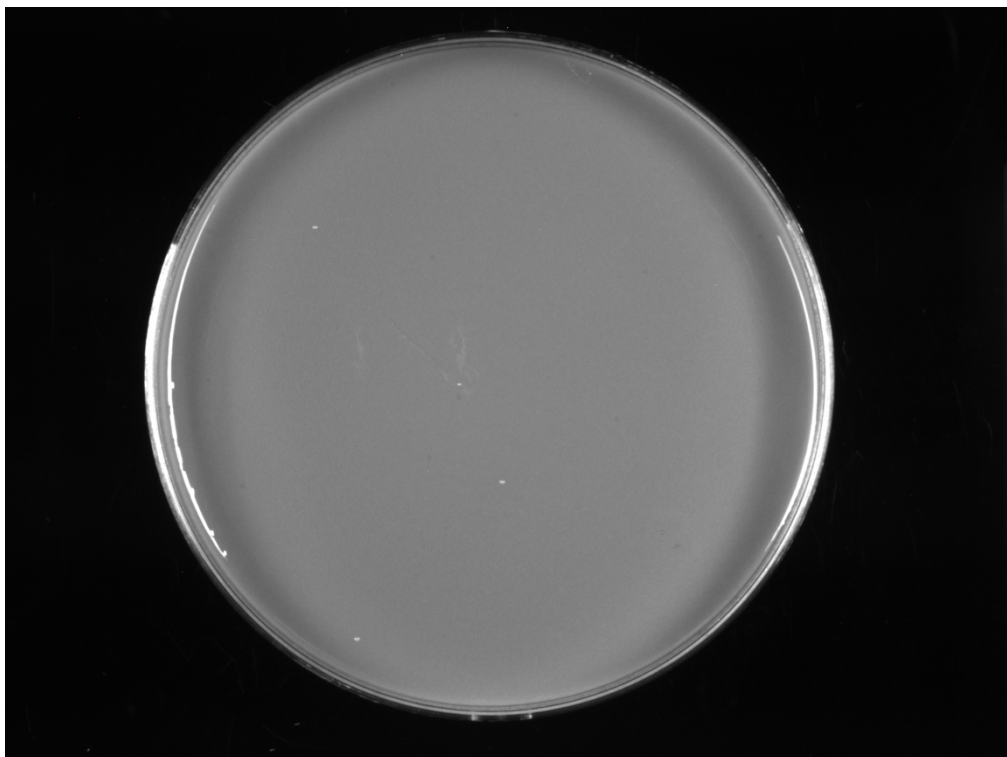


Figure 8. 10uL dilution of $\Delta rpsU1 \Delta rpsU2$ pF

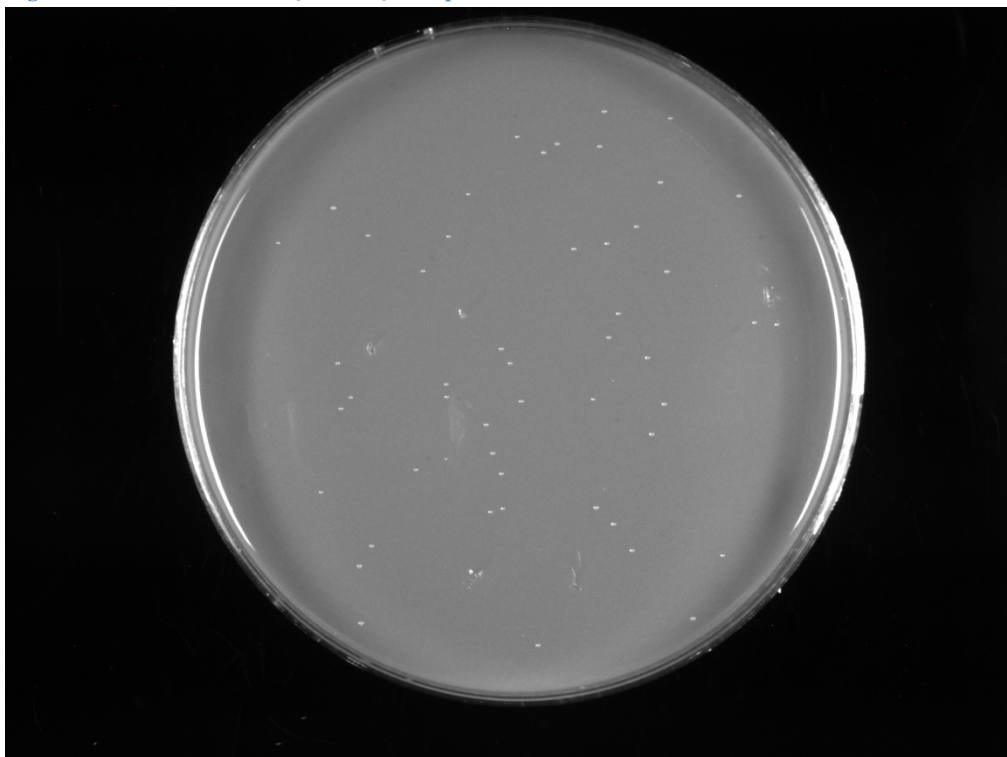


Figure 9. 100uL dilution of $\Delta rpsU1 \Delta rpsU2$ pF

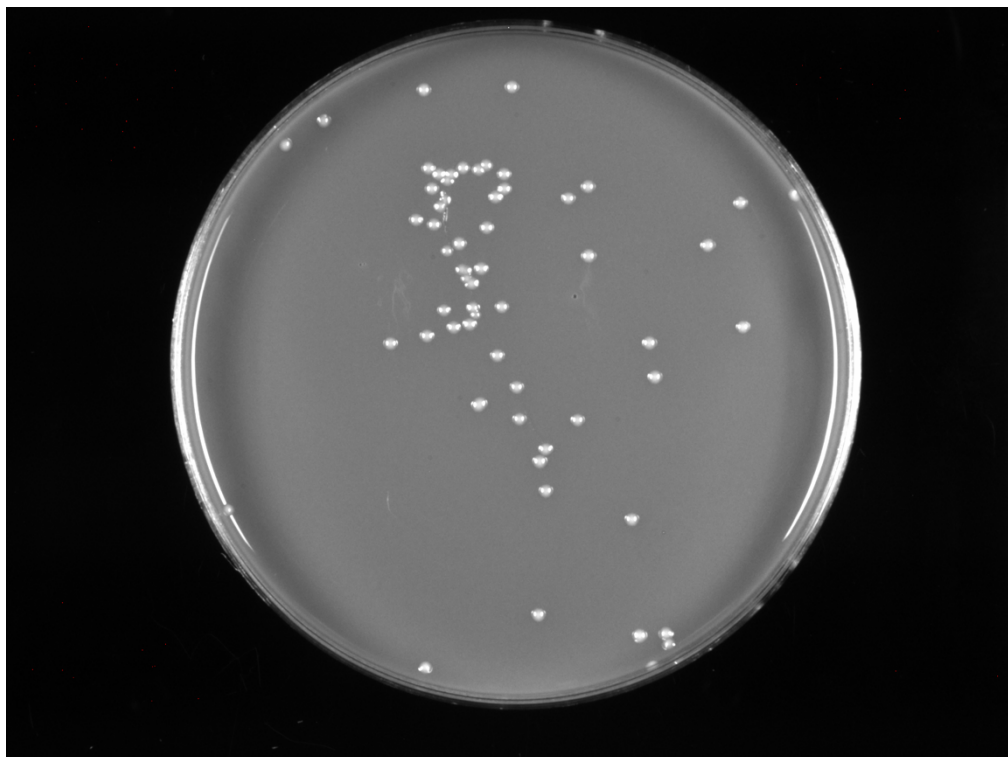


Figure 10. 10uL dilution of LVS pF

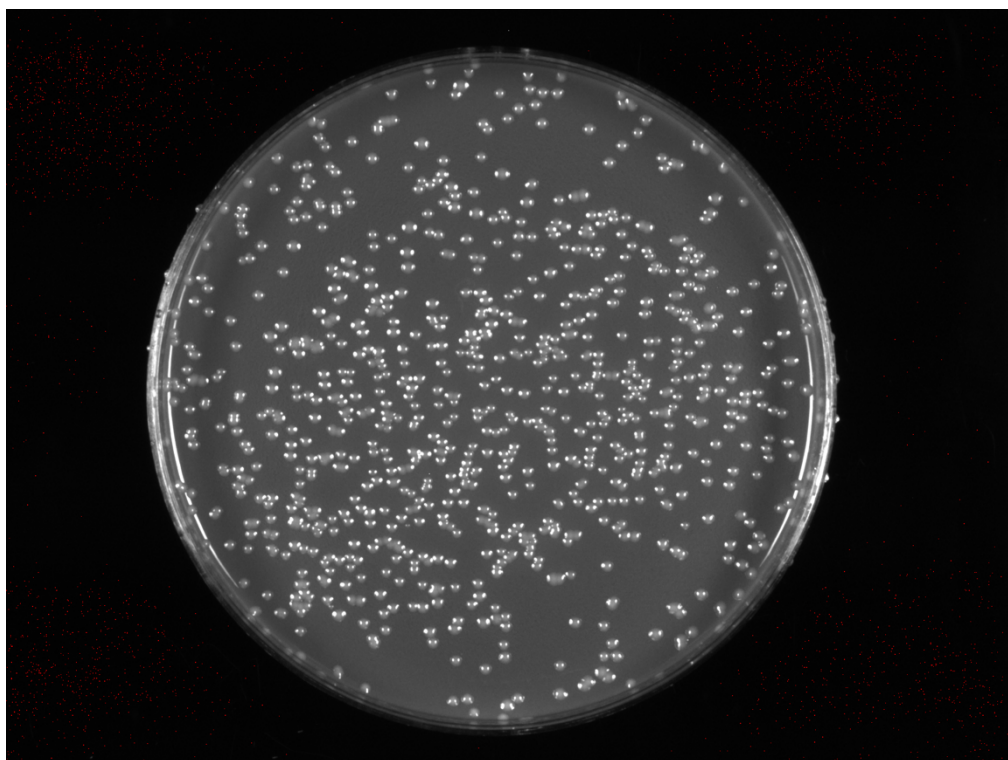


Figure 11. 100uL dilution of LVS pF

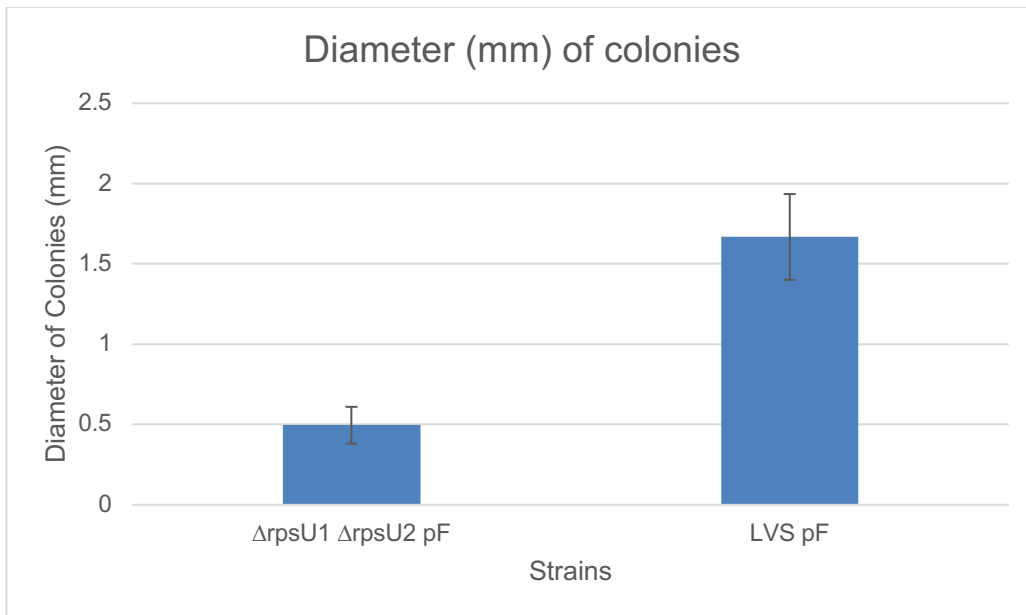


Figure 12. This graph compares the diameter of $\Delta rpsU1 \Delta rpsU2$ pF colonies to LVS pF colonies. LVS pF colonies are bigger than $\Delta rpsU1 \Delta rpsU2$ pF colonies as hypothesized. It is important to note that this did not change with or without the empty vector

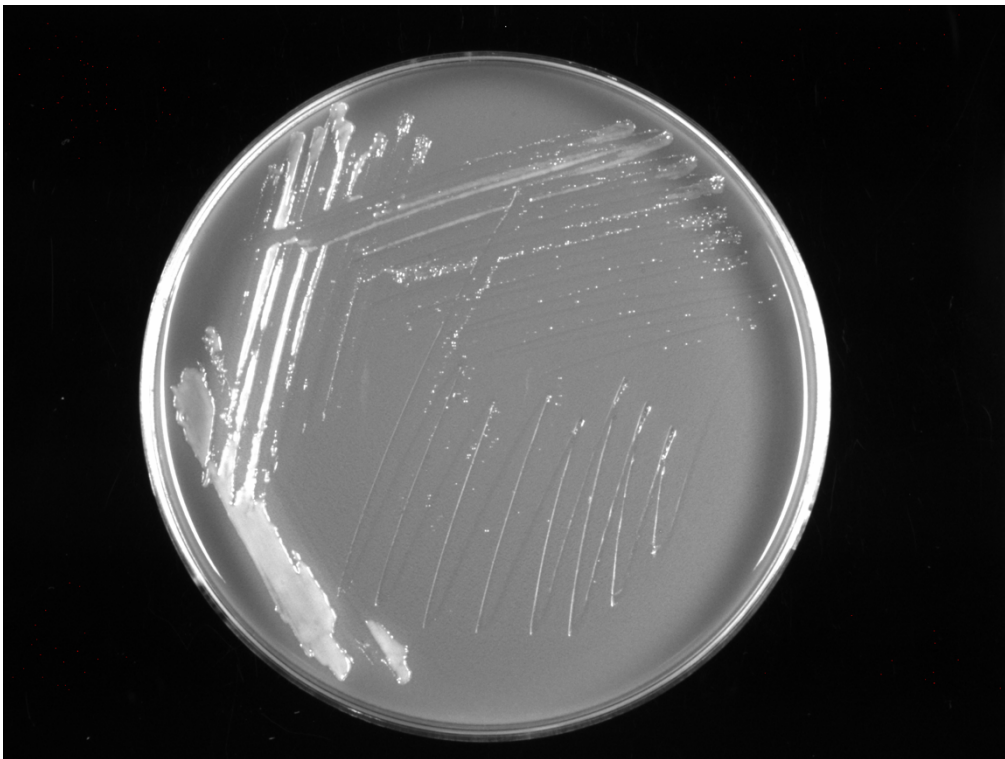


Figure 13. $\Delta rpsU1 \Delta rpsU2$ to compare to cloning library

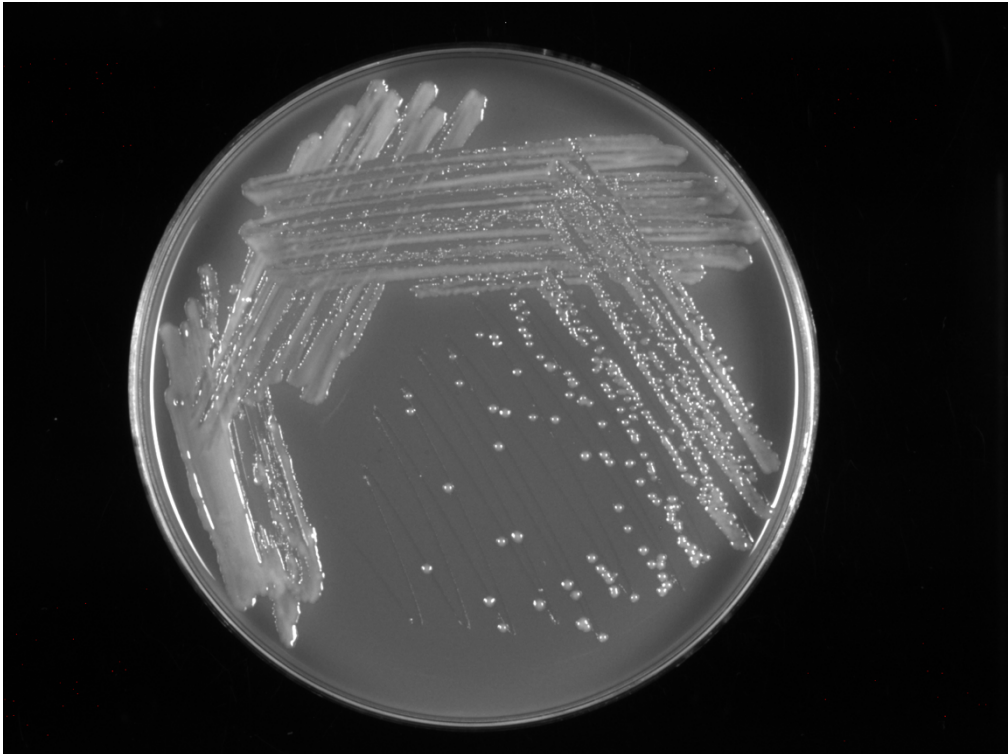


Figure 14. E2-21 clone

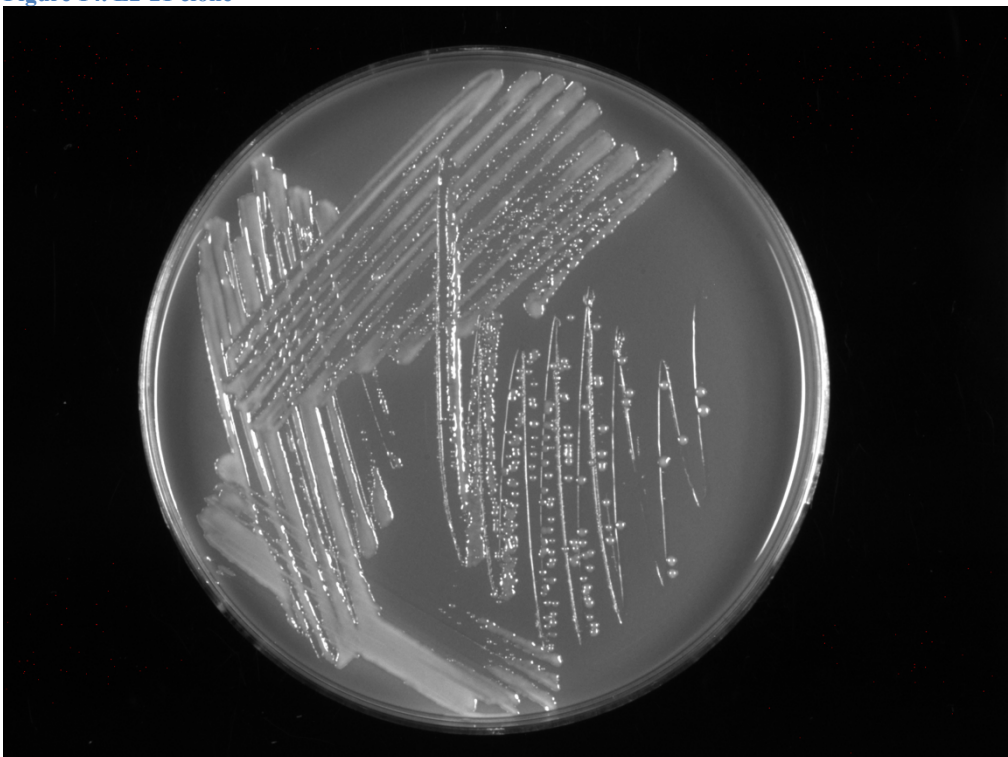


Figure 15. E2-27 clone



Figure 16. E2-29 clone

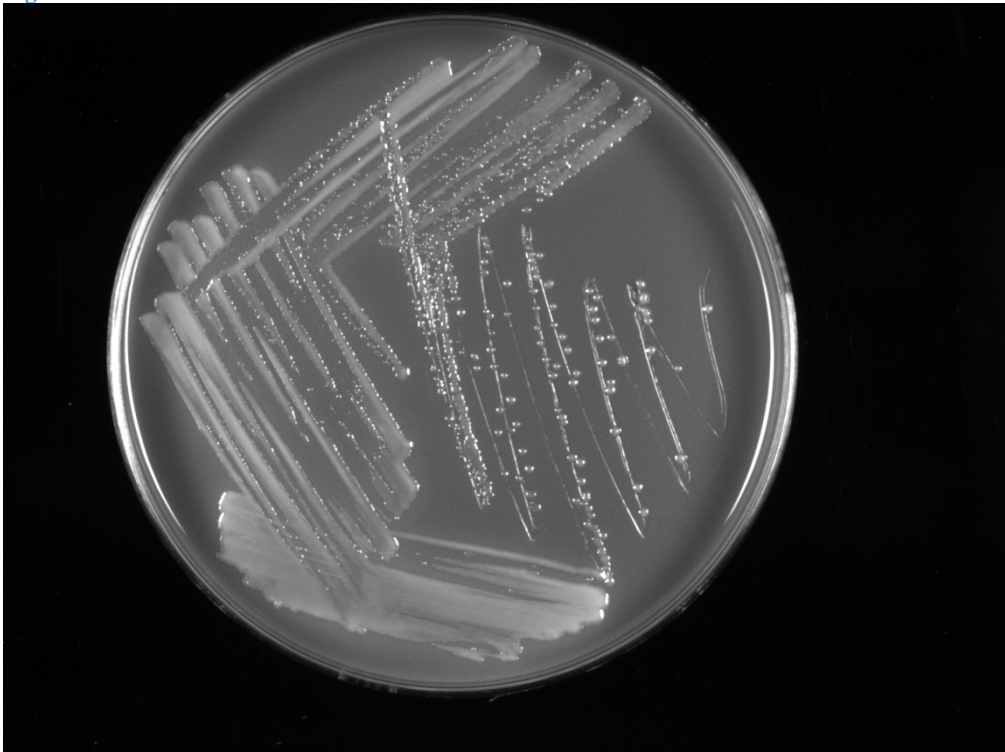


Figure 17. E2-30 clone

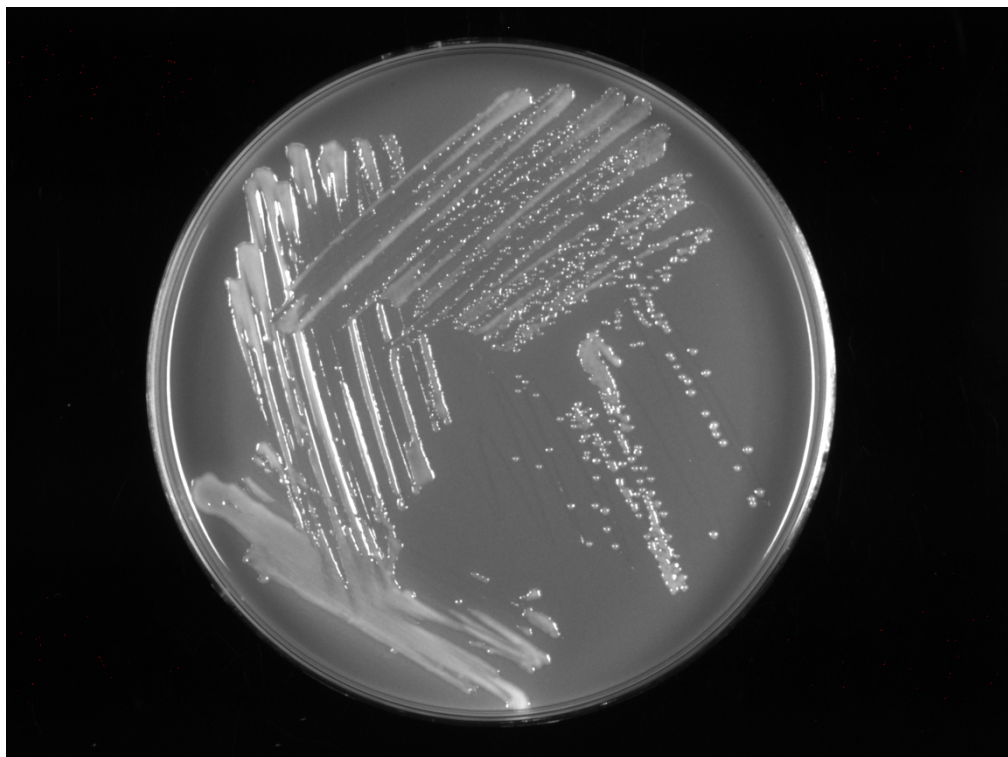


Figure 18. E2-38 clone

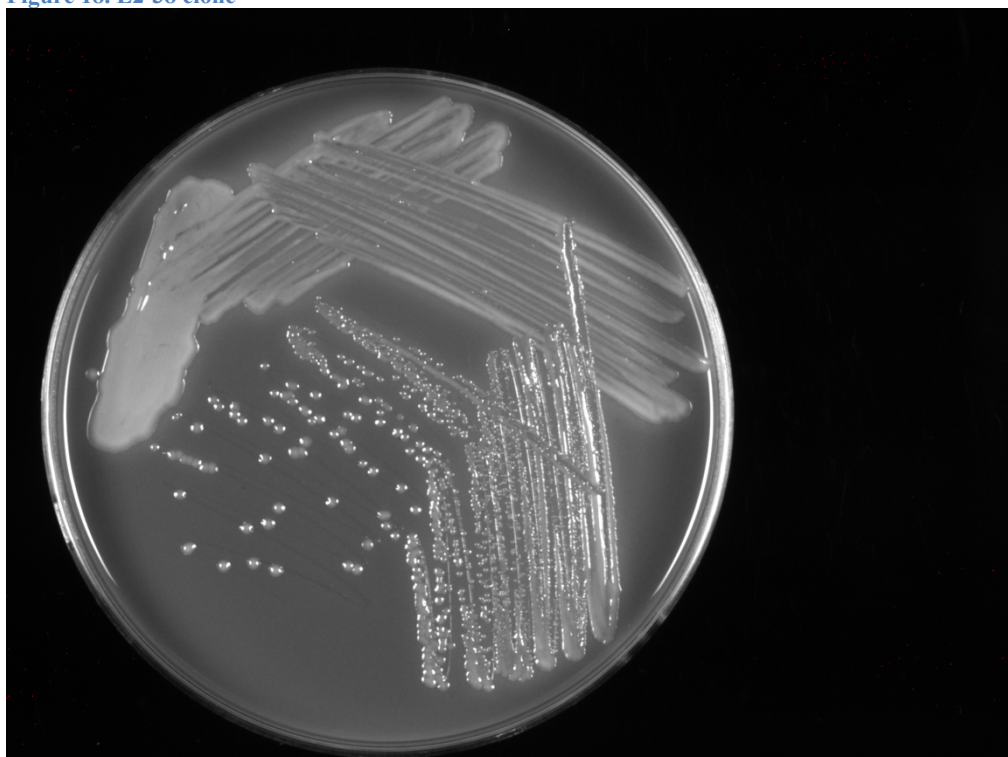


Figure 19. LVS pF colonies to compare to cloning library

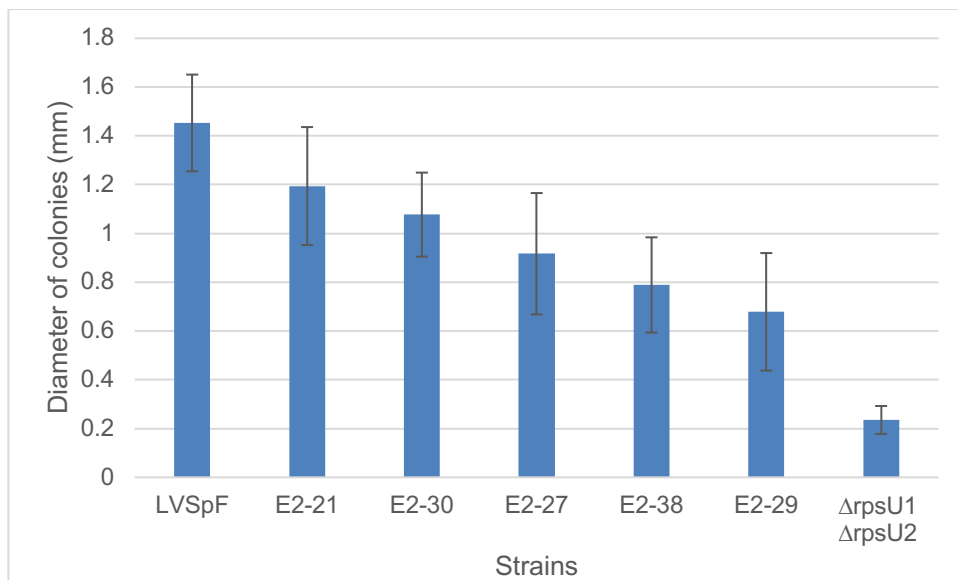


Figure 20. This graph is showing the diameter of colonies of some of the preliminary hits from the growth defect suppressor screen. The $\Delta rpsU1 \Delta rpsU2$ is supposed to have pF.

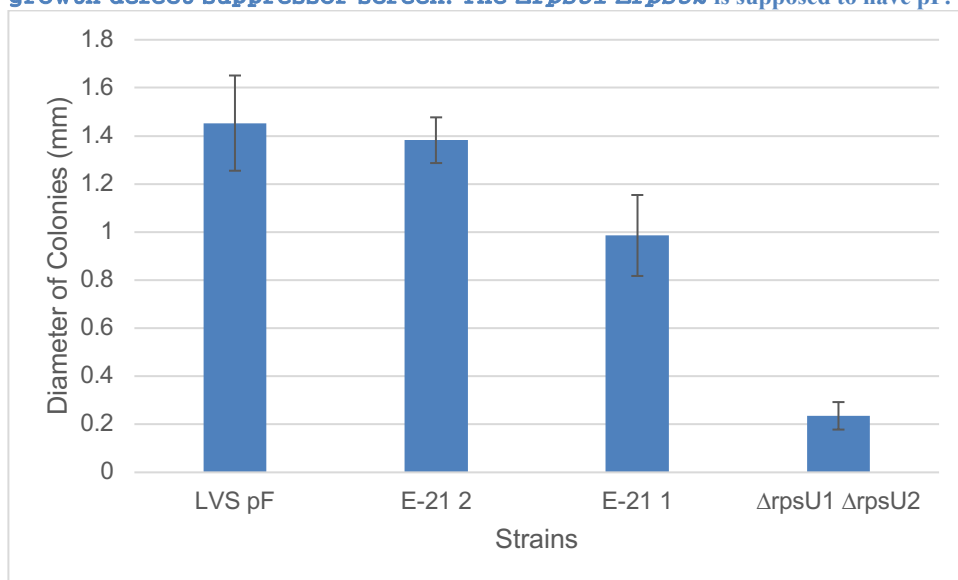


Figure 21. I split E2-21 into 1 and 2 based on their growth. By doing this, we can see that E2-21 2 colonies do not have significantly different growth from the wildtype. The $\Delta rpsU1 \Delta rpsU2$ is supposed to have pF.

Table 1. Here is the average and standard deviation for Figure 21

	LVS pF	E-21 2	E-21 1	$\Delta rpsU1 \Delta rpsU2$
average	1.453210526	1.3821	0.985666667	0.235263158
standard deviation	0.198096379	0.095081778	0.168435448	0.057194446

Friday, September 16, 2022

To Do:

1. Streak out from library in the -80C to single colony (other candidates)
2. Electroporate with pKR125
3. Do math for buffers

Results and Data:

Based on imaging results from yesterday, I electroporated pKR125 (pF-*era*) into LVS and d1/d2. Because we found that bigger colony on $\Delta rpsU1 \Delta rpsU2$ pF, Kathryn recommended that I also electroporate pF120 (plasmid isolated from E2-38 and other unknown genes) in $\Delta rpsU1 \Delta rpsU2$ to make sure it wasn't just the plasmid causing it.

Tube Number	Strain	Plasmid	Plasmid information
1	LVS	pKR125	pF- <i>era</i>
2	$\Delta rpsU1$ $\Delta rpsU2$	pKR125	pF- <i>era</i>
3	$\Delta rpsU1$ $\Delta rpsU2$	pKR120	Plasmid isolated from E2-38 (and other unknown genes)

$\Delta rpsU1 \Delta rpsU2$ pF = freeze down Monday (put in lab strains box)

$\Delta rpsU1 \Delta rpsU2$ pF (suppressor) = freeze down Monday (glycerol stocks?)

$\Delta rpsU1 \Delta rpsU2$ pKR120 = patch out and freeze down on Monday and Tuesday

$\Delta rpsU1 \Delta rpsU2$ pKR125 and LVS pKR125 = patch out and freeze down on Monday and Tuesday

I also streaked out from the cloning library. The goal is to streak all of them out to single colonies and image them and see if any of them grow to the same diameter of LVS pF.

KRLVS/Clone Number	Plate Type
E2-1	CHAH – Kan
E2-2	CHAH – Kan
E2-3	CHAH – Kan
E2-4	CHAH – Kan
E2-5	CHAH – Kan

Note: I forgot to do a positive (LVS pF) and negative control ($\Delta rpsU1 \Delta rpsU2$ pF) for this experiment so if I would want to use this data I would have to do the imaging again.

Monday, September 19, 2022

To Do:

1. Check electroporation plates first thing
 - a. Want to see if the $\Delta 1/\Delta 2$ with pKR125 is same as WT
 - b. Streak out big patch
2. Streak out slow growing strains on CHAH + Kan plates
 - a. $\Delta 1/\Delta 2$ with no pF
3. Check math for buffers, make sure we have everything needed for tomorrow
4. Freeze down strains
5. Image library plates
6. Quantify images
7. Make ppt slide for lab meeting

Results and Data:

I checked on the $\Delta rpsU1 \Delta rpsU2$ pKR125 and $\Delta rpsU1 \Delta rpsU2$ pKR120 that I electroporated on Friday. There was no growth on them, but there was on the LVS ones. Checked with Kathryn to see if we should push the schedule back or if we should continue with just LVS and $\Delta rpsU1 \Delta rpsU2$.

We decided to go forward with doing the gradient, but we are going to do $\Delta rpsU2$, $\Delta rpsU1 \Delta rpsU2$, and LVS. So, I streaked out two plates each of the deletion strains onto CHAH.

I streaked out the possible suppressor mutant onto CHAH-Kan. I want to check that after 3 days to see if it grows at the same rate as LVS pF or not. If it does, then we may want to sequence it to see what is mutated that has allowed it to recover its growth.

I also imaged E2-1 to E2-5, which I had streaked out on Friday.

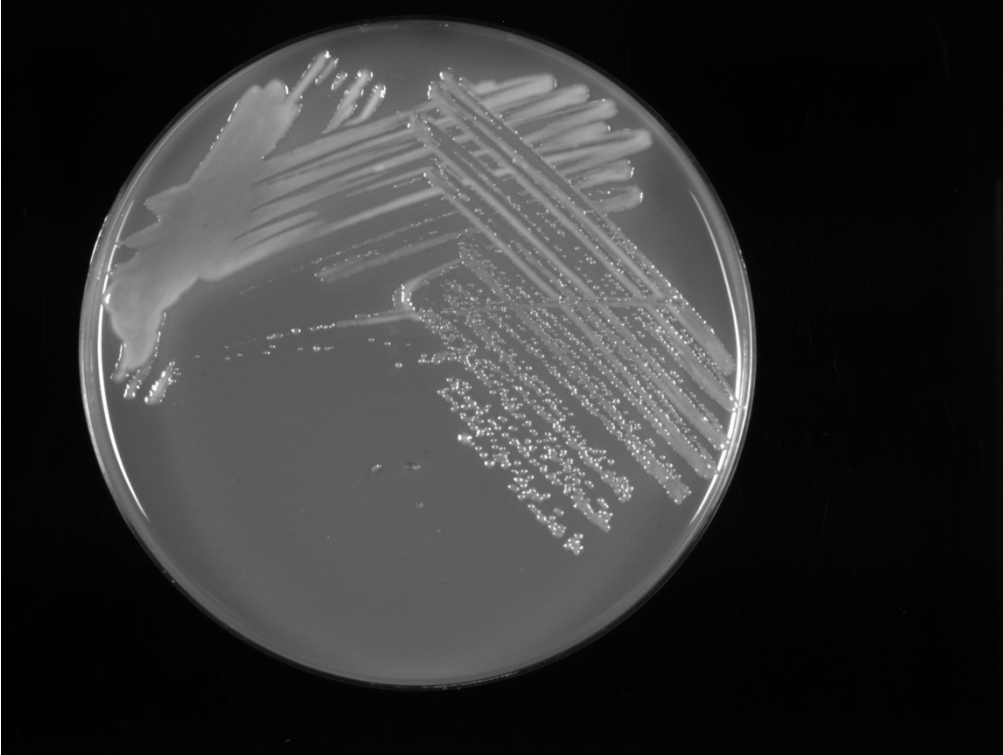


Figure 22. E2-1 Colonies

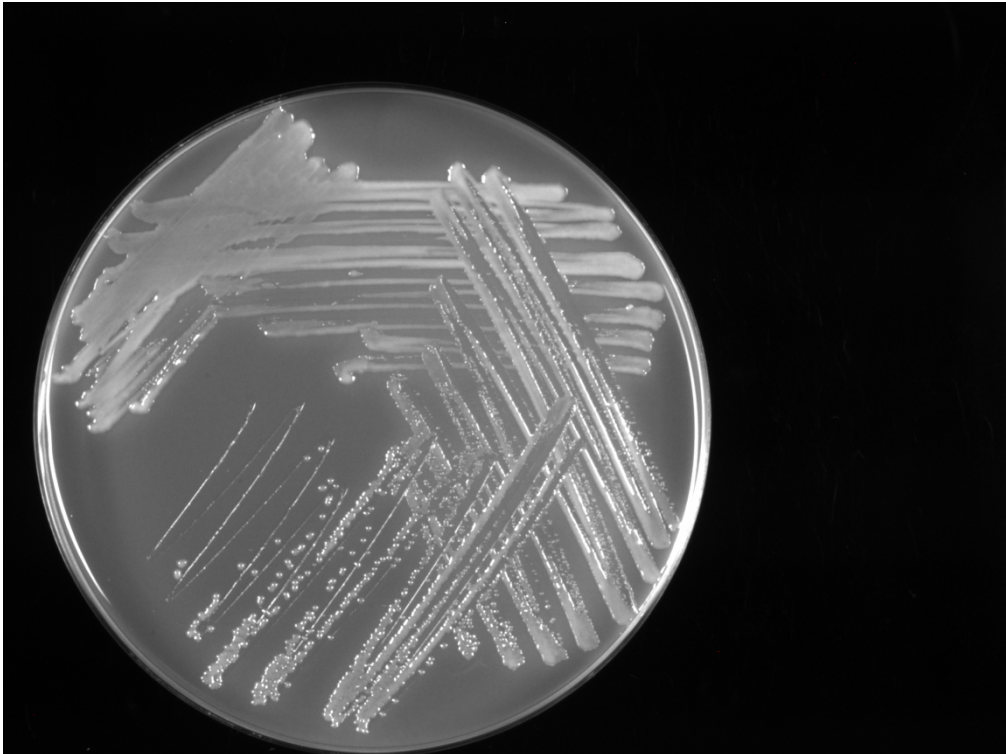


Figure 23. E2-2 Colonies

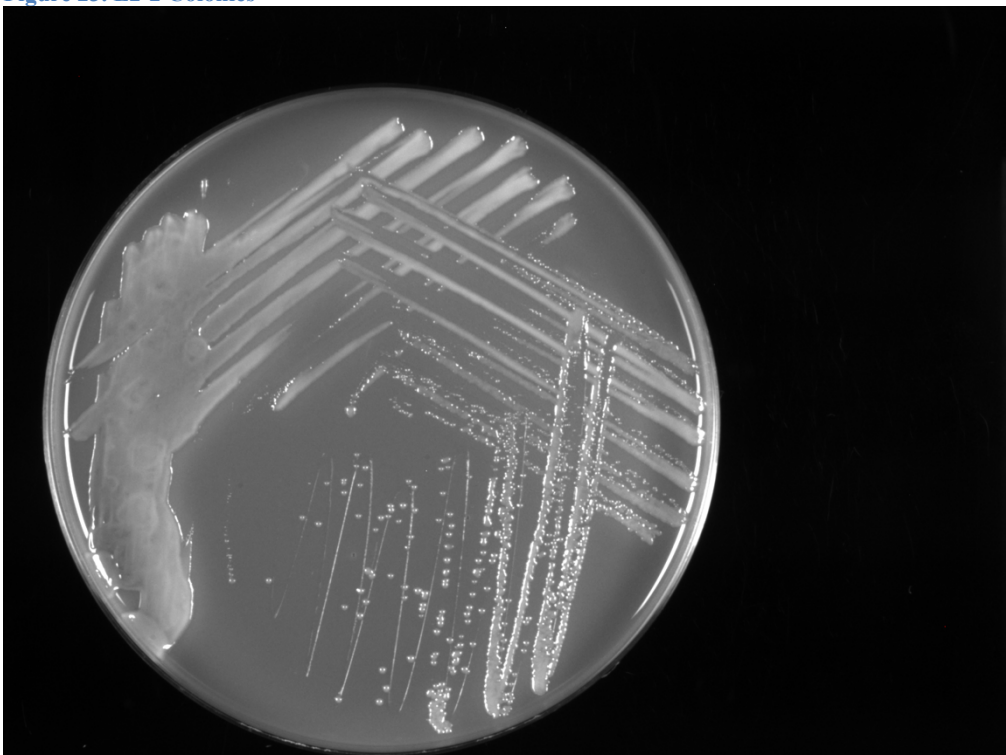


Figure 24. E2-3 Colonies

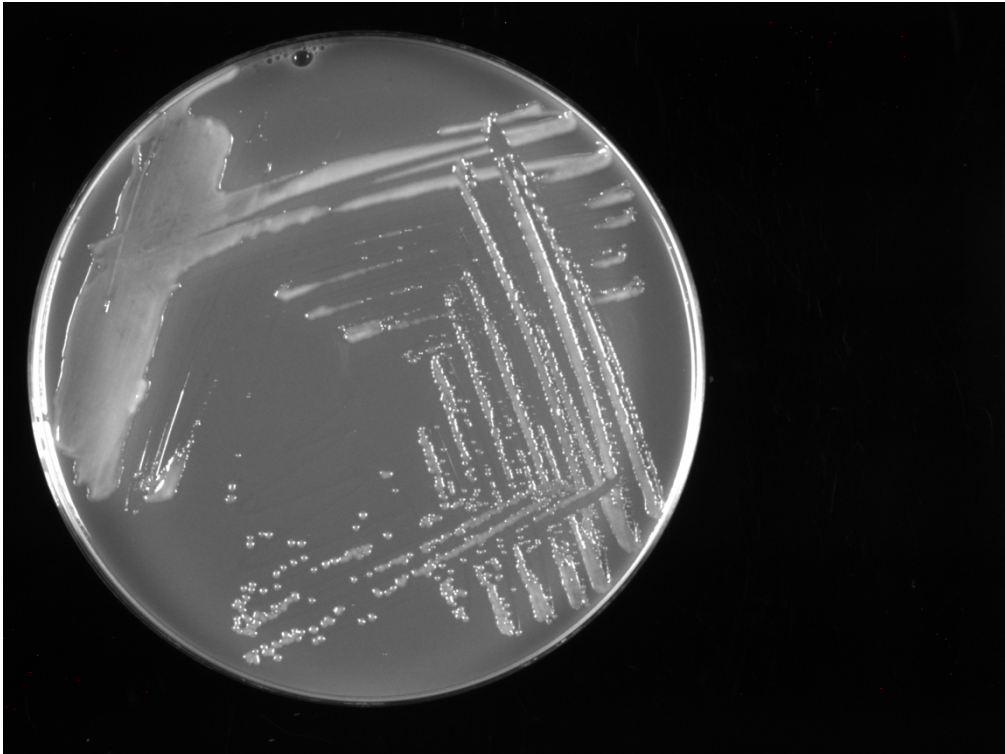


Figure 25. E2-4 Colonies

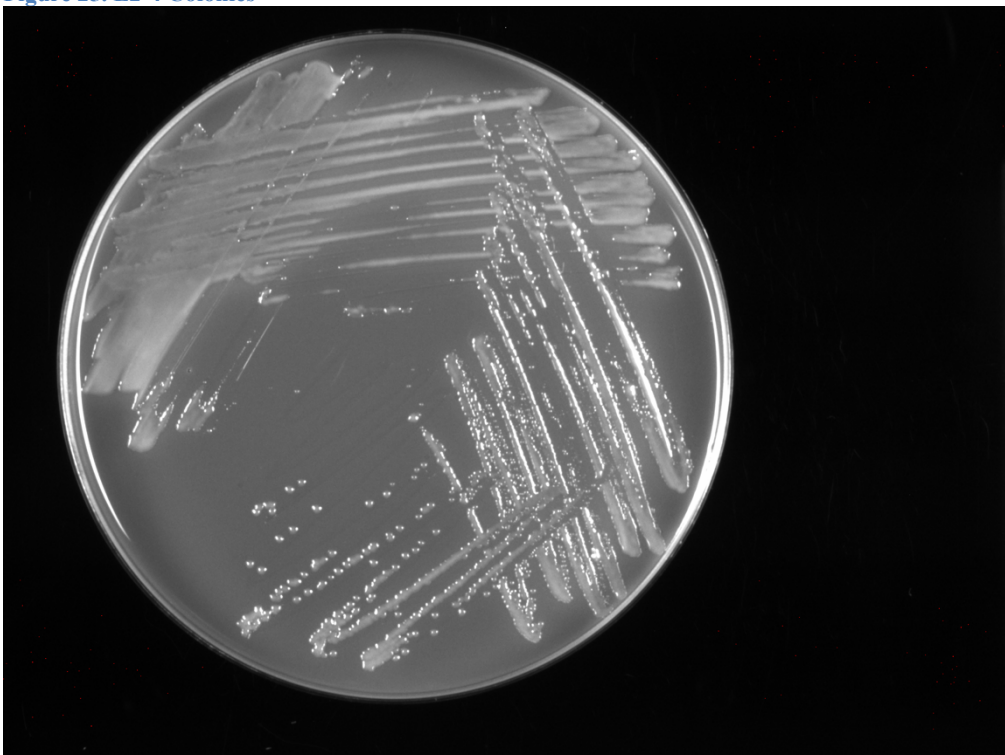


Figure 26. E2-5 Colonies

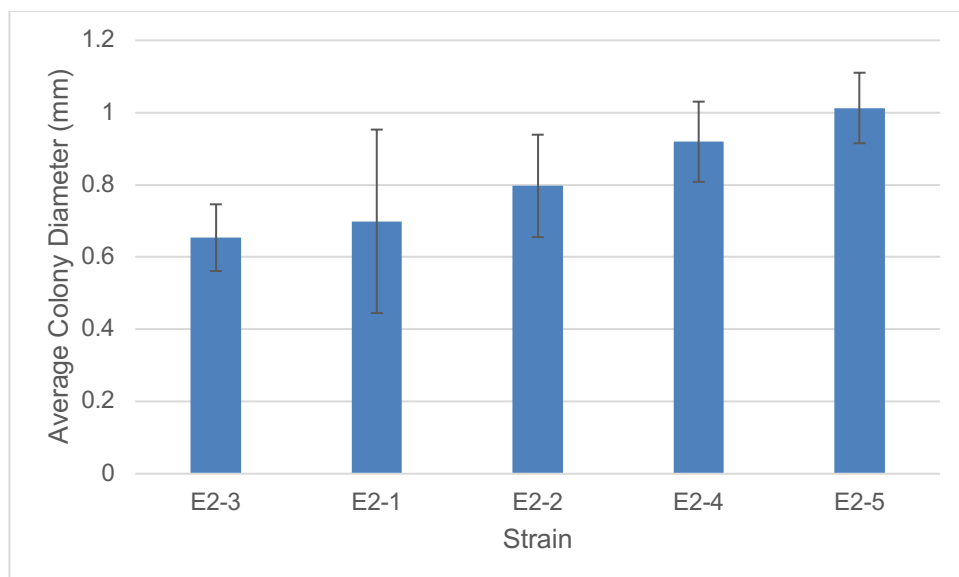


Figure 27. This shows the average colony diameter for 5 of the cloning library strains. Unfortunately, I forgot to streak out LVS pF (positive control) and $\Delta rpsU1 \Delta rpsU2$ pF (negative control), so I can't make any conclusions from this data.

Freezing permanent bacterial strain stocks

Add strain to strain list

If you are freezing down a strain generated by allelic exchange, remember to add the 1° integrant that generated the final strain. Include it on the strain list first.

For each strain you add, include:

- Genotype
- Notes (for example, FTL number corresponding to a gene name or vice versa)
- Location (Strain boxes are stored at -80°C)
- The genotype of the original strain you started with (Background)
- Any integrated plasmid (no plasmids in clean deletions, but plasmids are in 1° integrants)
- The descriptive name of any integrated plasmid
- Strain verification data: should include the name of the person who verified and the date for each type of verification
- Any additional notes (notable phenotypes, etc)

Freeze down strain

Use aseptic technique (strain = patch)

1. Per ~~strain~~ patch, label 2 cryotubes with strain number. Include LVS, strain number, genotype, date.
2. Add 200 uL sterile 75% glycerol to each tube (2 per strain).
3. In a sterile 2 mL tube (1 per strain or patch), add 400 uL of MHB
4. Resuspend patch (all of what you have) in MHB to homogeneity
5. Correct volume to 1700 uL (add 2x 650 uL MHB)
6. Transfer 800 uL to each cryotube (final volume should be 1 mL)
7. Vortex cryotube
8. Quickly spin (mini-fuge) to get liquid to the bottom of the tube
9. Freeze at -80°C in appropriate strain box

800uL MHB, add cells, 200uL of glycerol, and then take 10uL and streak to single colony (this is for the possible suppressor)

Tuesday, September 20, 2022

To Do:

1. ~~Streak LVS onto CHAH (with Kan?)~~
2. ~~Prep buffers~~

Results and Data:

I struck out two CHAH plates of LVS and then I prepped buffers for the upcoming experiment.

From the previous electroporations on Friday, September 16, I struck out LVS pKR125, *ΔrpsU1 ΔrpsU2* pKR125, and *ΔrpsU1 ΔrpsU2* pKR120. I patched them so that I could freeze them down at a later date. This will probably take 2 days to grow and then I can freeze them down.

Took *ΔrpsU1 ΔrpsU2* pF possible suppressor and made a lysate for it. The goal with this is that I will do a colony (?) PCR to see if it is *ΔrpsU1 ΔrpsU2* or if it is just LVS.

I also struck out 3 strains onto CHAH-Kan for imaging:

Strain	Plate	Control or Experimental
<i>ΔrpsU1 ΔrpsU2</i> pF (KRLVS196)	CHAH-Kan	Negative control
<i>ΔrpsU1 ΔrpsU2</i> possible mutant	CHAH-Kan	Experimental
LVS pF (KRLVS120)	CHAH-Kan	Positive control

To make lysate

1. Put 50uL of molecular grade water in a non-sterile tube
2. Used a small toothpick and picked just enough cells that you could see them on the toothpick and put those cells in the tube
3. Resuspended the cells by twirling the toothpick until all of the cells got off of it and by pipetting up and down a few times
4. Heat tube at 95C (heat block A is set to 95 C) for 10 minutes
5. Vortexed and spun down the lysate (for 5 minutes)
6. Stored it in -20C freezer (put in HT cloning box 2)

Buffers to Prep

Lysis buffer – 1 mL per sample x 3

Ingredient	Final Conc.	Stock Conc.	Volume (uL)
HEPES pH 7.6	25 mM	1 M	75
NH ₄ Cl	100 mM	1 M	300
MgCl ₂	0.6 mM	1 M	1.8
Triton X-100	0.4%	25%	48
NP-40	0.1%	10%	30
RNase-free DNase	100 U/mL	1 U/uL	300
Nuclease-free H ₂ O			2,245.2
Final volume			3000 uL

Don't add RNase-free DNase until the day of

Disassociation buffer – 500 ul per sample x 3

25 mM HEPES, 100 mM NH₄Cl, 0.6 mM MgCl₂, 0.5 mM EDTA, 2 mM DTT

Ingredient	Final Conc.	Stock Conc.	Volume (uL)
HEPES pH 7.6	25 mM	1 M	37.5
NH ₄ Cl	100 mM	1 M	150
MgCl ₂	0.6 mM	1 M	0.9
EDTA	0.5 mM	0.5M	1.5
DTT	2 mM	0.1 M	30
Nuclease-free H ₂ O			1280 (640 x2)
Final volume			1500 uL

Don't add DTT until the day of

Sucrose solutions – 15 mL per sample x 3

10%: 25 mM HEPES, 100 mM NH₄Cl, 0.6 mM MgCl₂ and 10% sucrose

Ingredient	Final Conc.	Stock Conc.	Volume (uL)
HEPES	25 mM	1 M	1,125 (562.5 x2)x
NH ₄ Cl	100 mM	1 M	4,500
MgCl ₂	0.6 mM	1 M	27
10% sucrose			4.5 g
Nuclease-free H ₂ O			39,348
Final volume			45,000

50%: 25 mM HEPES, 100 mM NH₄Cl, 0.6 mM MgCl₂ and 10% sucrose

Ingredient	Final Conc.	Stock Conc.	Volume (uL)
HEPES	25 mM	1 M	1,125
NH ₄ Cl	100 mM	1 M	4,500
MgCl ₂	0.6 mM	1 M	27
50% sucrose			22.5 g
Nuclease-free H ₂ O			39,348
Final volume			45,000

Use designated “ribosome” (i.e. nuclease-free) water, clean glassware, and filter sterilize all solutions

Wednesday, September 21, 2022

To Do:

1. Make overnight cultures in MHB

Results and Data:

Supplemented the MHB media for growing the strains overnight.

Strains	Hours Growing	OD	Start Time
<i>ΔrpsU1/ΔrpsU2</i>	18	0.05	2:30PM
<i>ΔrpsU2</i>	16	0.02	4:30PM
LVS	16	0.005	4:30PM

Math for the start time is based on starting at 8:30AM on Thursday.

Mueller-Hinton broth protocol

For 500 mL of MHB

1. Weigh out 10.5 g of Mueller-Hinton broth into 1L square-bottle
2. Add 500mL of ddiH₂O (type I)
3. Autoclave on 30' liquid cycle, filling the water bin up
4. Cool down to 37 C or cooler
5. Can keep this sterile media indefinitely without supplements
6. Add **5mL** of 10% glucose
7. Add **5mL** of 2.5% iron pyrophosphate
8. Add isovitalex (combine 10mL from liquid vial with solids, add entire volume to broth)
 - a. Found in the big freezer on the left, right next to the shaker incubator
9. Can keep this supplemented media for 2 weeks, storing at 4C

Inoculating test tube cultures of *Francisella tularensis* protocol

Note: Perform all following steps using aseptic technique

Reagents: sterile tubes, supplemented MHB, CHAH plates with heavily grown *Francisella tularensis*

1. Determine the starting OD600 you want for your test tube cultures
2. Prepare sterile test tubes with desired volume supplemented MHB (S-MHB)
3. Prepare a sterile 50mL conical with 3 mL S-MHB (large volumes) or a microfuge tube with 400 uL of S-MHB (small volumes)
4. Obtain CHAH plates with heavily grown bacteria (grown at least overnight, depending on the strain)
5. Using a loop, scrape cells off the plate until you have a clump
6. Swirl the loop in the tube of MHB and dislodge the clump of cells from the loop
7. Repeat steps 5 and 6, 2-3x to get enough bacteria into the culture
8. Resuspend the pellets/clumps of cells using a p1000 pipet until there are no more clumps
9. Once the cells are resuspended, take an OD600 of a 1:20 dilution of your culture (50uL culture, 950uL S-MHB)
10. If you need a higher starting OD, repeat steps 4-9 until you have reached the desired starting OD or close to it
11. Use $C1V1 = C2V2$ formula to calculate necessary volume to add to test tube of MHB
12. Take the OD600 of 1mL to confirm the starting OD
13. Allow to grow at 37C shaking

Thursday, September 22, 2022

To Do:

1. Harvest cells (need liquid nitrogen, buffer, big centrifuge, and bead beater)
2. Lysis
3. Freeze down LVS pKR125, d1/d2 pKR125, d1/d2 pKR120

Results and Data:

I harvested and lysed cells that I grew overnight. Unfortunately, *ΔrpsU1/ΔrpsU2* was contaminated, so I have to plan to do that at a separate time. I was able to complete the protocols for LVS and d2, but they were not ideal.

LVS had a starting OD600 of 0.421 and d2 had a starting OD600 of 0.114.

When I lysed the cells, I noticed that the supernatant of LVS was significantly smaller than that of d2 (**Figure 28**). I was still able to get ~250uL of supernatant from the LVS tube.

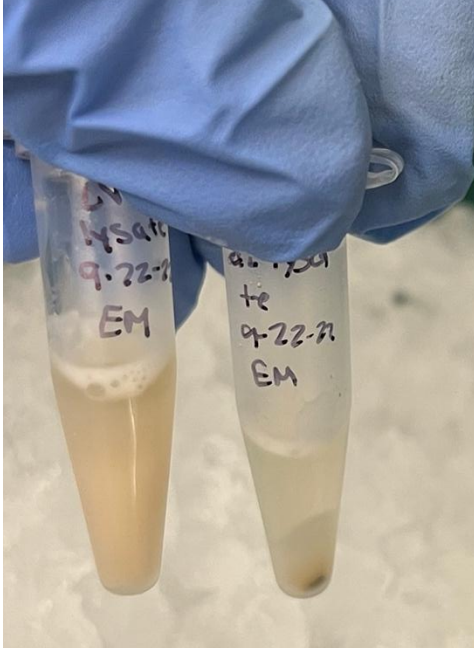


Figure 28. The tube on the left is LVS and the tube on the right is d2.

Cell harvesting protocol

- 500 mL of cells grown shaking aerobically for 16-18 hours at 250 rpm
 - Media: supplemented Mueller Hinton Broth (0.025% iron pyrophosphate, 0.1% glucose, and 2% isovitalax)
 - Grow until mid-log 0.3-0.4 OD
 - Starting ODs:
 - Growing *ΔrpsU1ΔrpsU2* – 18 hours with starting OD of 0.05
 - Growing LVS – 16 hours with a starting OD of 0.005
 - Growing *ΔrpsU2* – try 16 hours with starting OD of 0.02
- Harvest protocol
 - Let cultures sit at room temperature for 10 minutes
 - Spin down at 4000 x g for 10 minutes at 4C, remove supernatant
 - Takes centrifuge about 20 minutes to cool down, so try and cool it down first thing
 - Do half the first time, and then half the next and dump out the supernatant inbetween
 - Transfer pellet to a 50 mL conical (using disassociation buffer if necessary), spin down again same conditions and remove liquid
 - Option: move pellet to -80C freezer

Cell lysis protocol

- Lysis buffer: 25 mM HEPES pH 7.6, 100 mM NH₄Cl, 0.6 mM MgCl₂, 0.4% Triton X-100, 0.1% NP-40, 100 U/mL Dnase (RNase-free)
- Bead beating

- a. Dropwise 500 uL of lysis buffer into 50 mL conical tube filled with liquid nitrogen. wait for each pellet to sink before pipetting next pellet to prevent clumping
 - b. Clean 10mL mixer mill canister with Millipore water and EtOH. Use compressed air to blow dry. Make sure no residual water droplets are
 - c. Combine with frozen cell pellet in 10 mL mixer mill canister
 - d. Mixer mill 5 times at 15 Hz for 3 minutes, cooling in liquid nitrogen between each round
 - e. Thaw in shallow water bath, transfer to 1.5 mL tube
3. Spin cell lysate at 4C max speed for 5 minutes and save supernatant to remove cell debris
 4. Store lysate in -80C

Friday, September 23, 2022

To Do:

1. Work on rotation powerpoint presentation
2. ~~Image plates from Tuesday~~
3. ~~Freeze down d1/d2 pKR120~~

Results and Data:

I imaged plates from Tuesday – so I looked at d1/d2 pF possible suppressor, d1/d2 pF, and LVS pF. Because the suppressor colonies were bigger, I scraped up the cells from the original streak (1st streak) and mixed it in a microcentrifuge tube with 200uL 1X PBS. I spun the cells down to pellet them, removed the supernatant and froze it down (should have froze down the cell pellet not the supernatant). I put the d1/d2 suppressor supernatant in -20 freezer (HT cloning box 2). This will be used for colony PCR.

Update (9/27): Supernatant was just PBS so I saved the wrong thing – throw that out.

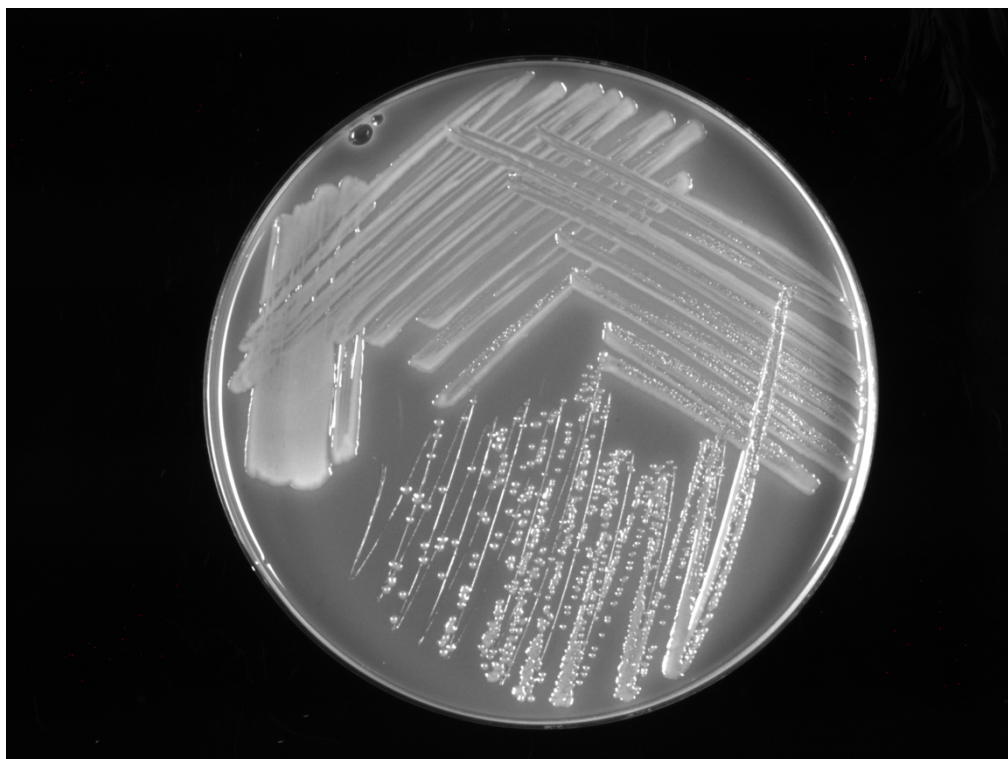


Figure 29. LVS pF colonies

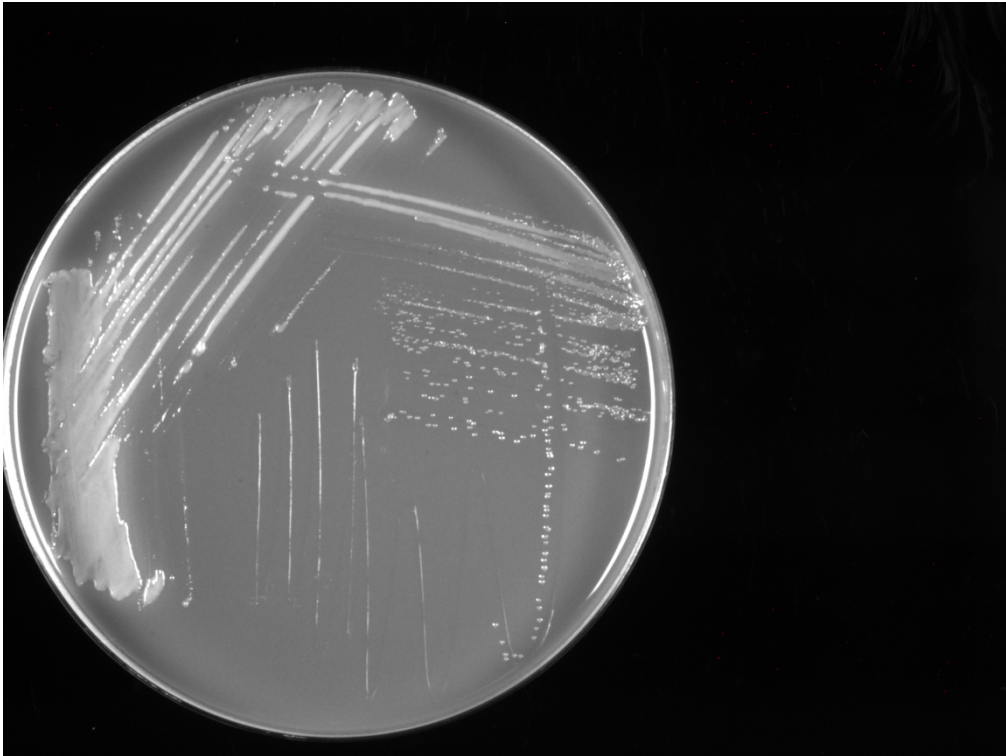


Figure 30. $\Delta rpsU1 \Delta rpsU2$ pF colonies

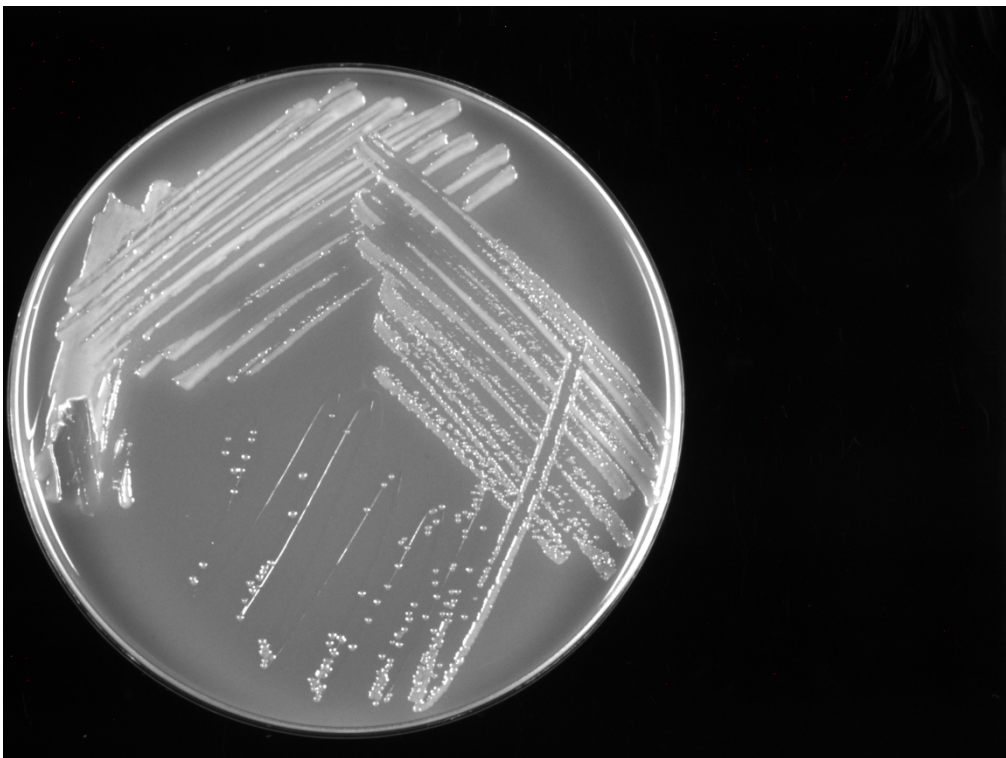


Figure 31. $\Delta rpsU1 \Delta rpsU2$ pF possible suppressor mutant colonies

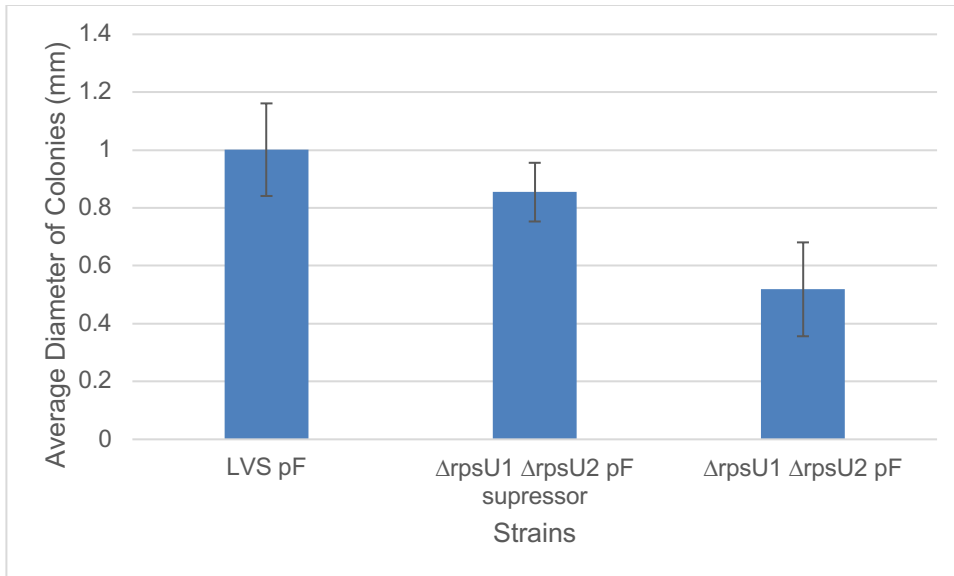


Figure 32. This graph is looking at the average diameter of colonies in LVS pF, $\Delta rpsU1 \Delta rpsU2$ pF suppressor mutants, and $\Delta rpsU1 \Delta rpsU2$ pF. The graph indicates that the suppressor mutant has bigger colony sizes compared to $\Delta rpsU1 \Delta rpsU2$ pF. The suppressor mutant colonies are smaller than the LVS pF colonies, with a p-value of 0.044.

Monday, September 26, 2022

To Do:

1. Run gradients and fractionate (need sucrose cushions, gradient maker, and ultracentrifuge)

Results and Data:

Get 60 tubes – 30 LVS f1-30 with date; 30 $\Delta 2$ f1-30 with date

For this one, fractionate into 30 per sample

Ran the gradients and fractionated LVS and $\Delta rpsU2$ to look at ribosomes and see if deleting *rpsU2* affects ribosomal assembly.

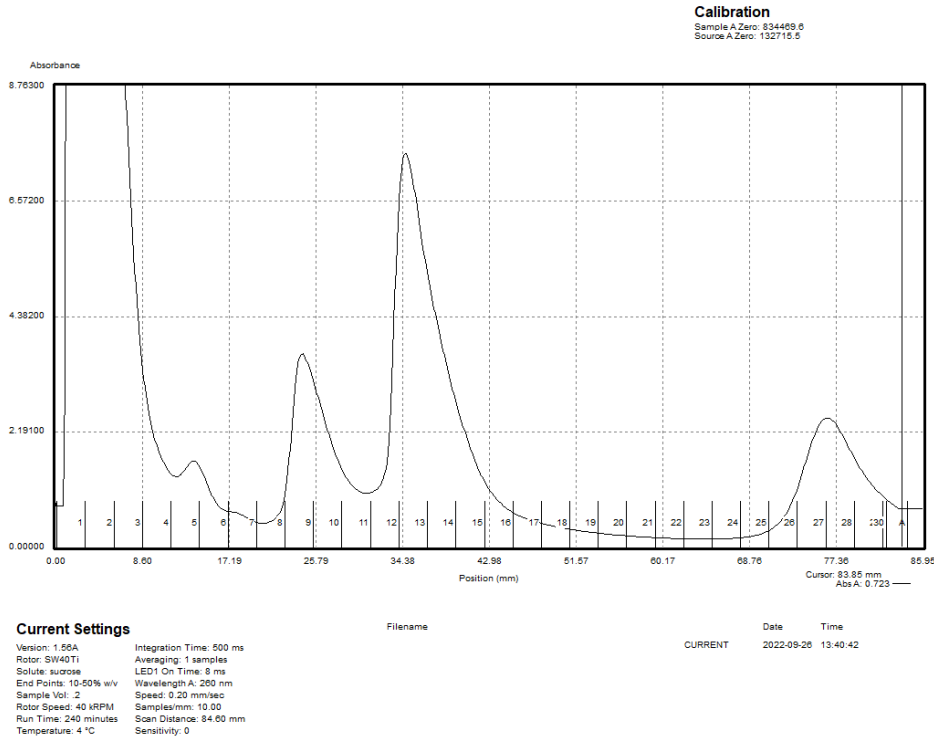


Figure 33. LVS Subunits

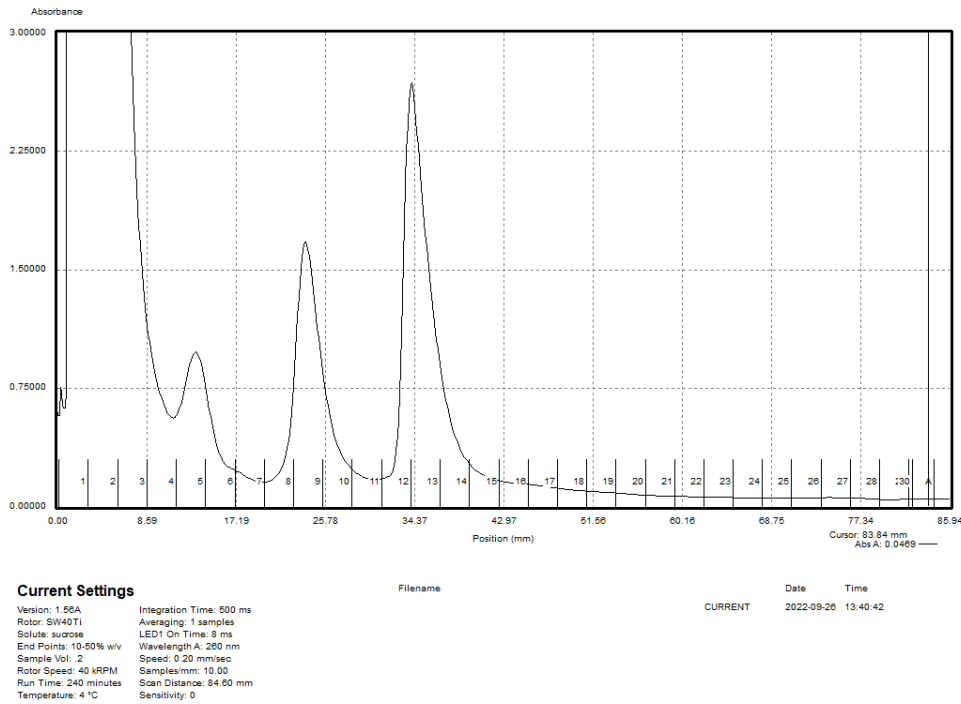


Figure 34. *ΔrpsU2* subunits

The top image is the fractioning of LVS. It shows that 30S starts between fraction 8 and ends around fraction 11. It also shows that the 50S starts at the beginning of the 11th fraction and ends around the 15th

fraction. The bottom image, $\Delta rpsU2$, also shows 30S starting between fraction 8, but it ends around fraction 10. The 50S for $\Delta rpsU2$ starts around fraction 11 and ends at fraction 14. This slight difference may be because there were more LVS cells to begin with, meaning there may be a wider range compared to $\Delta rpsU2$, which started with less cells.

From these images, I do not think that deleting $rpsU2$ influences ribosomal assembly. One thing that does interest me is the hump between fraction 4 and 6 in both images – it may just be cell debris, but I want to ask about that.


Sucrose gradient protocol

Note: Make sure you are keeping everything cool/on ice, this includes putting empty tubes in cold room or freezer before you use them.

1. Have prepared a light solution (i.e., 10% sucrose) and a heavy solution (50% sucrose) that has been autoclaved and filter-sterilized. Sucrose solutions should be in the same buffer as your sample. Also prepare about 205 μ L of your sample, diluted to 0.6 μ g/ μ L
2. Put a tube in the marker block and draw a line at the top ledge
3. With a 30mL syringe, push into light solution and pump the air bubbles out. Draw out light solution (about 8 mL per gradient; so, draw out 10mL to be safe) and plunge needle to the very bottom of the tube. Lift the needle as solution pours out, be careful to keep the tip of the needle just under the surface of the solution. Stop at just above the line.
4. With a 10mL syringe, push into heavy solution and pump the air bubbles out. Draw out heavy solution, then wipe the needle and push a drop out, dabbing onto a kimwipe (making sure there is no air). Holding the tube with thumb and middle finger, use forefinger as a guide as you plunge needle to bottom of the tube, resting the needle along the wall and push out gently just enough to form a pool. Keep pushing out solution as you draw up the needle, keeping the tip about 1cm below the interface of the layers until there is about 2-3 mm of space at the top. Make sure the needle is resting against the wall of the tube as you quickly draw it out. There should be a visible line between the layers.
5. Use light solution to adjust the top layers, making sure all samples are at the same level
6. Cap the tubes, making sure the hole is the last part to seal. Some liquid should be visible inside the cap, if there is visible air or you can't see liquid in cap add some more light solution
7. Turn on gradient station in back, select "GMST"
8. Use the up and down keys on the gradient station and the level to level the plate. When level, press "done"
9. Put tubes in tube holder (no need to balance); Go to exit \square gradient \square recent \square confirm rotor \square use (confirm 10:50) \square run (recent should be the 10-50% sucrose gradient because that's what we've been using. If you need to find a different one go to recent and scroll through)
10. Put back in tube rack and refrigerate for 45 minutes. Now is a good time to cool down the ultracentrifuge.
 - a. When cooling down the ultracentrifuge, also put tubes in the cold room
11. Take off caps, remove 40 μ L of top layer and weigh the samples. Using light solution, adjust as necessary until there is no more than 0.002 difference in weight.
 - a. Make sure to keep them on ice at this point

12. Use sample layering device (or just a micropipette) to add 200uL of sample to the top (put tube in tube holder, use both hands for the device, press the edge of the syringe or micropipette tip against farther tube wall and gently push the sample onto the top layer)
13. Put samples on ice and bring to INBRE to spin on ultracentrifuge with rotor SW40 Ti. 40,000 rpm for 4 hours at 4C, using 5 for acceleration and deceleration.
14. After spin, continue with fractionation protocol.

Fractionating Protocol

1. An hour or so before the ultracentrifuge is done, want to set up the fractionator
2. Before launching the Triax software, turn fractionator on via button on the side, then set fractionator to “scan”
3. Choose the username, click “single UV OD scan”. Channel A Wavelength should be 260 nm
4. On the bottom of the screen fill out Gradients necessary info (i.e., sample volume, gradient type, speed, etc.)
5. Under the ~~rotor~~ scan settings (SW40Ti) type the number of fractions you want ~~select “number fractions” as the mode to fractionate by~~
6. On the LED Power screen, make sure Channel A is reading between 800,000 and 900,000. Push water through the cell if it needs to be adjusted
7. Go to scan set up, make sure everything is filled out correctly
8. Go to graph. Pump water through the cell again to calibrate. Y axis should be close to 0, with at least two zeros following the decimal point (ex// 0.002)
 - a. Push water through the system and then give it some time. Come back about 10 minutes before the ultracentrifuge is done and make sure that the reading has at least two zeros following the decimal point
9. At this point is when the ultracentrifuge should be done, so you can grab your samples while making sure that they stay on ice
10. Purge air through the system, then load the first sample. Cap the sample with the flow cell cap by turning and pushing down and put sample in the flow cell, using bottom lock. Slide it on the fractionator then rotate 90 degrees to fix it in place (window should face front)
11. Load microcentrifuge tubes in rack in the middle of two rows, then put the rack on the fractionator. The 29 should face the back. The dispenser will make a U moving back then left then forwards, so load tubes accordingly
12. On software, hit “start scan”. When all of the tubes are filled, hit “end” to move the tubing to the back. Flush with water for 10 seconds then air for 10 seconds. Repeat for a total of 3 washes. Rinse screw on cap with DI water
13. Save run as csv when prompted and graph via file  save graph image
14. Repeat step 9 for the remaining samples
15. When all of the samples are complete, push water through the flow cell, then flush 5 times with water, 3 times with 70% isopropyl alcohol, and 5 times with air only using the dry syringe. Switch metal lever down to assist with air flushes
16. Flush the line your sample goes through with DI water
17. Save fractions at -20C if interested in proteins, -80C if interested in RNA

Tuesday, September 27, 2022**To Do:**

1. Run colony PCR
2. Take 1-2 hours and work on powerpoint
3. Streak out $\Delta 1/\Delta 2$ and $\Delta 1/\Delta 2$ suppressor on CHAH Kan
4. Streak out pKR125 and 120 along with LVS pF and $\Delta 1/\Delta 2$ pF

Results and Data:

The PCR did not end up working, I think I messed up the tubes as in I put the wrong DNA in one or I put the tubes in the wrong lanes. Because of this, I need to redo the PCR on another day. The plan is to redo it on Thursday with gDNA for the $\Delta rpsU1 \Delta rpsU2$ and $\Delta rpsU1 \Delta rpsU2 \Delta$ mutant samples.

Plates for isolating gDNA (doing on Wednesday)

Strain Number	Genotype
KRLVS196.1 (box 7)	$\Delta rpsU1 \Delta rpsU2$ pF
In EM Rotation -80C box	$\Delta rpsU1 \Delta rpsU2$ pF mutant suppressor

Plates for imaging (doing on Friday)

Strain Number	Genotype
KRLVS203.1 (box 7)	$\Delta rpsU1 \Delta rpsU2$ pKR125
KRLVS205.1 (box 7)	$\Delta rpsU1 \Delta rpsU2$ pKR120
KRLVS196.1 (box 7)	$\Delta rpsU1 \Delta rpsU2$ pF
KRLVS120.1 (box 5)	LVS pF

Primer Descriptions

Primer Number	Description
P742 and P743	For rpsU1 deletion Expect 1352 bps if rpsU1 intact Expect 1167 bps if rpsU1 deleted
KROL147 and KROL148	For rpsU2 deletion Expect 1275 if rpsU2 is intact Expect 1090 if rpsU2 is deleted

PCR Tube Labels

Strain/Labe l	Description	Primer	Control or Experimenta l
1	Ladder		

MM1 = rpsU1 master mix

MM2 = rpsU2 master mix

Get own aliquots of everything since PCR is very prone to contamination

6. Use PCR program:
 - a. 94°C 2'
 - b. 94°C 20"
 - c. 50°C 30"
 - d. 68°C (1' per kb)
 - e. Go to step 2, rep 32x
 - f. 68°C 5'
 - g. Hold 12°C
7. For PCR program:
 - a. Use STN1
 - b. Step 2 is denaturing, step 3 is annealing, and step 4 is extension (1 min per kB, so calculate this based on your biggest expected bp)
8. If necessary, PCR purify and digest only LVS gDNA control and your potential mutants. Diagnostic digest should be 20 uL per reaction with 0.5 uL of enzyme.
9. For cross-outs which appear to have the desired mutation (maximum 4), streak to single colony on CHAH plates, incubate at 37° for 3 days (or until single colonies appear).
10. Did 10 uL of ladder for gel
11. For gels, put 4uL of buffer onto parafilm and it will bead. This way you can just mix the buffer and your PCR samples on the parafilm and you will use less tips!

Wednesday, September 28, 2022

To Do:

1. Isolate genomic DNA for suppressor and $\Delta 1/\Delta 2$
2. ~~Work on powerpoint~~

Results and Data:

Was not able to complete the isolation for gDNA because I had to leave early, but I ended by having issues with ethanol contamination. Because of this, Kathryn talked about having me make a 100 ng/uL aliquot of the gDNAs and then checking them by nanodrop.

gDNA extraction protocol

Need MasterPure Complete DNA and RNA Purification Kit for some of the solutions

Make sure cell block heater is at 65C

1. Dilute 1uL of **Proteinase K** into 300uL of **Tissue and Cell Lysis Solution** for each sample
2. Pellet cells in PBS by centrifugation and discard the supernatant, leaving approximately 25uL of liquid
3. Vortex for 10 seconds to resuspend the pellet
4. Add 300 uL of Tissue and Cell Lysis Solution containing the Proteinase K and mix thoroughly
5. Incubate at 65C for 15 minutes; vortex every 5 minutes
6. Cool the sample to 37C and add 4 uL of 5 mg/mL RNase (or 2 uL of 20 mg/mL) to the sample; mix thoroughly
 - a. Since RNase is pretty stable, don't really need to let it cool down

7. Incubate at 37 C for 30 minutes
 - a. At this point, want to turn on 4C centrifuge
 - b. Get ice
8. Place the samples on ice for 3-5 minutes
9. Add 150uL of MPC Protein Precipitation Reagent to 300 uL of lysed sample and vortex vigorously for 10 seconds
10. Pellet the debris by centrifugation at 4C for 10 minutes $\geq 10,000 \times g$ in a microcentrifuge. If the resultant pellet is clear, small, or loose, add an additional 25uL of MPC Protein Precipitation Reagent, mix, and pellet the debris again
 - a. Spin at max speed
11. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet
 - a. Give lots of room for the cell pellet, make sure that the supernatant is clear
12. Add 500uL of isopropanol to the recovered supernatant. Invert the tube 30-40 times.
 - a. Want to make sure that you see that they are mixed – may see some white strings and that is the DNA
13. Pellet the DNA by centrifugation at 4C for 10 minutes in a microcentrifuge.
14. Carefully pour off the isopropanol without dislodging the DNA pellet
 - a. Just make sure you keep an eye on the DNA pellet when you do this
 - b. Don't want to jostle it too much, so just tip the microcentrifuge tube
15. Rinse twice with 70% ethanol, being careful to not dislodge the pellet. Centrifuge briefly if the pellet is dislodged. Remove all of the residual ethanol with a pipet – let air dry
 - a. Let air dry for 5 minutes, when dry the DNA pellet should be basically clear
16. Resuspend the DNA in 35 uL of 0.1X EB

Make sure to turn off 4C centrifuge

I messed up the beginning of the protocol, using PBS instead of Tissue and Cell Lysis Solution for steps 1-7. I tried to save it by transferring the DNA to another tube and then doing the protocol over correctly. Because of this, I had to change some of the amounts of stuff I put in.

Did 450uL of TCLS instead of 300, 250 uL of MPC instead of 150 (added an additional 40uL MPC at step 10), and 835 uL of isopropanol instead of 500 uL.

Plates for isolating gDNA (doing on Friday/Monday)

Strain Number	Genotype
KRLVS196.1 (box 7)	$\Delta rpsU1 \Delta rpsU2$ pF
In EM Rotation -80C box	$\Delta rpsU1 \Delta rpsU2$ pF mutant suppressor

Friday, September 30, 2022

To Do:

1. Image plates
2. Start gDNA extraction (freeze down pellets)
3. Work on corrections for powerpoint

Results and Data:

I imaged $\Delta rpsU1 \Delta rpsU2$ pKR120 cells compared to wildtype LVS pF cells and $\Delta rpsU1 \Delta rpsU2$ pF cells to see if the plasmid is what is causing the partial restoration of growth or if there is a chromosomal mutation that is causing a partial restoration of growth. I was not able to image the $\Delta rpsU1 \Delta rpsU2$ pKR125 cells because they ended up contaminated.

Based on the results of the imaging, I struck out LVS pKR120 with the other three strains to see if the growth defect is specific to *rpsU* or not.

Plates for imaging (doing on Monday)

Strain Number	Genotype
KRLVS136.1 (box 5)	LVS pKR120
KRLVS205.1 (box 7)	$\Delta rpsU1 \Delta rpsU2$ pKR120
KRLVS196.1 (box 7)	$\Delta rpsU1 \Delta rpsU2$ pF
KRLVS120.1 (box 5)	LVS pF

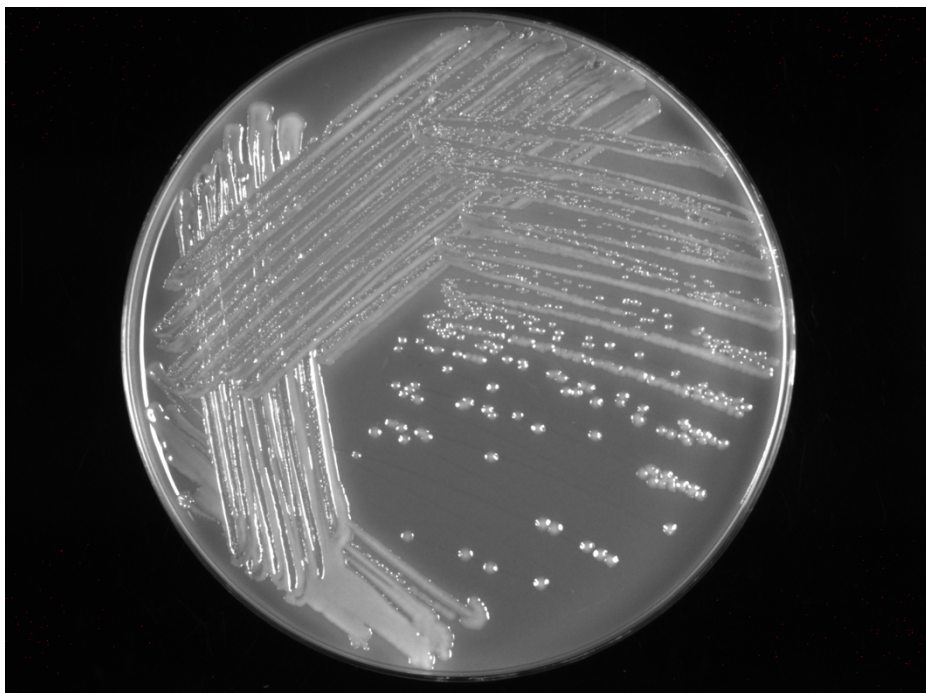


Figure 35. LVS pF colonies

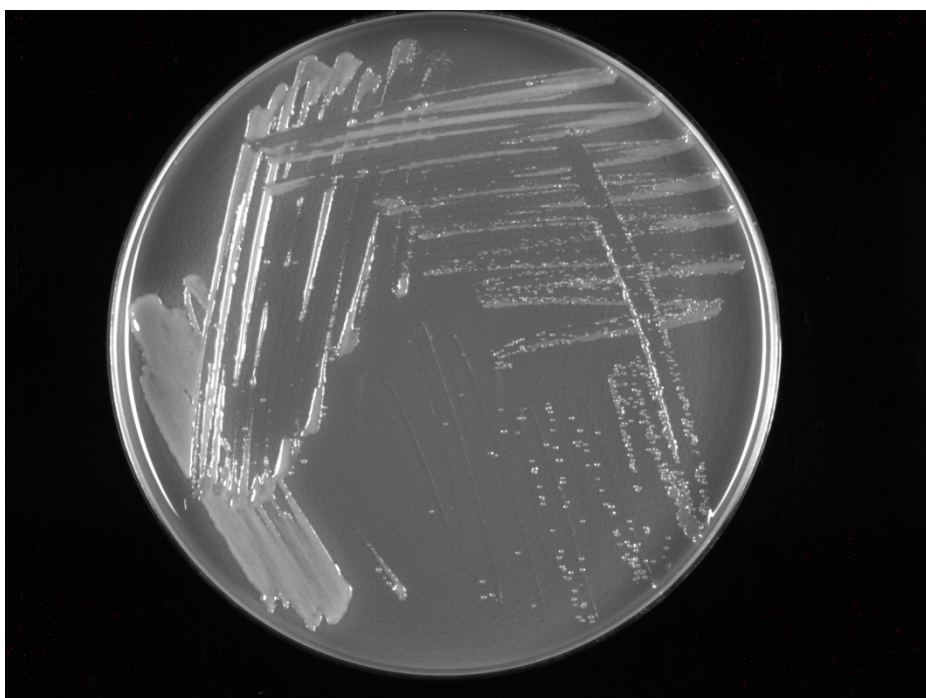


Figure 36. $\Delta rpsU1 \Delta rpsU2$ pF colonies

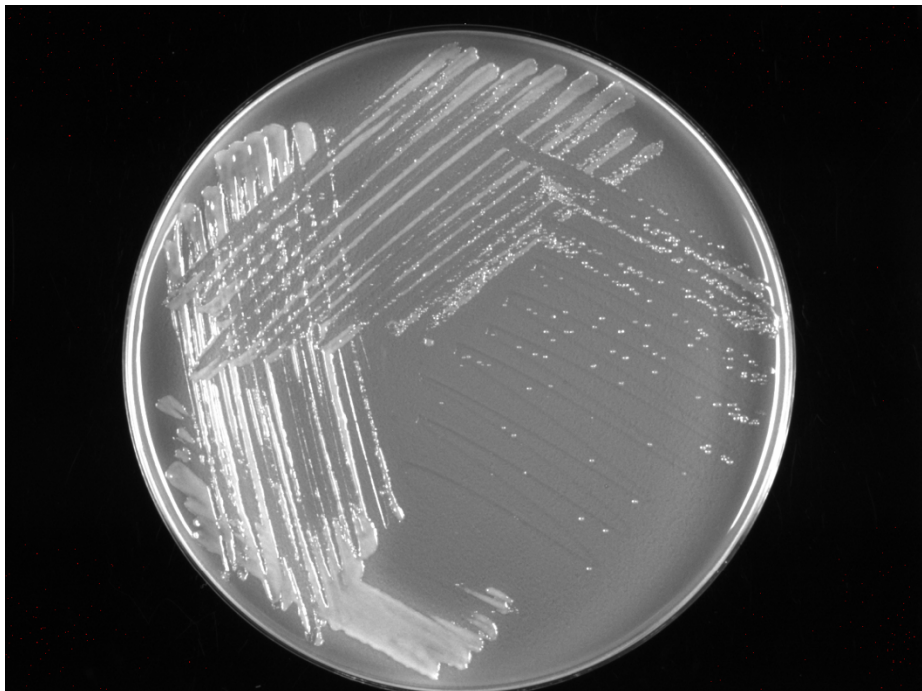


Figure 37. *ΔrpsU1 ΔrpsU2 pKR120* colonies

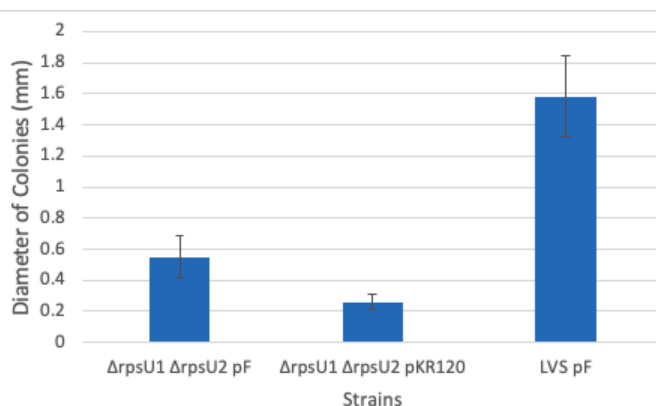


Figure 38. This graph is looking at the diameter of colonies after growth for 3 days. The graph shows that *ΔrpsU1 ΔrpsU2 pKR120* cells grow worse than both the wildtype (LVS) cells and the *ΔrpsU1 ΔrpsU2 pF* cells.

The results of the imaging indicate that the *ΔrpsU1 ΔrpsU2 pKR120* cells grow at a slower rate than both the LVS pF and *ΔrpsU1 ΔrpsU2 pF* cells. This indicates that the partial growth restoration seen previously (Thursday September 15 data) is not caused by the plasmid, rather by something else – possibly a chromosomal mutation. Remember, pKR120 is a plasmid that include *era* and other unknown genes. Because of this, it would be interesting to see the growth of *ΔrpsU1 ΔrpsU2 pKR125* and see if that one has the same result since pKR125 only has *era*.

Monday, October 3, 2022

To Do:

1. gDNA extraction

2. PCR
3. Image plates
4. Finish powerpoint and practice talk

Results and Data:

I went through the protocol for gDNA extraction (September 28). Initially, there is still that A230/260 contamination – this time the number were both below 1.0 and they are supposed to be around 2.0. I went through the protocol with Hannah to see if I had made a mistake during the protocol but I did not. I used a rotovap (may be called something ask, ask Dr. Rowley) to get any extra ethanol off – if ethanol contamination is an issue then this would solve that issue. It did not work and so tomorrow Kathryn is going to walk me through the purification to see what is happening.

I did end up imaging plates from Friday.

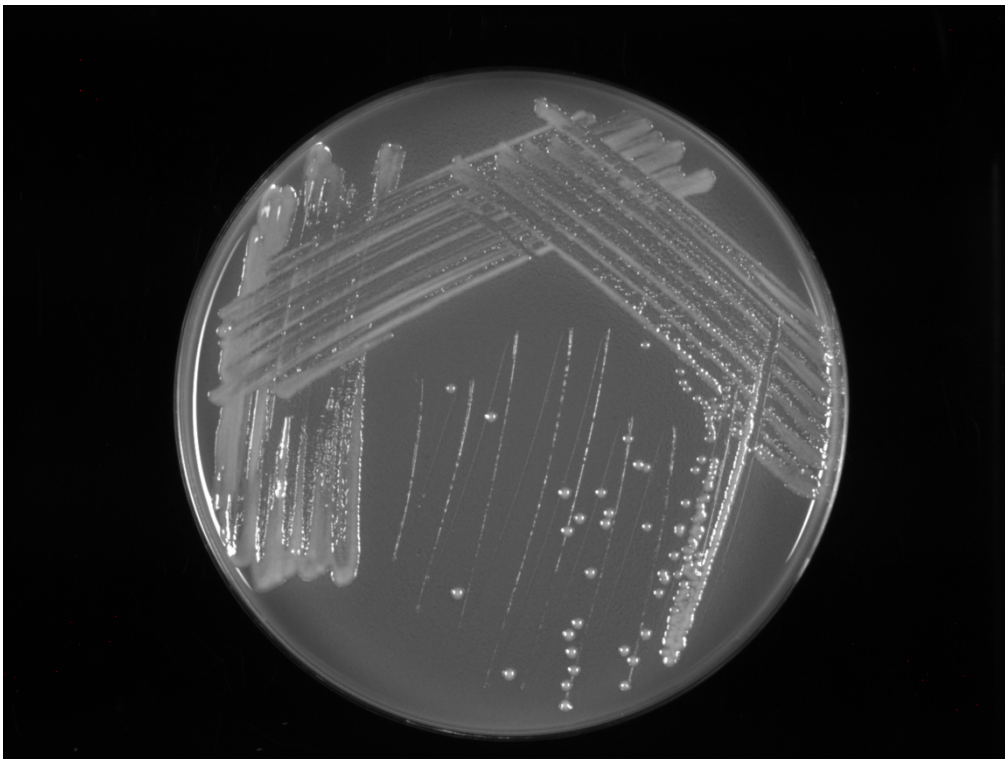


Figure 39. LVS pF colonies

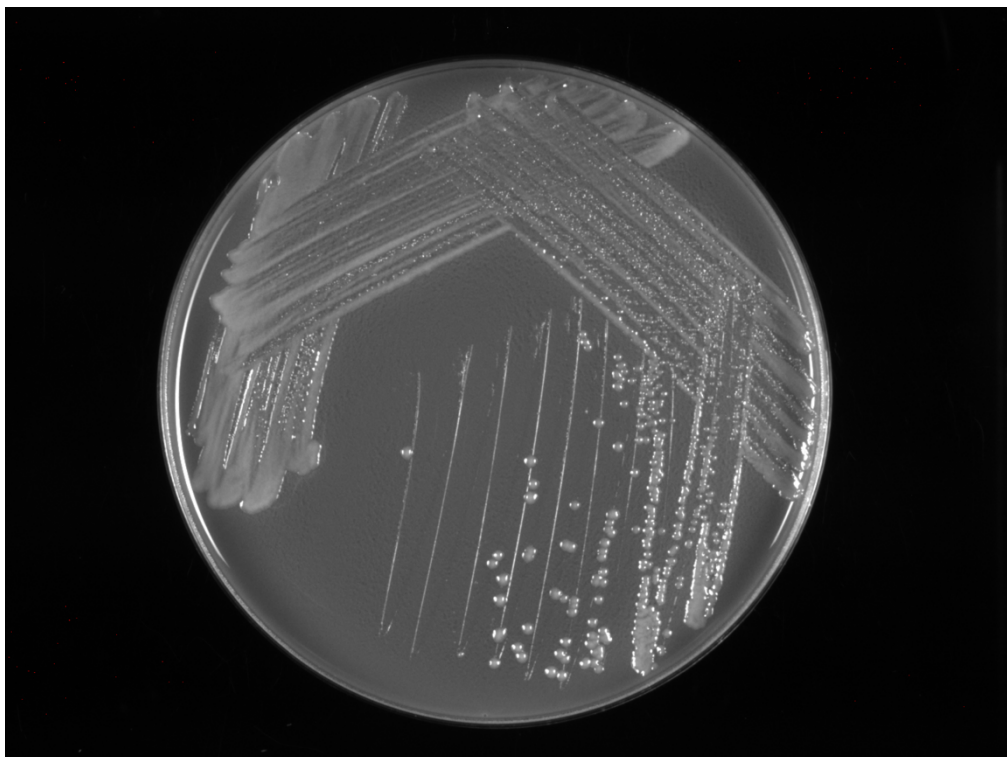


Figure 40. LVS pKR120

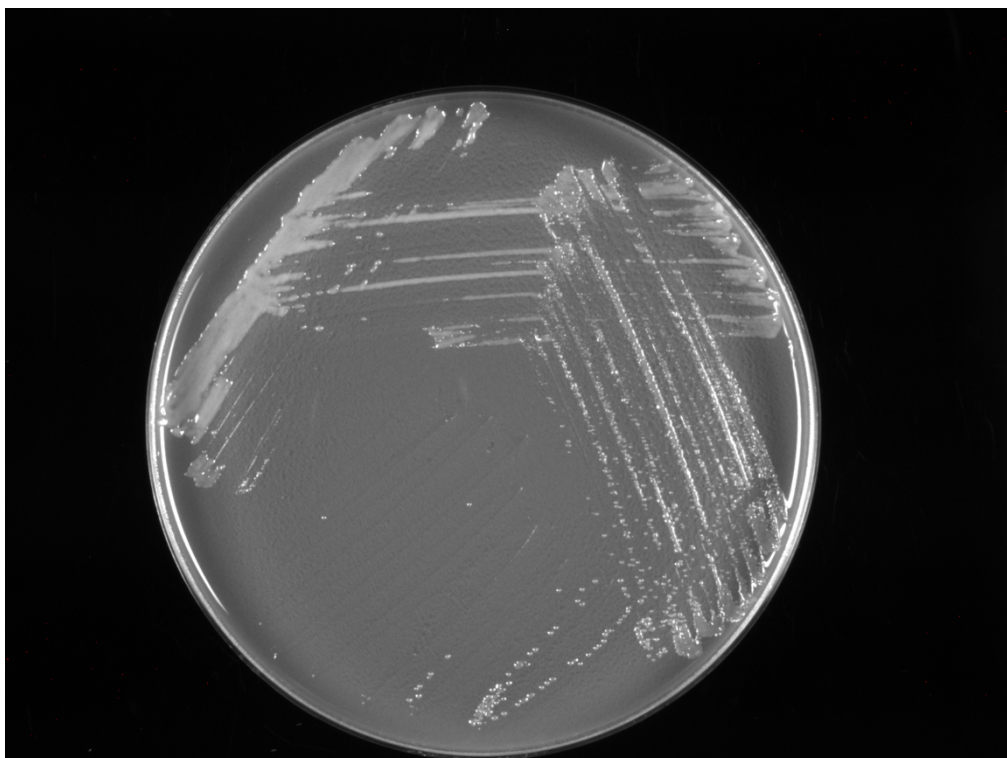


Figure 41. $\Delta rpsU1 \Delta rpsU2$ pF

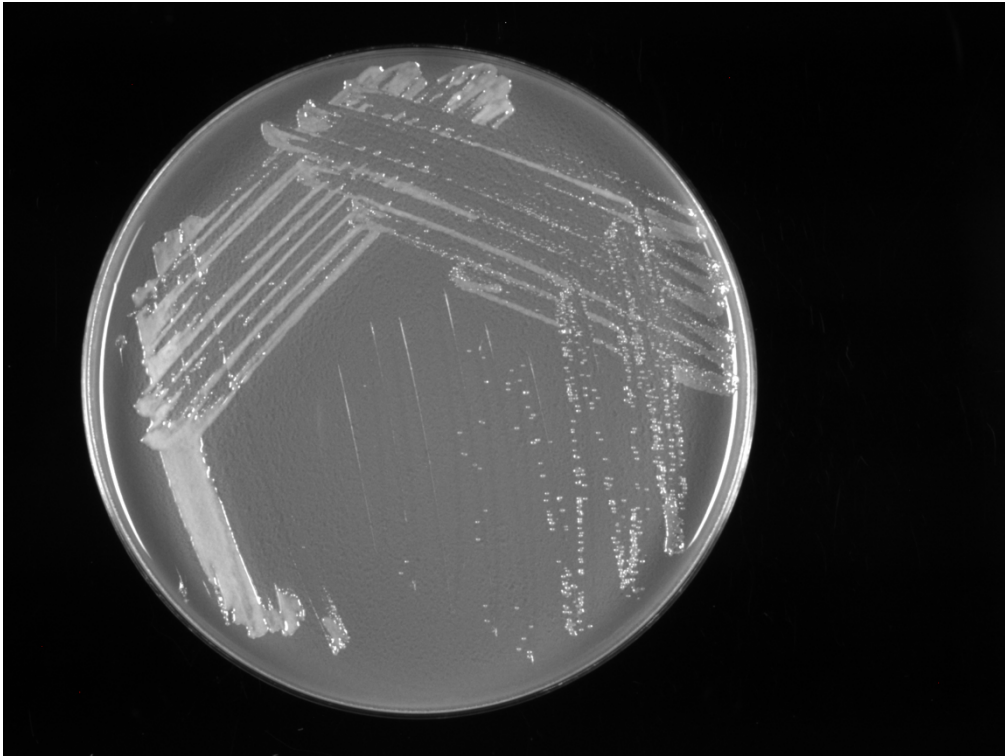


Figure 42. $\Delta rpsU1 \Delta rpsU2$ pKR120

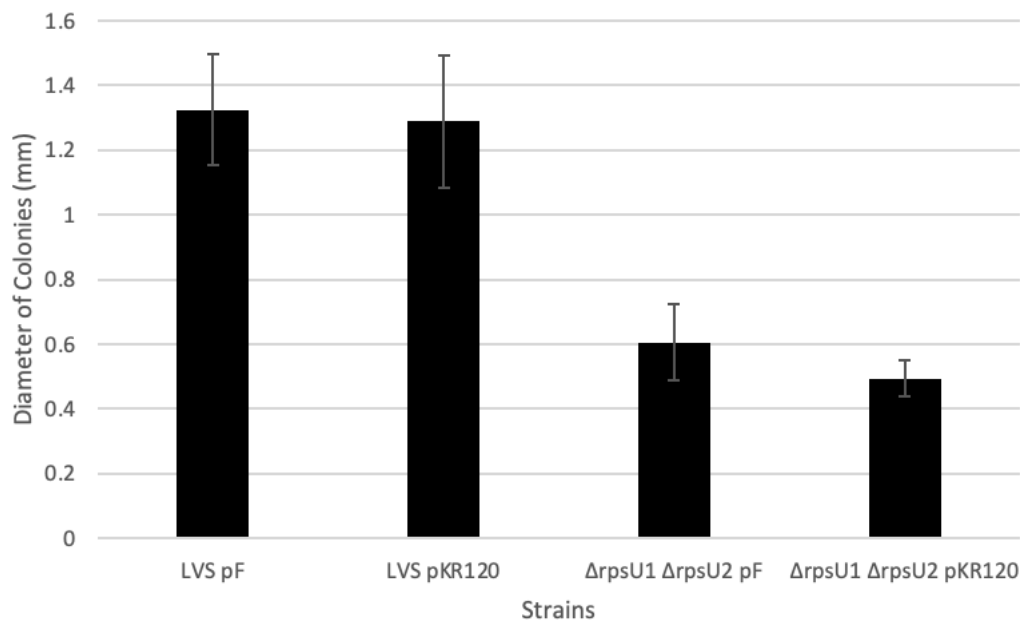


Figure 43. This graph indicates that the growth effect is strain specific and that the growth restoration in $\Delta rpsU1 \Delta rpsU2$ was not caused by the plasmid.

These results tell us two things – that the plasmid is not causing the growth restoration that we saw with the E-38 colony and that the effect is strain specific since inserted the plasmid into LVS does not change growth of the cells.

Tuesday, October 4, 2022

To Do:

1. Meet with Kathryn at 10
2. gDNA extraction

Results and Data:

Did gDNA extraction and was able to get it right this time. The A230/260 was still around 1.4, but Kathryn said we should just go for it. Started PCR - made tubes and put them in the thermocycler. I will run the gel tomorrow.

#	Sample ID	User name	Date and Time	Nucleic Acid	Unit	A260 (Abs)	A280 (Abs)	260/280	260/230	Sample Type	Factor
1		1 Science	10/4/2022 12:00:07 PM		590.3 ng/ul	11.896	6.202		1.9	1.4 DNA	50
2		2 Science	10/4/2022 12:01:31 PM		891.5 ng/ul	17.83	9.358		1.91	1.41 DNA	50

Want to dilute it to 100 ng/ul in 50 ul

Sample 1: $(100\text{ng/ul}) / (590.3\text{ng/ul}) * 50\text{ul} = 8.5 \text{ ul}$ of sample with 41.5 ul of 0.1X EB

Sample 2: $(100\text{ng/ul}) / (891.5\text{ng/ul}) * 50\text{ul} = 5.6 \text{ ul}$ of sample with 44.4 ul of 0.1X EB

For PCR:

Primer Descriptions

Primer Number	Description
P742 and P743	For rpsU1 deletion Expect 1352 bps if rpsU1 intact Expect 1167 bps if rpsU1 deleted
KROL147 and KROL148	For rpsU2 deletion Expect 1275 if rpsU2 is intact Expect 1090 if rpsU2 is deleted

MM1: P742/3 primers (rpsU1)

MM2: KROL147/8 primers (rpsU2)

Number of samples 4
Master mix factor 5

Components	Volumes in 1 reaction (uL)	Volumes in Master Mix (uL)
H ₂ O	11.8	59
5X PrimeStar GXL buffer	4	20
2.5 mM dNTPS	1.6	8
Forward primer (10 uM)	0.6	3
Reverse primer (10 uM)	0.6	3
PrimeStar DNA polymerase	0.4	2
Cell lysate/gDNA	1	
Total	20	95

PCR

Label	Lane	Description	Primer	Control or Experimental
	1	Ladder (10uL)		
1	2	Water with rpsU1 primer	P742, P744	Negative Control
2	3	LVS gDNA with rpsU1 primer	P742, P744	Positive Control
3	4	$\Delta rpsU1 \Delta rpsU2$ lysate with rpsU1 primer	P742, P744	Negative Control
4	5	$\Delta rpsU1 \Delta rpsU2$ suppressor mutant lysate with rpsU1 primer	P742, P744	Experimental
5	6	Water with rpsU2 primer	KROL147, KROL148	Negative Control
6	7	LVS gDNA with rpsU2 primer	KROL147, KROL148	Positive Control
7	8	$\Delta rpsU1 \Delta rpsU2$ lysate with rpsU2 primer	KROL147, KROL148	Negative Control
8	9	$\Delta rpsU1 \Delta rpsU2$ suppressor mutant lysate with rpsU2 primer	KROL147, KROL148	Experimental

Wednesday, October 5, 2022

To Do:

1. Finish PCR
2. Rearrange box 7
3. Work on powerpoint presentation

Results and Data:

I was finally able to organize KRLVS strains box #7 after I had dropped it. I then made my PCR gel and ran it based on the PCR table from yesterday (lane numbers and what's in each lane is important)

Making and running PCR gel

1. There is already gel that is usually premade, so you want to heat that until it melts at 300C
 - a. Want to make sure it does not overboil
2. Once its melted, need to cool it to 57C which takes 15-20 minutes (either cool it in the water bath or on the plate heater)
3. Once the gel is ready, want to pour it into the gel rig
 - a. Want to use the wider teeth to pour gel
 - b. Want to put 5uL of Sybro Safe DNA Gel Stain and spread it on the gel rig before pour the gel (this way it will be spread throughout the gel)
 - c. Want to pour it a bit more over halfway up the wide teeth
 - d. Need to wait about 30 minutes for it to solidify
4. Once it solidifies, want to pour used TE buffer on either sides of the gel rig and then on top of the gel so it covers it

5. After that, you can load your samples
 - a. 10 uL of ladder (?)
 - b. 4uL of orange dye per reaction (20uL of sample and then want to get the dye to 1X
 - i. For this, you can put the dots on a piece of parafilm and then suck it up and mix it with each PCR sample – saves time and micropipette tips
6. Want to run it for about 50 minutes

Bibliography