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Tuesday, February 2, 2021**To Do:**

1. Patch out Tn7:*rpsu1* Tn7:*rpsu2* Tn7:*rpsu3* for single use aliquots
2. Patch out strains for RNA
3. Pour square CHA plates

Methods:

The plates I made from the frozen glycerol stocks on Saturday look great (no contamination!) and I patched them to make single use aliquots tomorrow. The plates I made on Saturday for RNA culture were good except for some contamination that popped up in the first quadrant of Tn7:*rpsu1*. I imagine that something happened when I made the last batch of single use aliquots since I've been having such issues with contamination every time I streak from frozen, but I feel good about the new batch because as I said, no contamination has popped up on the plates that I streaked from glycerol. The plates that I made on Sunday also look good so far, so I hope to patch those out tomorrow so that I can do the RNA on Thursday. I poured square CHA plates for another track dilution later this week.

Wednesday, February 3, 2021**To Do:**

1. ~~Patch out Tn7:*rpsu1* Tn7:*rpsu2* Tn7:*rpsu3* for single use aliquots~~
2. ~~Pour square CHA plates~~
3. Make single use aliquots
4. Patch out strains for RNA

Methods:

I had contamination on Tn7:*rpsu1* again. Fortunately, because I had streaked out plates from glycerol frozen stock to make new aliquots, I had a non-contaminated plate to work with and therefore I patched out all my strains so that I can move forward with RNA isolation tomorrow. It is also ok (only in a pinch!) to streak directly from the glycerol stock if my aliquots are contaminated or I run out.

I also talked to Kathryn about my latest OD to CFU/mL curve. I had set up the dilution incorrectly (not the serial dilution, the dilution of tube 1). When I set it up next time, I will again put 700uL of culture into 700uL of PBS in Tube 1, then take out 700uL to put in the next tube in the series. From the 700 I have left over I will take 250uL out to make a 1:2 dilution, and measure that for the OD. Depending on the reading I can then determine what the ODs should be for the rest of the dilution series.

Thursday, February 4, 2021**To Do:**

1. ~~Make single use aliquots~~
2. ~~Patch out strains for RNA~~
3. Set up culture for RNA
4. RNA isolation

Methods and Results:

RNA culture set up:

| Sample # | Measured OD600 | Dilution Factor | Actual OD600 | Desired Volume mL | Desired OD | Volume of Resuspended Cells μ L | Resuspension on T0 (0940) | OD T2 (1143) | OD T4 (1329) | Final OD (1518) |
|----------|----------------|-----------------|--------------|-------------------|------------|-------------------------------------|---------------------------|--------------|--------------|-----------------|
| KB24-1 | 0.255 | 10 | 2.55 | 8 | 0.08 | 251 | 0.079 | 0.156 | 0.271 | 0.395 |
| KB24-2 | 0.254 | 10 | 2.54 | 8 | 0.08 | 252 | 0.078 | 0.152 | 0.266 | 0.392 |
| KB24-3 | 0.324 | 10 | 3.24 | 8 | 0.08 | 198 | 0.079 | 0.137 | 0.226 | 0.33 |
| KB24-4 | 0.281 | 10 | 2.81 | 8 | 0.08 | 228 | 0.076 | 0.14 | 0.229 | 0.337 |
| KB24-5 | 0.31 | 10 | 3.1 | 8 | 0.08 | 206 | 0.075 | 0.138 | 0.233 | 0.339 |
| KB24-6 | 0.287 | 10 | 2.87 | 8 | 0.08 | 223 | 0.077 | 0.146 | 0.25 | 0.364 |
| KB24-7 | 0.416 | 10 | 4.16 | 8 | 0.08 | 154 | 0.078 | 0.144 | 0.228 | 0.336 |
| KB24-8 | 0.348 | 10 | 3.48 | 8 | 0.08 | 184 | 0.072 | 0.133 | 0.217 | 0.326 |

I performed the first part of the RNA isolation in lab 470 because Bertin Lab is using their chem fume hood. Everything proceeded as expected.

Friday, February 5, 2021

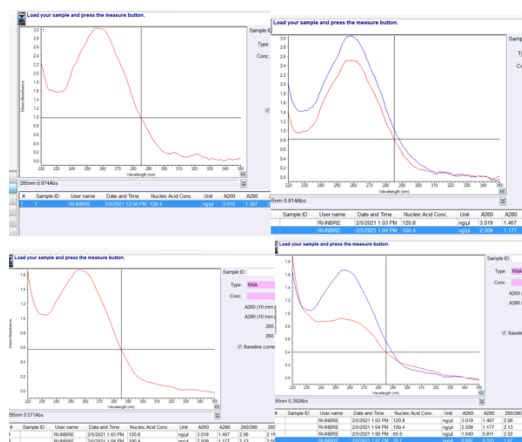
To Do:

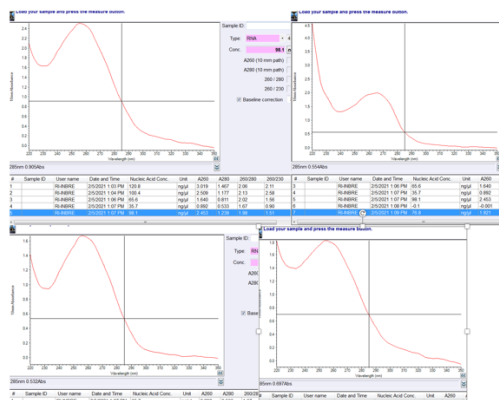
1. ~~Set up culture for RNA~~
2. ~~RNA isolation~~
3. DNase
4. Run gel
5. Maybe track dilution for T6SS assay

Methods and Results:

I was able to set up the track dilution although some of the plates were in rather rough shape (not entirely smooth), and therefore some of the tracks bled together.

Before performing the DNase portion of RNA isolation, I checked the concentrations of the genetic material to make sure the contamination/degradation that happened last time didn't happen in the first part.





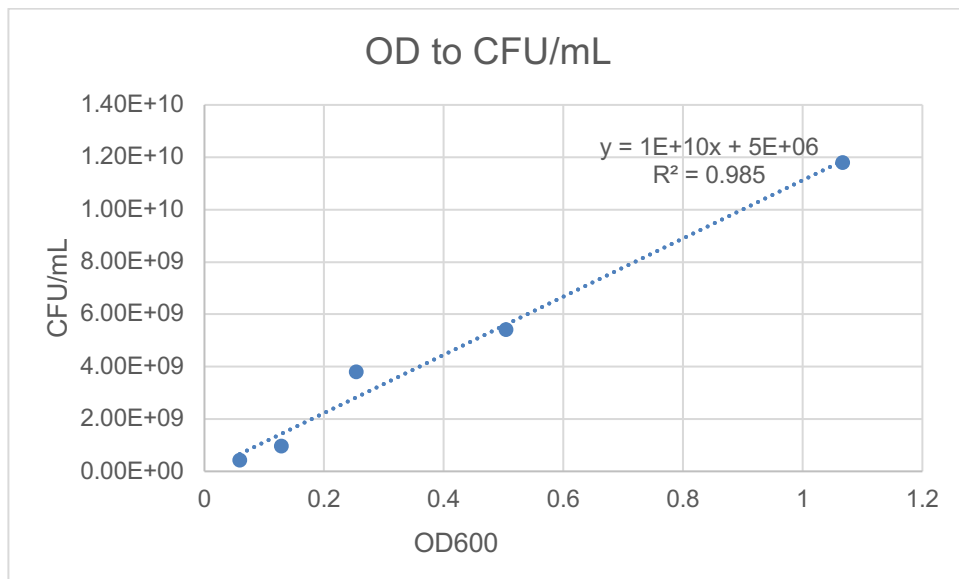
There is still some degradation.

Monday, February 8, 2021

To Do:

1. Read track dilutions

| Dilution | Measured OD (undiluted) | Measured OD (1:4) | Actual OD | CFU per mL |
|----------|-------------------------|-------------------|-----------|------------|
| Tube 1 | | 0.2667 | 1.0668 | 1.18E+10 |
| Tube 2 | 0.504 | | 0.504 | 5.40E+09 |
| Tube 3 | 0.254 | | 0.254 | 3.80E+09 |
| Tube 4 | 0.128 | | 0.128 | 9.70E+08 |
| Tube 5 | 0.059 | | 0.059 | 4.20E+08 |



R^2 looks good! At some point I will repeat this to make sure it's consistent.

Tuesday, February 9, 2021

To Do:

1. Set up cultures
2. RNA isolation
3. Check concentrations

Methods and Results:

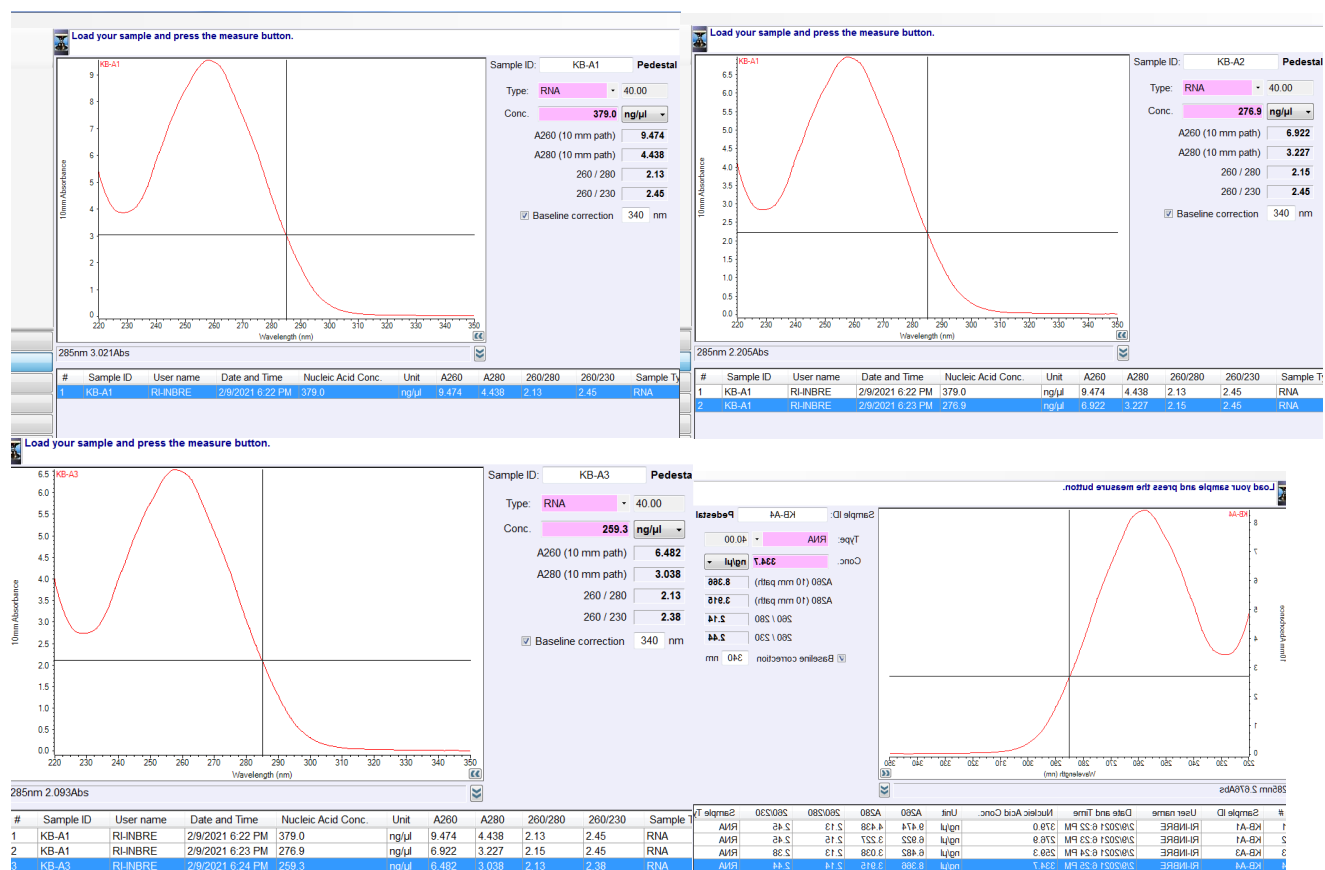
RNA Isolation

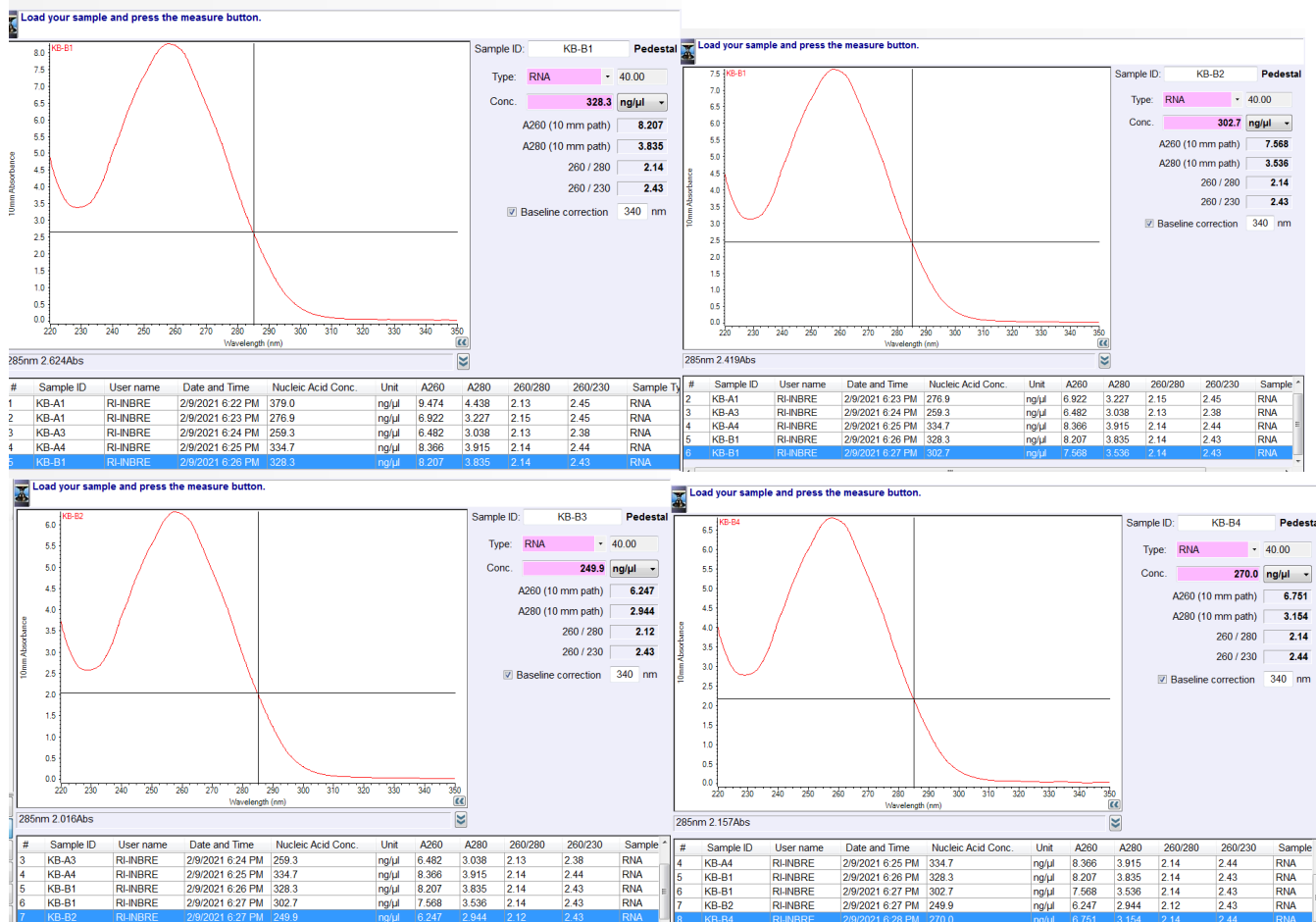
In order to determine what is going wrong in the procedure that is causing low concentrations and contamination, I set up two sets of cultures for only LVS and Tn7::rpsU1. I streaked to single colony for each strain and then made two patches per strain. From those I set up 2 cultures per patch per strain for a total of 8 cultures. Both A and B went through the first protocol.

| Sample # | Strain |
|----------|-----------|
| KB-A1 | LVS WT |
| KB-A2 | LVS WT |
| KB-A3 | Tn7:rpsU1 |
| KB-A4 | Tn7:rpsU1 |
| KB-B1 | LVS WT |
| KB-B2 | LVS WT |
| KB-B3 | Tn7:rpsU1 |
| KB-B4 | Tn7:rpsU1 |

| Sample # | Measured OD600 | Dilution Factor | Actual OD600 | Desired Volume mL | Desired OD | Volume of Resuspended Cells μ L | Resuspension T0 (1030) | OD T2 (1249) | OD T4 (1513) | Final OD (1545) |
|----------|----------------|-----------------|--------------|-------------------|------------|-------------------------------------|------------------------|--------------|--------------|-----------------|
| KB-A1 | 0.193 | 10 | 1.93 | 8 | 0.08 | 332 | 0.086 | 0.156 | 0.313 | 0.353 |
| KB-A2 | 0.172 | 10 | 1.72 | 8 | 0.08 | 372 | 0.082 | 0.157 | 0.309 | 0.347 |
| KB-A3 | 0.204 | 10 | 2.04 | 8 | 0.08 | 314 | 0.086 | 0.143 | 0.268 | 0.306 |
| KB-A4 | 0.164 | 10 | 1.64 | 8 | 0.08 | 390 | 0.085 | 0.16 | 0.297 | 0.334 |
| KB-B1 | 0.227 | 10 | 2.27 | 8 | 0.08 | 282 | 0.083 | 0.16 | 0.313 | 0.352 |
| KB-B2 | 0.177 | 10 | 1.77 | 8 | 0.08 | 362 | 0.082 | 0.161 | 0.322 | 0.359 |
| KB-B3 | 0.217 | 10 | 2.17 | 8 | 0.08 | 295 | 0.083 | 0.15 | 0.285 | 0.321 |
| KB-B4 | 0.166 | 10 | 1.66 | 8 | 0.08 | 386 | 0.085 | 0.16 | 0.308 | 0.35 |

After the first part of the protocol, I checked the concentrations on the Nanodrop to be sure that everything was working according to plan. The peaks all look good.





| # | Sample | User name | Date and Time | Nucleic | Unit | A260 | A280 | 260/280 | 260/230 | Sample Type | Factor |
|---|--------|-----------|---------------------|---------|-------|-------|-------|---------|---------|-------------|--------|
| 1 | KB-A1 | RI-INBRE | 2/9/2021 6:22:31 PM | 379 | ng/ul | 9.474 | 4.438 | 2.13 | 2.45 | RNA | 40 |
| 2 | KB-A1 | RI-INBRE | 2/9/2021 6:23:49 PM | 276.9 | ng/ul | 6.922 | 3.227 | 2.15 | 2.45 | RNA | 40 |
| 3 | KB-A3 | RI-INBRE | 2/9/2021 6:24:57 PM | 259.3 | ng/ul | 6.482 | 3.038 | 2.13 | 2.38 | RNA | 40 |
| 4 | KB-A4 | RI-INBRE | 2/9/2021 6:25:45 PM | 334.7 | ng/ul | 8.366 | 3.915 | 2.14 | 2.44 | RNA | 40 |
| 5 | KB-B1 | RI-INBRE | 2/9/2021 6:26:30 PM | 328.3 | ng/ul | 8.207 | 3.835 | 2.14 | 2.43 | RNA | 40 |
| 6 | KB-B1 | RI-INBRE | 2/9/2021 6:27:10 PM | 302.7 | ng/ul | 7.568 | 3.536 | 2.14 | 2.43 | RNA | 40 |
| 7 | KB-B2 | RI-INBRE | 2/9/2021 6:27:57 PM | 249.9 | ng/ul | 6.247 | 2.944 | 2.12 | 2.43 | RNA | 40 |
| 8 | KB-B4 | RI-INBRE | 2/9/2021 6:28:46 PM | 270 | ng/ul | 6.751 | 3.154 | 2.14 | 2.44 | RNA | 40 |

Wednesday, February 10, 2021

To Do:

1. ~~Set up cultures~~
2. ~~RNA isolation~~
3. ~~DNase~~
4. ~~Run gel~~

Methods and Results:

For the DNase I'm only setting up the A group, the B group will remain frozen. We are checking to see if something is wrong with the DNase buffer and enzyme; if so, I will have B to work with when we get new materials.

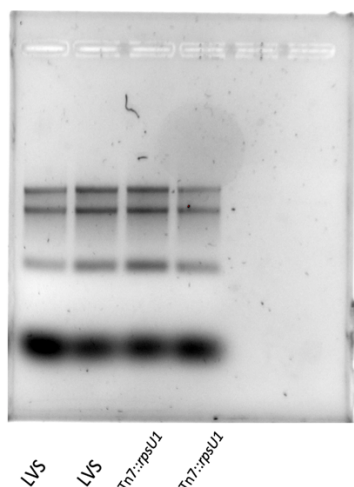
| # | Sample ID | User name | Date and Time | Nucleic Acid Conc. | Unit | A260 | A280 | 260/280 | 260/230 | Sample Type | Factor |
|---|-----------|-----------|----------------------|--------------------|-------|-------|-------|---------|---------|-------------|--------|
| 1 | KB-A1 | RI-INBRE | 2/10/2021 3:25:05 PM | 248.6 | ng/μl | 6.215 | 2.858 | 2.18 | 2.43 | RNA | 40 |
| 2 | KB-A2 | RI-INBRE | 2/10/2021 3:26:19 PM | 182.5 | ng/μl | 4.562 | 2.108 | 2.16 | 2.38 | RNA | 40 |
| 3 | KB-A3 | RI-INBRE | 2/10/2021 3:26:56 PM | 175.9 | ng/μl | 4.398 | 2.078 | 2.12 | 2.41 | RNA | 40 |
| 4 | KB-A4 | RI-INBRE | 2/10/2021 3:27:48 PM | 222.3 | ng/μl | 5.557 | 2.599 | 2.14 | 2.46 | RNA | 40 |

For the gel:

| Sample # | RNA concentration (ng/μL) | Amount RNA (μg) | Volume RNA (μL) | Volume H2O (μL) | Total volume (μL) |
|----------|---------------------------|-----------------|-----------------|-----------------|-------------------|
| KB-A1 | 248.6 | 1.5 | 6.02 | 5.98 | 12 |
| KB-A2 | 182.5 | 1.5 | 8.2 | 3.8 | 12 |
| KB-A3 | 175.9 | 1.5 | 8.52 | 3.48 | 12 |
| KB-A4 | 222.3 | 1.5 | 6.76 | 5.24 | 12 |

Plus 3uL
of loading
dye

Gel looks good!



Thursday, February 11, 2021

To Do:

1. DNase

~~2. Run gel~~

3. Grow strains for duplicate RNA isolation next week

| Measured OD (undiluted) | Measured OD (1:4) | Measured OD (1:8) | Actual OD |
|-------------------------|-------------------|-------------------|-----------|
| | 0.627 | | 2.508 |
| | 0.318 | | 1.272 |
| 0.572 | | | 0.572 |
| 0.294 | | | 0.294 |
| 0.147 | | | 0.147 |

Friday, February 12, 2021

To Do:

~~1. DNase~~

~~2. Run gel~~

3. Grow strains for duplicate RNA isolation next week

4. Set up T6SS OD curve

Methods and Results:

T6SS

I harvested cells in 4 ml of PBS, then set up a 1/2 dilution in 5 tubes with 700uL of culture going into tube 1 with 700 uL PBS, then 700uL of tube 1 going into tube 2 with 700uL PBS, etc. For tube 1 I also diluted it 1:4 by taking 250uL of tube 1 (after it was diluted) and adding it to 750uL PBS in a cuvette.

*I also took a measurement at 1:8 for tube 1 by adding 125uL of tube1 to 875uL PBS in a cuvette but the math doesn't seem to work so I must be doing something wrong.

| Dilution | Measured OD (undiluted) | Measured OD (1:4) | Measured OD (1:8) | Actual OD |
|----------|-------------------------|-------------------|-------------------|-----------|
| Tube 1 | | 0.627 | | 2.508 |
| Tube 2 | | 0.318 | | 1.272 |
| Tube 3 | 0.572 | | | 0.572 |
| Tube 4 | 0.294 | | | 0.294 |
| Tube 5 | 0.147 | | | 0.147 |

| Dilution | Measured OD (undiluted) | Measured OD (1:4) | Measured OD (1:8) | Actual OD |
|----------|-------------------------|-------------------|-------------------|-----------|
| Tube 1 | | 0.627 | 0.153 | 1.224 |
| Tube 2 | | 0.318 | | 1.272 |
| Tube 3 | 0.572 | | | 0.572 |
| Tube 4 | 0.294 | | | 0.294 |
| Tube 5 | 0.147 | | | 0.147 |

I then proceeded with the 1:10 dilution in a 96 well plate and inoculated ten plates (2x each sample) with 10uL of wells 4-10 and incubated them over the weekend. All of the tracks were good except one of the Tube 4 plates so I added another one just in case.

Sunday, February 14, 2021

To Do:

1. Patch strains for RNA isolation
2. Check track plates

I patched the strains and looked at the track plates, the colonies were very tiny, so I will check them again tomorrow.

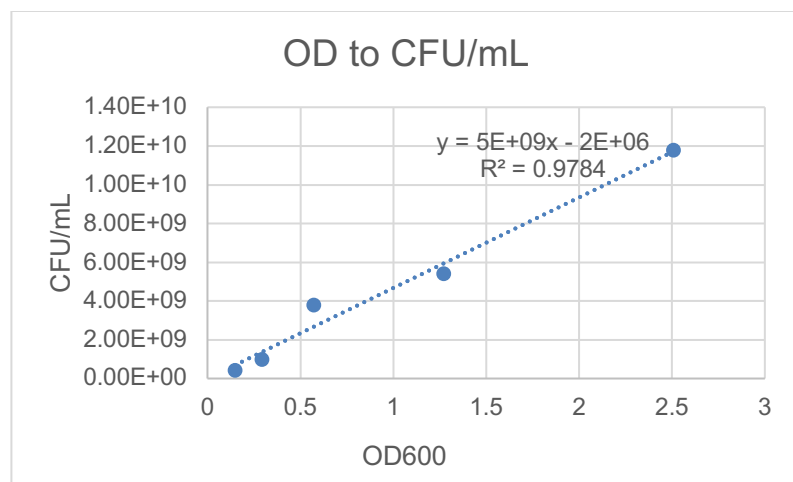
Monday, February 15, 2021

To Do:

- ~~1. Patch strains for RNA isolation~~
2. Check track plates
- ~~3. Count track plates~~
- ~~4. Set up RNA culture~~
- ~~5. RNA isolation~~

Methods and Results:

T6SS:



I set up the RNA cultures:

| Sample # | Strain |
|----------|------------|
| KB215-1 | LVS WT |
| KB215-2 | LVS WT |
| KB215-3 | Tn7::rpsU1 |
| KB215-4 | Tn7::rpsU1 |
| KB215-5 | Tn7::rpsU2 |
| KB215-6 | Tn7::rpsU2 |
| KB215-7 | Tn7::rpsU3 |
| KB215-8 | Tn7::rpsU3 |

| Sample # | Measured OD600 | Dilution Factor | Actual OD600 | Desired Volume mL | Desired OD | Volume of Resuspended Cells μ L | Resuspension T0 (1100) | OD T2 (1300) | OD T5 (1600) | Final OD (1630) |
|----------|----------------|-----------------|--------------|-------------------|------------|-------------------------------------|------------------------|--------------|--------------|-----------------|
| KB215-1 | 0.28 | 10 | 2.8 | 8 | 0.08 | 229 | 0.081 | 0.147 | 0.352 | 0.395 |
| KB215-2 | 0.223 | 10 | 2.23 | 8 | 0.08 | 287 | 0.083 | 0.149 | 0.362 | 0.406 |
| KB215-3 | 0.253 | 10 | 2.53 | 8 | 0.08 | 253 | 0.080 | 0.136 | 0.278 | 0.318 |
| KB215-4 | 0.278 | 10 | 2.78 | 8 | 0.08 | 230 | 0.083 | 0.142 | 0.301 | 0.343 |
| KB215-5 | 0.255 | 10 | 2.55 | 8 | 0.08 | 251 | 0.081 | 0.14 | 0.311 | 0.344 |
| KB215-6 | 0.235 | 10 | 2.35 | 8 | 0.08 | 272 | 0.081 | 0.152 | 0.339 | 0.382 |
| KB215-7 | 0.212 | 10 | 2.12 | 8 | 0.08 | 302 | 0.079 | 0.134 | 0.272 | 0.31 |
| KB215-8 | 0.224 | 10 | 2.24 | 8 | 0.08 | 286 | 0.081 | 0.153 | 0.323 | 0.366 |

I proceeded with the RNA isolation, nothing notable to report.

Tuesday, February 16, 2021

To Do:

1. ~~Count track plates~~
2. ~~Set up RNA culture~~
3. ~~RNA isolation~~
4. DNase
5. Run gel

Methods and Results:

RNA

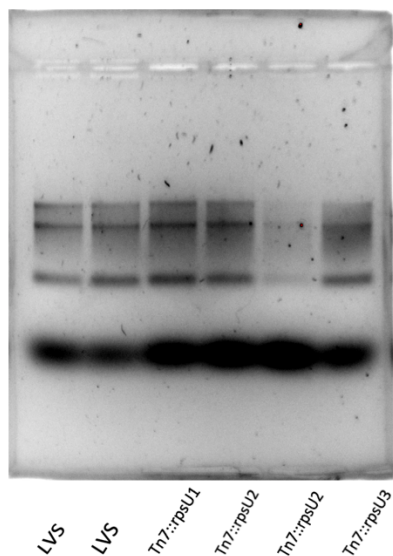
I continued the RNA isolation with the DNase portion. Something went wrong for some of the samples:

| # | Sample ID | User name | Date and Time | Nucleic Acid | Unit | A260 | A280 | 260/280 | 260/230 | Sample Type | Factor |
|---|-----------|-----------|----------------------|--------------|-------|-------|-------|---------|---------|-------------|--------|
| 1 | KB215-1 | RI-INBRE | 2/16/2021 6:24:29 PM | 261.6 | ng/μl | 6.54 | 2.998 | 2.18 | 2.4 | RNA | 40 |
| 2 | KB215-2 | RI-INBRE | 2/16/2021 6:25:17 PM | 258.1 | ng/μl | 6.453 | 3.003 | 2.15 | 2.06 | RNA | 40 |
| 3 | KB215-3 | RI-INBRE | 2/16/2021 6:25:57 PM | 175.6 | ng/μl | 4.389 | 2.091 | 2.1 | 1.89 | RNA | 40 |
| 4 | KB215-4 | RI-INBRE | 2/16/2021 6:26:36 PM | 237.6 | ng/μl | 5.941 | 2.97 | 2 | 1.3 | RNA | 40 |
| 5 | KB215-5 | RI-INBRE | 2/16/2021 6:27:21 PM | 194.2 | ng/μl | 4.856 | 2.239 | 2.17 | 2.37 | RNA | 40 |
| 6 | KB215-6 | RI-INBRE | 2/16/2021 6:27:57 PM | 415.8 | ng/μl | 10.4 | 6.343 | 1.64 | 0.82 | RNA | 40 |
| 7 | KB215-7 | RI-INBRE | 2/16/2021 6:29:28 PM | 240.2 | ng/μl | 6.004 | 3.099 | 1.94 | 1.26 | RNA | 40 |
| 8 | KB215-8 | RI-INBRE | 2/16/2021 6:30:31 PM | 202.4 | ng/μl | 5.06 | 2.359 | 2.15 | 2.14 | RNA | 40 |

Gel Calculation:

| Sample | RNA conc ng/ul | | RNA amt | RNA Volume (ul) | H2O Volume (ul) | Total Volume (ul) |
|---------|----------------|-------|---------|-----------------|-----------------|-------------------|
| KB215-1 | 261.6 | 0.262 | 1.5 | 5.73 | 6.27 | 12 |
| KB215-2 | 258.1 | 0.258 | 1.5 | 5.81 | 6.19 | 12 |
| KB215-3 | 175.6 | 0.176 | 1.5 | 8.54 | 3.46 | 12 |
| KB215-4 | 237.6 | 0.238 | 1.5 | 6.31 | 5.69 | 12 |
| KB215-5 | 194.2 | 0.194 | 1.5 | 7.72 | 4.28 | 12 |
| KB215-6 | 415.8 | 0.416 | 1.5 | 3.61 | 8.39 | 12 |
| KB215-7 | 240.2 | 0.240 | 1.5 | 6.24 | 5.76 | 12 |
| KB215-8 | 202.4 | 0.202 | 1.5 | 7.41 | 4.59 | 12 |

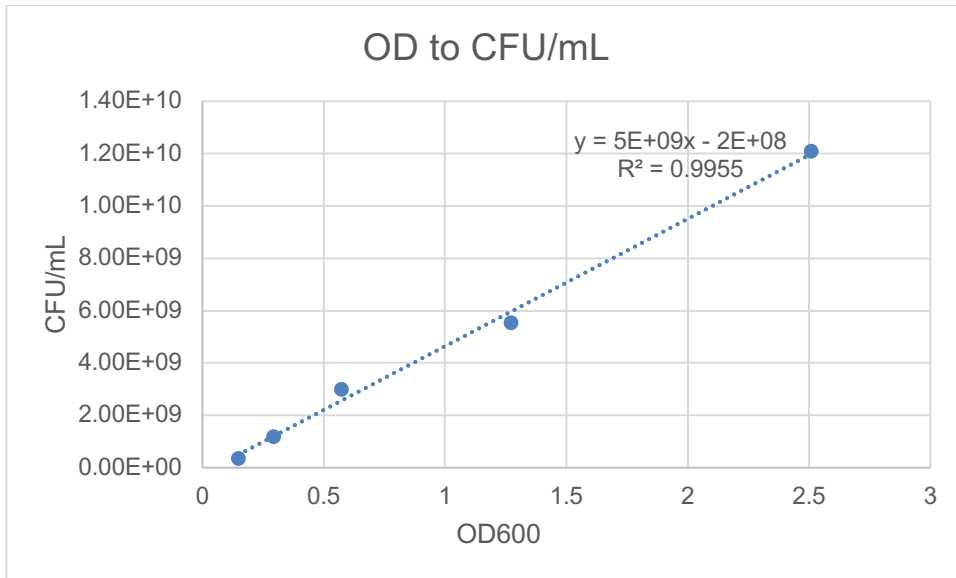
Even though some of the samples are contaminated I'm still proceeding with the gel. I only have 6 lanes instead of 8 because I took the wrong comb out and didn't realize until it was too late, so I chose to load the 5 good samples (KB215-1,-2,-3, -5, -8) and one of the contaminated ones (KB215-6).



T6SS

I realized on the way home last night that I never counted the track plates so I did it today. The colonies were big and crowding each other, so I tried to be as accurate as possible. The ideal day for me to read them is Day 3.

| | | Track Plate 1 | | | | | | | Track Plate 2 | | | | | | | Dilution factor counted | Average Cells | CFU per mL |
|---------------|--------|---------------|----------|-----------|---|---|---|---|---------------|---------|----------|-----------|---|---|---|-------------------------|---------------|------------|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | | | |
| Tube 1 | | | | 102 | | | | | | | 140 | | | | | 0.000001 | 121 | 1.21E+10 |
| Tube 2 | | | | 60 | | | | | | | 51 | | | | | 0.000001 | 56 | 5.55E+09 |
| Tube 3 | | | | 30 | | | | | | | 30 | | | | | 0.000001 | 30 | 3.00E+09 |
| Tube 4 | | | 109 | | | | | | | 130 | | | | | | 0.000001 | 120 | 1.20E+09 |
| Tube 5 | | | 32 | | | | | | | 39 | | | | | | 0.000001 | 36 | 3.55E+08 |
| Dilution Fact | 0.0001 | 0.00001 | 0.000001 | 0.0000001 | | | | | 0.0001 | 0.00001 | 0.000001 | 0.0000001 | | | | | | |



Really good R^2 !

Wednesday, February 17, 2021

To Do:

1. ~~DNase~~
2. ~~Run gel~~

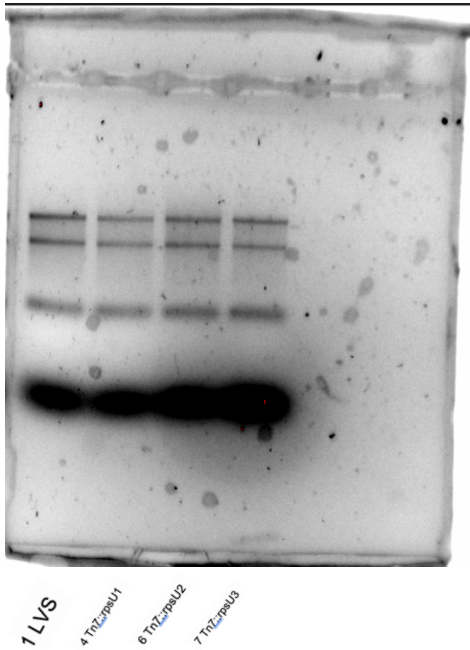
I spoke to Kathryn about the contaminated samples. We agree that the contamination is organic material. She suggests I let those samples thaw and then vent them to see if that will get rid of any extra phenol or ethanol. I will re-run them on Nanodrop and if they're good, run them on a gel, and if they're bad, scrap the whole thing and start over.

| # | Sample ID | User name | Date and Time | Nucleic Acid C | Unit | A260 | A280 | 260/280 | 260/230 | Sample Type | Factor |
|---|-----------|-----------|----------------------|----------------|-------|-------|-------|---------|---------|-------------|--------|
| 1 | 4 | RI-INBRE | 2/17/2021 3:48:10 PM | 245.7 | ng/μl | 6.144 | 3.085 | 1.99 | 1.29 | RNA | 40 |
| 2 | 6 | RI-INBRE | 2/17/2021 3:50:10 PM | 145.7 | ng/μl | 3.643 | 1.761 | 2.07 | 1.89 | RNA | 40 |
| 3 | 7 | RI-INBRE | 2/17/2021 3:51:00 PM | 187 | ng/μl | 4.675 | 2.244 | 2.08 | 1.91 | RNA | 40 |

The trick worked for # 6 and 7 but not 4 (I repeated it, data shown is from the first time because there was no change). I am going to move onto the gel with #1 as a control.

| Sample | RNA conc ng/ul | RNA amt | RNA Volume (u | H2O Volume | Totoal |
|--------|----------------|---------|---------------|------------|--------|
| 1 | 261.6 | 0.262 | 1.5 | 5.73 | 6.27 |
| 4 | 245.7 | 0.2457 | 1.5 | 6.11 | 5.89 |
| 6 | 145.7 | 0.1457 | 1.5 | 10.30 | 1.70 |
| 7 | 187 | 0.187 | 1.5 | 8.02 | 3.98 |
| | | | | | 12 |

Note: The TAE buffer is foamy while the gel is running.



Interestingly #4 (lane 2) looks pretty good, although it is a little fainter than the rest.

Thursday, February 18, 2021

To Do:

1. Run gel
2. Make cDNA
3. Purify cDNA

Methods and Results:

cDNA Generation:

Since we can't really trust sample #4, I will proceed with cDNA with 2 sets: One will be LVS and Tn7::*rpsU1* from last week, and the other will be LVS, Tn7::*rpsU2*, and Tn7::*rpsU3* from the most recent isolation for a total of 10 samples.

Generate cDNA (half protocol)

REACTION SIZE CUT IN HALF from Lory lab microarray protocol

Combine the first components for primer annealing:

| Component | Volume or Amount | Final Concentration |
|--------------------------------------|------------------|---------------------|
| RNA | 3 ug | 267 - 333 ng/ ul |
| (NS) ₅ Primer (250 ng/ul) | 1.5 ul | 25 ng/ul |
| RNase-free water | up to 15 ul | |

| Sample # | Strain | Date Isolated | RNA conc (ng/ul) | Volume RNA (ul) | Volume H2O (ul) | Total Desired RNA (ug) | Actual RNA (ul) | Total Volume RNA and H2O(ul) | NS5 Volume (ul) | Total Volume (ul) |
|----------|------------------|---------------|------------------|-----------------|-----------------|------------------------|-----------------|------------------------------|-----------------|-------------------|
| 1 | LVS (A-1) | 2/5/21 | 248.6 | 8.05 | 5.45 | 2 | | 13.5 | 1.5 | 15 |
| 2 | LVS (A-2) | 2/5/21 | 182.5 | 10.96 | 2.54 | 2 | | 13.5 | 1.5 | 15 |
| 3 | Tn7::rpsU1 (A-3) | 2/5/21 | 175.9 | 11.37 | 2.13 | 2 | | 13.5 | 1.5 | 15 |
| 4 | Tn7::rpsU1 (A-4) | 2/5/21 | 222.3 | 9.00 | 4.50 | 2 | | 13.5 | 1.5 | 15 |
| 5 | LVS (B-1) | 2/16/21 | 245.7 | 8.14 | 5.36 | 2 | | 13.5 | 1.5 | 15 |
| 6 | LVS (B-2) | 2/16/21 | 258.1 | 7.75 | 5.75 | 2 | | 13.5 | 1.5 | 15 |
| 7 | Tn7::rpsU2 (B-5) | 2/16/21 | 194.2 | 10.30 | 3.20 | 2 | | 13.5 | 1.5 | 15 |
| 8 | Tn7::rpsU2 (B-6) | 2/16/21 | 145.7 | 13.73 | -0.23 | 2 | 13 | 13.5 | 1.5 | 15 |
| 9 | Tn7::rpsU3 (B-7) | 2/16/21 | 187 | 10.70 | 2.80 | 2 | | 13.5 | 1.5 | 15 |
| 10 | Tn7::rpsU3 (B-8) | 2/16/21 | 202.4 | 9.88 | 3.62 | 2 | | 13.5 | 1.5 | 15 |

*NS5 is stored in -80

Incubate using program JSScDNA1:

| Step | Temp | Time |
|------|------|------|
| 1 | 70°C | 10' |
| 2 | 25°C | 10' |
| 3 | 4°C | hold |

Master Mix Table

| Component | Final Concentration | Volume | x 11.5 |
|----------------------------|---------------------|--------|--------|
| 5X 1st strand buffer | 1x | 6 | 69 |
| RNase-free water | | 2.88 | 33.12 |
| 100 mM DTT | 10 mM | 3 | 34.5 |
| 10 mM dNTPs | 0.5 mM | 1.5 | 17.25 |
| Superscript III (200 U/ul) | 10.8 U/ul | 1.63 | 18.75 |

Aliquot 15uL to each tube from first reaction for a total volume of 30uL.

Incubate using program JSScDNA2

| Step | Temp | Time |
|------|------|------|
| 1 | 25°C | 10' |
| 2 | 37°C | 60' |
| 4 | 42°C | 60' |
| 5 | 70°C | 10' |
| 6 | 4°C | hold |

Move samples to -80°C until I can purify them

*I left them on infinite hold overnight

Friday, February 19, 2021

To Do:

1. ~~Make cDNA~~
2. Purify cDNA and check concentration
3. Make cDNA stocks for PCR

Add 10 ul of 1N NaOH

Incubate 65°C for 30'

Neutralize with 10 ul of 1N HCl

Final volume is 50 ul

Purify cDNA using Qiagen PCR clean-up column

Elute in 60 ul of 0.1x EB

***Note I added the NaOH out of the wrong container, not the “RNA only” container.
Remember it’s the large plastic container with the blue top.**

Check concentration by Nanodrop

| # | Sample ID | User name | Date and Time | Nucleic Acid | Unit | A260 | A280 | 260/280 | 260/230 | Sample Type | Factor |
|----|-----------|-----------|----------------------|--------------|-------|-------|-------|---------|---------|-------------|--------|
| 1 | 1 | RI-INBRE | 2/19/2021 3:00:27 PM | 57.3 | ng/μl | 1.147 | 0.589 | 1.95 | 1.85 | DNA | 50 |
| 2 | 2 | RI-INBRE | 2/19/2021 3:01:45 PM | 50.3 | ng/μl | 1.006 | 0.53 | 1.9 | 1.78 | DNA | 50 |
| 3 | 3 | RI-INBRE | 2/19/2021 3:02:41 PM | 49.2 | ng/μl | 0.983 | 0.505 | 1.95 | 2.15 | DNA | 50 |
| 4 | 4 | RI-INBRE | 2/19/2021 3:03:32 PM | 52.1 | ng/μl | 1.042 | 0.531 | 1.96 | 2.35 | DNA | 50 |
| 5 | 5 | RI-INBRE | 2/19/2021 3:04:31 PM | 46.3 | ng/μl | 0.927 | 0.469 | 1.98 | 2.24 | DNA | 50 |
| 6 | 6 | RI-INBRE | 2/19/2021 3:05:27 PM | 59 | ng/μl | 1.179 | 0.592 | 1.99 | 2.35 | DNA | 50 |
| 7 | 7 | RI-INBRE | 2/19/2021 3:06:20 PM | 51.1 | ng/μl | 1.021 | 0.525 | 1.94 | 2.46 | DNA | 50 |
| 8 | 8 | RI-INBRE | 2/19/2021 3:07:12 PM | 61.7 | ng/μl | 1.233 | 0.647 | 1.91 | 2.3 | DNA | 50 |
| 9 | 9 | RI-INBRE | 2/19/2021 3:08:02 PM | 65.9 | ng/μl | 1.318 | 0.696 | 1.89 | 2.52 | DNA | 50 |
| 10 | 10 | RI-INBRE | 2/19/2021 3:08:54 PM | 48.9 | ng/μl | 0.977 | 0.484 | 2.02 | 2.56 | DNA | 50 |

| | | |
|-----------------|----------|------|
| KROL377/KROL378 | FTL_0097 | 1.87 |
| KROL379/KROL380 | FTL_0097 | 1.96 |
| KROL381/KROL382 | FTL_0097 | 1.98 |
| KROL383/KROL384 | FTL_1181 | 2.01 |
| KROL385/KROL386 | FTL_1181 | 2.00 |
| KROL387/KROL388 | FTL_1181 | 1.98 |
| KROL389/KROL390 | FTL_1883 | 2.01 |
| KROL391/KROL392 | FTL_1883 | 2.00 |
| KROL393/KROL394 | FTL_1883 | 1.99 |
| <i>tul4</i> | control | 1.97 |
| <i>tul4_2</i> | control | 1.98 |

Those that are highlighted are the primer sets I will use for the PCR, plus *tul4* as a control

Sunday, February 21, 2021

To Do:

1. Purify cDNA and check concentration
2. Make cDNA stocks for PCR
3. Run qPCR on cDNA
4. Streak strains for RNA isolation

A KROL381/382 (FTL_0097)
 B KROL383/384 (FTL_1181)
 C KROL389/390 (FTL_1883)
 D KROL63/KROL64 (*tul4*)

1 LVS (A)
 2 LVS (A)
 3 Tn7::*rpsU1* (A)
 4 Tn7::*rpsU1* (A)
 5 LVS (B)
 6 LVS (B)
 7 Tn7::*rpsU2* (B)
 8 Tn7::*rpsU2* (B)
 9 Tn7::*rpsU3* (B)

10 Tn7::*rpsU3* (B)

| Plate 1 | | | | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 1-A | 1-A | 1-A | 1-B | 1-B | 1-B | 1-C | 1-C | 1-C | 1-D | 1-D | 1-D |
| B | 2-A | 2-A | 2-A | 2-B | 2-B | 2-B | 2-C | 2-C | 2-C | 2-D | 2-D | 2-D |
| C | 3-A | 3-A | 3-A | 3-B | 3-B | 3-B | 3-C | 3-C | 3-C | 3-D | 3-D | 3-D |
| D | 4-A | 4-A | 4-A | 4-B | 4-B | 4-B | 4-C | 4-C | 4-C | 4-D | 4-D | 4-D |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

| Plate 2 | | | | | | | | | | | | |
|---------|------|------|------|------|------|------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | 5-A | 5-A | 5-A | 5-B | 5-B | 5-B | 5-C | 5-C | 5-C | 5-D | 5-D | 5-D |
| B | 6-A | 6-A | 6-A | 6-B | 6-B | 6-B | 6-C | 6-C | 6-C | 6-D | 6-D | 6-D |
| C | 7-A | 7-A | 7-A | 7-B | 7-B | 7-B | 7-C | 7-C | 7-C | 7-D | 7-D | 7-D |
| D | 8-A | 8-A | 8-A | 8-B | 8-B | 8-B | 8-C | 8-C | 8-C | 8-D | 8-D | 8-D |
| E | 9-A | 9-A | 9-A | 9-B | 9-B | 9-B | 9-C | 9-C | 9-C | 9-D | 9-D | 9-D |
| F | 10-A | 10-A | 10-A | 10-B | 10-B | 10-B | 10-C | 10-C | 10-C | 10-D | 10-D | 10-D |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

| Plate 1 | | | | | | | | | | | | |
|--------------------|--------------------------|-----|-----|--------------------------|-----|-----|--------------------------|-----|-----|------------------------|-----|-----|
| | A KROL381/382 (FTL 0097) | | | B KROL383/384 (FTL 1181) | | | C KROL389/390 (FTL 1883) | | | D KROL63/KROL64 (tul4) | | |
| LVS-A | 1-A | 1-A | 1-A | 1-B | 1-B | 1-B | 1-C | 1-C | 1-C | 1-D | 1-D | 1-D |
| LVS-A | 2-A | 2-A | 2-A | 2-B | 2-B | 2-B | 2-C | 2-C | 2-C | 2-D | 2-D | 2-D |
| Tn7:: <i>rpsU1</i> | 3-A | 3-A | 3-A | 3-B | 3-B | 3-B | 3-C | 3-C | 3-C | 3-D | 3-D | 3-D |
| Tn7:: <i>rpsU1</i> | 4-A | 4-A | 4-A | 4-B | 4-B | 4-B | 4-C | 4-C | 4-C | 4-D | 4-D | 4-D |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |

| Plate 2 | | | | | | | | | | | | |
|--------------------|--------------------------|------|------|--------------------------|------|------|--------------------------|------|------|------------------------|------|------|
| | A KROL381/382 (FTL 0097) | | | B KROL383/384 (FTL 1181) | | | C KROL389/390 (FTL 1883) | | | D KROL63/KROL64 (tul4) | | |
| LVS-B | 5-A | 5-A | 5-A | 5-B | 5-B | 5-B | 5-C | 5-C | 5-C | 5-D | 5-D | 5-D |
| LVS-B | 6-A | 6-A | 6-A | 6-B | 6-B | 6-B | 6-C | 6-C | 6-C | 6-D | 6-D | 6-D |
| Tn7:: <i>rpsU2</i> | 7-A | 7-A | 7-A | 7-B | 7-B | 7-B | 7-C | 7-C | 7-C | 7-D | 7-D | 7-D |
| Tn7:: <i>rpsU2</i> | 8-A | 8-A | 8-A | 8-B | 8-B | 8-B | 8-C | 8-C | 8-C | 8-D | 8-D | 8-D |
| Tn7:: <i>rpsU3</i> | 9-A | 9-A | 9-A | 9-B | 9-B | 9-B | 9-C | 9-C | 9-C | 9-D | 9-D | 9-D |
| Tn7:: <i>rpsU3</i> | 10-A | 10-A | 10-A | 10-B | 10-B | 10-B | 10-C | 10-C | 10-C | 10-D | 10-D | 10-D |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |

10 samples x 4 primer pairs = 40 reactions x triplicate = 120 wells

Master mix for single reaction (20uL): 10uL sybr green, 1uL primer pair (F and R are in same tube), 8uL H₂O (accounting for 1uL DNA in the strip tube)

10 x 3.5 + 3.5 = 38.5uL

10uL sybr green x 38.5 = 385 uL
1uL primer pair x 38.5 = 38.5 uL
8uL H₂O x 38.5 = 308 uL
Total = 731.5 uL

A KROL381/382
B KROL383/384
C KROL389/390
D *tul4* (KROL63/KROL64)

Put 3.5uL DNA in strip tubes and add 66.5uL of Master Mix for a total volume of 70uL.

I am worried that I set up plate 1 wrongly, but I am very confident that I set up plate 2 correctly.

Monday, February 22, 2021

To Do:

1. ~~Run qRT-PCR~~
2. Analyze qPCR

In GSC on the computer:

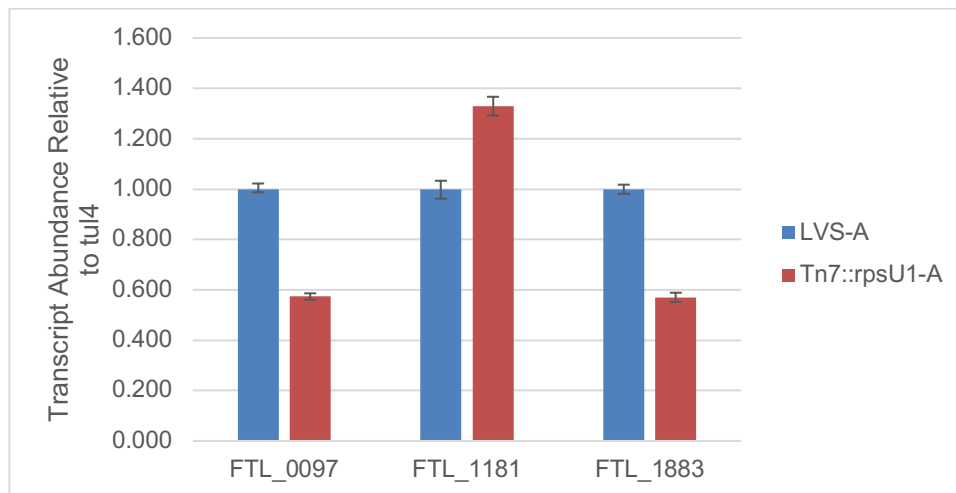
Go into analysis, abs quant, all samples, calculate, save

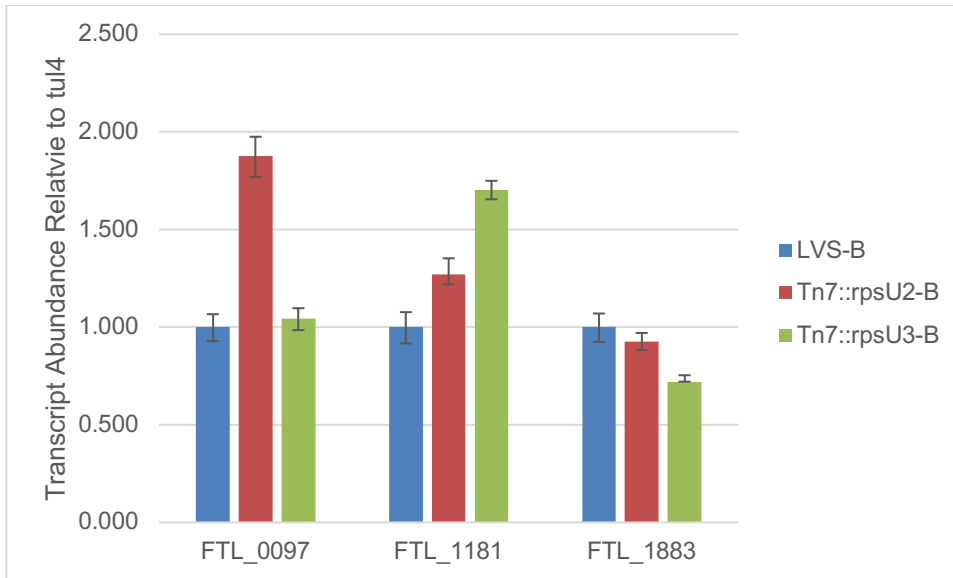
Navigation, export batch results, next, next, select abs quant, select file

Tuesday, February 23, 2021

To Do:

1. Analyze qPCR
2. Patch strains for RNA isolation





Wednesday, February 24, 2021

To Do:

- ~~1. Analyze qPCR~~
- ~~2. Patch strains for RNA isolation~~
- ~~3. Compare qPCR data to mass spec data~~

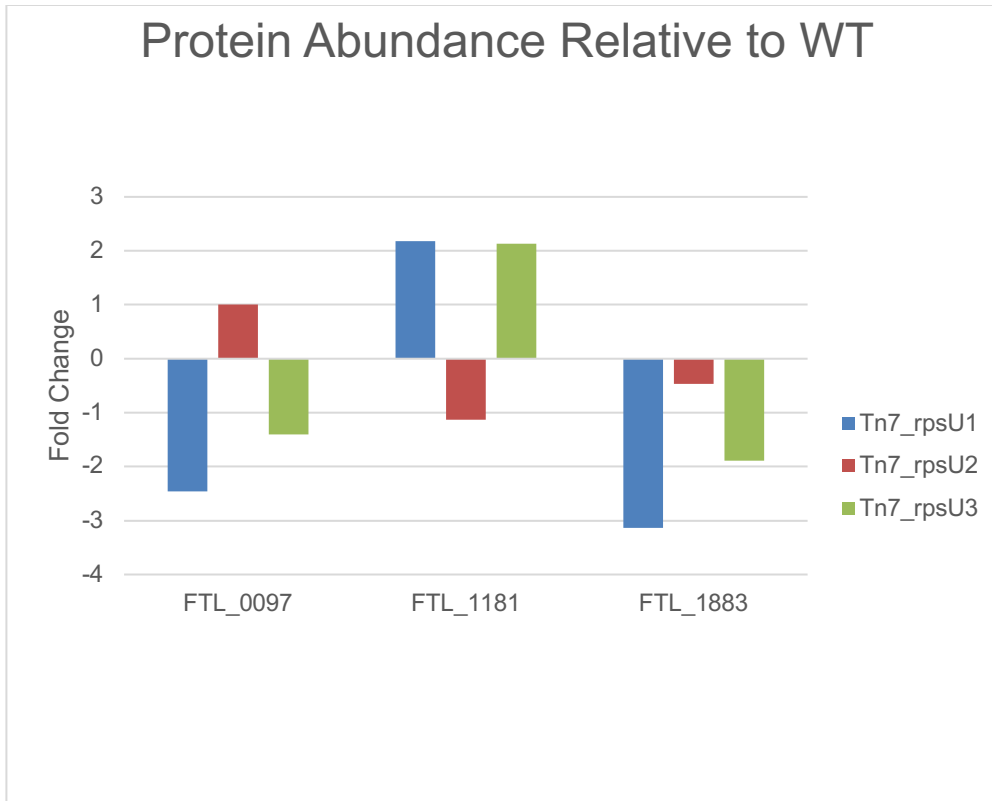
I now have to compare my data to mass spec data. I have to calculate log2 fold change. Fold change is the ratio that measures how quantity changes from initial value to final value. In proteomics it measures change in level of gene expression.

It is calculated as difference between initial value (A) and final value (B) over initial value (A):

$(B-A)/A$ or $B/A-1$

log2 of 2 = 1, log2 of 0.5 = -1

In excel it's =LOG(cell,2)



Some highlights: the FTL_1181 is upregulated in both rpsU1 and 3 at about the same FC, but the level of transcript is higher in rpsU3 than in rpsU1.

FTL_0097 is downregulated in rpsu1 by about 2.5 FC, the level of transcript is just below 0.6 compared to LVS at 1.

Similarly, FTL_1883 is downregulated by 3 FC in rpsU1, but show the same level of transcript as FTL_0097 compared to LVS

Thursday, February 25, 2021

To Do:

4. Fractionator training

Friday, February 26, 2021

To Do:

1. ~~Fractionator training~~
2. Data analysis

Monday, March 1, 2021**To Do:**

1. Data analysis
2. Patch strains

Tuesday, March 2, 2021**To Do:**

1. Data analysis
2. Patch strains
3. RNA culture and isolation

Methods and Results:

| Sample # | Strain |
|----------|------------|
| 1 | LVS WT |
| 2 | LVS WT |
| 3 | LVS WT |
| 4 | Tn7::rpsU1 |
| 5 | Tn7::rpsU1 |
| 6 | Tn7::rpsU1 |
| 7 | Tn7::rpsU2 |
| 8 | Tn7::rpsU2 |
| 9 | Tn7::rpsU2 |
| 10 | Tn7::rpsU3 |
| 11 | Tn7::rpsU3 |
| 12 | Tn7::rpsU3 |

| Sample # | Measured OD600 | Dilution Factor | Actual OD600 | Desired Volume mL | Desired OD | Volume of Resuspended Cells μ L | Resuspension T0 (1100) | OD T2 (1315) | OD T4 (1520) | OD T5 (1620) | Final OD (1700) |
|----------|----------------|-----------------|--------------|-------------------|------------|-------------------------------------|------------------------|--------------|--------------|--------------|-----------------|
| 1 | 0.359 | 10 | 3.59 | 8 | 0.08 | 178 | 0.084 | 0.152 | 0.261 | 0.335 | 0.386 |
| 2 | 0.276 | 10 | 2.76 | 8 | 0.08 | 232 | 0.084 | 0.146 | 0.263 | 0.34 | 0.389 |
| 3 | 0.332 | 10 | 3.32 | 8 | 0.08 | 193 | 0.077 | 0.129 | 0.23 | 0.301 | 0.357 |
| 4 | 0.325 | 10 | 3.25 | 8 | 0.08 | 197 | 0.075 | 0.129 | 0.227 | 0.292 | 0.348 |
| 5 | 0.298 | 10 | 2.98 | 8 | 0.08 | 215 | 0.081 | 0.129 | 0.223 | 0.28 | 0.33 |
| 6 | 0.311 | 10 | 3.11 | 8 | 0.08 | 206 | 0.083 | 0.136 | 0.244 | 0.327 | 0.355 |
| 7 | 0.324 | 10 | 3.24 | 8 | 0.08 | 198 | 0.080 | 0.139 | 0.241 | 0.301 | 0.345 |
| 8 | 0.364 | 10 | 3.64 | 8 | 0.08 | 176 | 0.085 | 0.156 | 0.258 | 0.323 | 0.37 |
| 9 | 0.328 | 10 | 3.28 | 8 | 0.08 | 195 | 0.090 | 0.154 | 0.257 | 0.329 | 0.372 |
| 10 | 0.351 | 10 | 3.51 | 8 | 0.08 | 182 | 0.070 | 0.131 | 0.207 | 0.259 | 0.305 |
| 11 | 0.482 | 10 | 4.82 | 8 | 0.08 | 133 | 0.074 | 0.125 | 0.212 | 0.266 | 0.307 |
| 12 | 0.42 | 10 | 4.2 | 8 | 0.08 | 152 | 0.085 | 0.15 | 0.246 | 0.311 | 0.36 |

Wednesday, March 3, 2021**To Do:**

1. RNA culture and isolation
2. Dnase
3. Run a gel

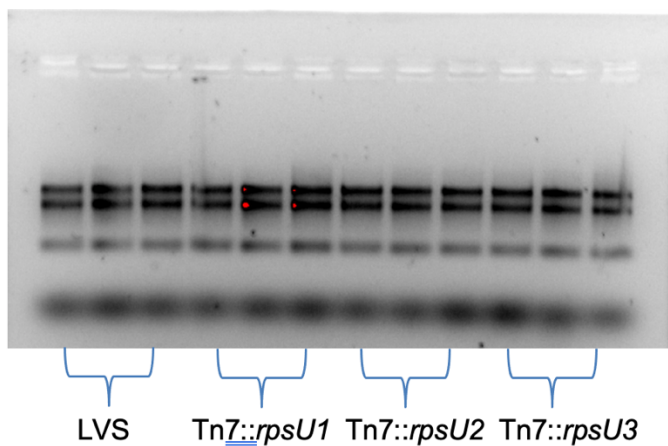
Methods and Results:

| # | Sample ID | User name | Date and Time | Nucleic Acid | Unit | A260 (Abs) | A280 (Abs) | 260/280 | 260/230 | Sample Type | Factor |
|----|-----------|-----------|---------------------|--------------|-------|------------|------------|---------|---------|-------------|--------|
| 1 | 1 | Science | 3/3/2021 1:14:47 PM | 214.2 | ng/ul | 5.355 | 2.523 | 2.12 | 2.47 | RNA | 40 |
| 2 | 2 | Science | 3/3/2021 1:15:54 PM | 223.6 | ng/ul | 5.591 | 2.639 | 2.12 | 2.47 | RNA | 40 |
| 3 | 3 | Science | 3/3/2021 1:16:38 PM | 255.9 | ng/ul | 6.397 | 2.976 | 2.15 | 2.49 | RNA | 40 |
| 4 | 4 | Science | 3/3/2021 1:17:18 PM | 216 | ng/ul | 5.4 | 2.538 | 2.13 | 2.46 | RNA | 40 |
| 5 | 5 | Science | 3/3/2021 1:17:57 PM | 221.6 | ng/ul | 5.539 | 2.586 | 2.14 | 2.48 | RNA | 40 |
| 6 | 6 | Science | 3/3/2021 1:18:33 PM | 181.5 | ng/ul | 4.538 | 2.18 | 2.08 | 2.18 | RNA | 40 |
| 7 | 7 | Science | 3/3/2021 1:19:20 PM | 248.7 | ng/ul | 6.216 | 2.942 | 2.11 | 2.16 | RNA | 40 |
| 8 | 8 | Science | 3/3/2021 1:19:54 PM | 154.3 | ng/ul | 3.857 | 1.816 | 2.12 | 2.39 | RNA | 40 |
| 9 | 9 | Science | 3/3/2021 1:27:31 PM | 277.8 | ng/ul | 6.946 | 3.26 | 2.13 | 2.34 | RNA | 40 |
| 10 | 10 | Science | 3/3/2021 1:22:17 PM | 185.9 | ng/ul | 4.646 | 2.173 | 2.14 | 2.4 | RNA | 40 |
| 11 | 11 | Science | 3/3/2021 1:25:56 PM | 178 | ng/ul | 4.45 | 2.098 | 2.12 | 2.41 | RNA | 40 |
| 12 | 12 | Science | 3/3/2021 1:26:36 PM | 183.1 | ng/ul | 4.577 | 2.185 | 2.1 | 2.27 | RNA | 40 |

Note-use 10ul sybr safe for big gel rig

RNA concentration for gel:

| Sample | RNA conc ng | Desired RNA | Volume RNA | Volume H2O | Total Volume |
|--------|-------------|-------------|------------|------------|--------------|
| 1 | 214.2 | 1.5 | 7.00 | 5.00 | 12 |
| 2 | 223.6 | 1.5 | 6.71 | 5.29 | 12 |
| 3 | 255.9 | 1.5 | 5.86 | 6.14 | 12 |
| 4 | 216 | 1.5 | 6.94 | 5.06 | 12 |
| 5 | 221.6 | 1.5 | 6.77 | 5.23 | 12 |
| 6 | 181.5 | 1.5 | 8.26 | 3.74 | 12 |
| 7 | 248.7 | 1.5 | 6.03 | 5.97 | 12 |
| 8 | 154.3 | 1.5 | 9.72 | 2.28 | 12 |
| 9 | 277.8 | 1.5 | 5.40 | 6.60 | 12 |
| 10 | 185.9 | 1.5 | 8.07 | 3.93 | 12 |
| 11 | 178 | 1.5 | 8.43 | 3.57 | 12 |
| 12 | 183.1 | 1.5 | 8.19 | 3.81 | 12 |



Thursday, March 4, 2021

To Do:

1. ~~Dnase~~
2. ~~Run a gel~~
3. Make and purify cDNA
4. qRT-PCR (if time)

Methods and Results:

cDNA Generation:

Reaction 1:

| Component | Volume or Amount | Final Concentration |
|--------------------------------------|------------------|---------------------|
| RNA | 3 ug | 267 - 333 ng/ ul |
| (NS) ₅ Primer (250 ng/ul) | 1.5 ul | 25 ng/ul |
| RNase-free water | up to 15 ul | |

| Sample # | Strain | Date Isolated | RNA conc (ng/ul) | Volume RNA (ul) | Volume H ₂ O (ul) | Total Desired RNA (ug) | Total Volume RNA and H ₂ O (uL) | NS5 Volume (ul) | Total Volume (ul) |
|----------|--------------|---------------|------------------|-----------------|------------------------------|------------------------|--|-----------------|-------------------|
| 1 | LVS-1 | 3/3/21 | 214.2 | 9.34 | 4.16 | 2 | 13.5 | 1.5 | 15 |
| 2 | LVS-2 | 3/3/21 | 223.6 | 8.94 | 4.56 | 2 | 13.5 | 1.5 | 15 |
| 3 | LVS-3 | 3/3/21 | 255.9 | 7.82 | 5.68 | 2 | 13.5 | 1.5 | 15 |
| 4 | Tn7::rpsU1-1 | 3/3/21 | 216 | 9.26 | 4.24 | 2 | 13.5 | 1.5 | 15 |
| 5 | Tn7::rpsU1-2 | 3/3/21 | 221.6 | 9.03 | 4.47 | 2 | 13.5 | 1.5 | 15 |
| 6 | Tn7::rpsU1-3 | 3/3/21 | 181.5 | 11.02 | 2.48 | 2 | 13.5 | 1.5 | 15 |
| 7 | Tn7::rpsU2-1 | 3/3/21 | 248.7 | 8.04 | 5.46 | 2 | 13.5 | 1.5 | 15 |
| 8 | Tn7::rpsU2-2 | 3/3/21 | 154.3 | 12.96 | 0.54 | 2 | 13.5 | 1.5 | 15 |
| 9 | Tn7::rpsU2-3 | 3/3/21 | 277.8 | 7.20 | 6.30 | 2 | 13.5 | 1.5 | 15 |
| 10 | Tn7::rpsU3-1 | 3/3/21 | 185.9 | 10.76 | 2.74 | 2 | 13.5 | 1.5 | 15 |
| 11 | Tn7::rpsU3-2 | 3/3/21 | 178 | 11.24 | 2.26 | 2 | 13.5 | 1.5 | 15 |
| 12 | Tn7::rpsU3-3 | 3/3/21 | 183.1 | 10.92 | 2.58 | 2 | 13.5 | 1.5 | 15 |

Reaction 2:

Master Mix Table:

| Component | Final Concentration | Volume | x 13.5 |
|----------------------------|---------------------|--------|--------|
| 5X 1st strand buffer | 1x | 6 | 81 |
| RNase-free water | | 2.88 | 38.88 |
| 100 mM DTT | 10 mM | 3 | 40.5 |
| 10 mM dNTPs | 0.5 mM | 1.5 | 20.25 |
| Superscript III (200 U/ul) | 10.8 U/ul | 1.63 | 22.01 |

Aliquot 15uL to each tube from first reaction for a total volume of 30uL

Add 10 ul of 1N NaOH

Incubate 65°C for 30'

Neutralize with 10 ul of 1N HCl

Final volume is 50 ul

Purify cDNA using Qiagen PCR clean-up column (250 ul buffer)

Elute in 60 ul of 0.1x EB

Check concentration by Nanodrop

| # | Sample ID | User name | Date and Time | Nucleic Ac | Unit | A260 (Abs) | A280 (Abs) | 260/280 | 260/230 | Sample Type | Factor |
|----|-----------|-----------|----------------------|------------|-------|------------|------------|---------|---------|-------------|--------|
| 1 | 1 | Science | 3/4/2021 12:20:15 PM | 26.1 | ng/μl | 0.79 | 0.422 | 1.87 | 1.46 | ssDNA | 33 |
| 2 | 2 | Science | 3/4/2021 12:21:02 PM | 24.5 | ng/μl | 0.741 | 0.407 | 1.82 | 1.61 | ssDNA | 33 |
| 3 | 3 | Science | 3/4/2021 12:21:29 PM | 26.2 | ng/μl | 0.794 | 0.475 | 1.67 | 1.38 | ssDNA | 33 |
| 4 | 4 | Science | 3/4/2021 12:21:58 PM | 21.9 | ng/μl | 0.662 | 0.373 | 1.78 | 1.41 | ssDNA | 33 |
| 5 | 5 | Science | 3/4/2021 12:22:37 PM | 20.9 | ng/μl | 0.632 | 0.34 | 1.86 | 1.42 | ssDNA | 33 |
| 6 | 6 | Science | 3/4/2021 12:23:11 PM | 18.2 | ng/μl | 0.553 | 0.292 | 1.89 | 1.33 | ssDNA | 33 |
| 7 | 7 | Science | 3/4/2021 12:23:40 PM | 15.9 | ng/μl | 0.483 | 0.255 | 1.9 | 1.56 | ssDNA | 33 |
| 8 | 8 | Science | 3/4/2021 12:24:03 PM | 13.4 | ng/μl | 0.405 | 0.221 | 1.84 | 1.39 | ssDNA | 33 |
| 9 | 9 | Science | 3/4/2021 12:24:32 PM | 19.8 | ng/μl | 0.6 | 0.32 | 1.88 | 1.59 | ssDNA | 33 |
| 10 | 10 | Science | 3/4/2021 12:24:59 PM | 19.1 | ng/μl | 0.58 | 0.32 | 1.81 | 1.89 | ssDNA | 33 |
| 11 | 11 | Science | 3/4/2021 12:25:26 PM | 18.5 | ng/μl | 0.56 | 0.305 | 1.83 | 1.87 | ssDNA | 33 |
| 12 | 12 | Science | 3/4/2021 12:25:53 PM | 20.1 | ng/μl | 0.61 | 0.321 | 1.9 | 2.23 | ssDNA | 33 |

These are low concentration and have poor absorbance ratios. I made 1.5 stock concentrations just in case.

| Sample # | Strain | cDNA stock c | cDNA new c | Desired Volu | Total Volume | 0.1xEB (ul) |
|----------|------------|--------------|------------|--------------|--------------|-------------|
| 1 | LVS | 26.1 | 1.5 | 2 | 34.8 | 32.8 |
| 2 | LVS | 24.5 | 1.5 | 2 | 32.7 | 30.7 |
| 3 | LVS | 26.2 | 1.5 | 2 | 34.9 | 32.9 |
| 4 | Tn7::rpsU1 | 21.9 | 1.5 | 2 | 29.2 | 27.2 |
| 5 | Tn7::rpsU1 | 20.9 | 1.5 | 2 | 27.9 | 25.9 |
| 6 | Tn7::rpsU1 | 18.2 | 1.5 | 2 | 24.3 | 22.3 |
| 7 | Tn7::rpsU2 | 15.9 | 1.5 | 2 | 21.2 | 19.2 |
| 8 | Tn7::rpsU2 | 13.4 | 1.5 | 2 | 17.9 | 15.9 |
| 9 | Tn7::rpsU2 | 19.8 | 1.5 | 2 | 26.4 | 24.4 |
| 10 | Tn7::rpsU3 | 19.1 | 1.5 | 2 | 25.5 | 23.5 |
| 11 | Tn7::rpsU3 | 18.5 | 1.5 | 2 | 24.7 | 22.7 |
| 12 | Tn7::rpsU3 | 20.1 | 1.5 | 2 | 26.8 | 24.8 |

After consulting with Kathryn, I am going to try to repurify these.

Monday, March 8, 2021

To Do:

1. Repurify cDNA

Methods and Results:

To repurify the cDNA, I added 5 PB buffer and spun, and then washed with 750 PE buffer ul 2X and then spun for 4 minutes. I eluted in 35 ul 0.1XEB, let sit for 1 minute, spun for 1 minute, put elution back on the column, let sit for 1 minute, and spun again for 1 minute.

Nanodrop:

| # | Sample ID | User name | Date and Time | Nucleic Acid (Unit) | A260 | A280 | 260/280 | 260/230 | Sample | Factor |
|----|-----------|-----------|----------------------|---------------------|------|------|---------|---------|--------|--------|
| 1 | 1 | RI-INBRE | 3/8/2021 10:14:35 AM | 8.9 ng/μl | 0.27 | 0.15 | 1.83 | 1.74 | ssDNA | 33 |
| 2 | 2 | RI-INBRE | 3/8/2021 10:15:42 AM | 11.5 ng/μl | 0.35 | 0.19 | 1.81 | 1.11 | ssDNA | 33 |
| 3 | 3 | RI-INBRE | 3/8/2021 10:16:40 AM | 10.4 ng/μl | 0.32 | 0.2 | 1.6 | 1.03 | ssDNA | 33 |
| 4 | 4 | RI-INBRE | 3/8/2021 10:17:27 AM | 13.2 ng/μl | 0.4 | 0.23 | 1.75 | 1.13 | ssDNA | 33 |
| 5 | 5 | RI-INBRE | 3/8/2021 10:18:00 AM | 17.5 ng/μl | 0.53 | 0.31 | 1.73 | 0.89 | ssDNA | 33 |
| 6 | 6 | RI-INBRE | 3/8/2021 10:18:32 AM | 12.1 ng/μl | 0.37 | 0.21 | 1.72 | 1.21 | ssDNA | 33 |
| 7 | 7 | RI-INBRE | 3/8/2021 10:19:19 AM | 10.1 ng/μl | 0.31 | 0.17 | 1.81 | 1.61 | ssDNA | 33 |
| 8 | 8 | RI-INBRE | 3/8/2021 10:19:50 AM | 14.7 ng/μl | 0.45 | 0.26 | 1.72 | 0.92 | ssDNA | 33 |
| 9 | 9 | RI-INBRE | 3/8/2021 10:20:17 AM | 9.6 ng/μl | 0.29 | 0.16 | 1.77 | 1.4 | ssDNA | 33 |
| 10 | 10 | RI-INBRE | 3/8/2021 10:20:48 AM | 59.5 ng/μl | 1.8 | 5.91 | 0.31 | 5.41 | ssDNA | 33 |
| 11 | 11 | RI-INBRE | 3/8/2021 10:21:40 AM | 32 ng/μl | 0.97 | 1.04 | 0.94 | -1.09 | ssDNA | 33 |
| 12 | 12 | RI-INBRE | 3/8/2021 10:22:15 AM | 12.9 ng/μl | 0.39 | 0.23 | 1.7 | 1.24 | ssDNA | 33 |

These are even worse. I will go back to RNA to generate new cDNA.

cDNA Generation

Reaction 1:

| Component | Volume or Amount | Final Concentration |
|--------------------------------------|------------------|---------------------|
| RNA | 3 ug | 267 - 333 ng/ul |
| (NS) ₅ Primer (250 ng/ul) | 1.5 ul | 25 ng/ul |
| RNase-free water | up to 15 ul | |

| Sample # | Strain | Date Isolated | RNA conc (ng/ul) | Volume RNA (ul) | Volume H2O (ul) | Total Desired RNA (ug) | Total Volume RNA and H2O (ul) | NS5 Volume (ul) | Total Volume (ul) |
|----------|--------------|---------------|------------------|-----------------|-----------------|------------------------|-------------------------------|-----------------|-------------------|
| 1 | LVS-1 | 3/3/21 | 214.2 | 9.34 | 4.16 | 2 | 13.5 | 1.5 | 15 |
| 2 | LVS-2 | 3/3/21 | 223.6 | 8.94 | 4.56 | 2 | 13.5 | 1.5 | 15 |
| 3 | LVS-3 | 3/3/21 | 255.9 | 7.82 | 5.68 | 2 | 13.5 | 1.5 | 15 |
| 4 | Tn7::rpsU1-1 | 3/3/21 | 216 | 9.26 | 4.24 | 2 | 13.5 | 1.5 | 15 |
| 5 | Tn7::rpsU1-2 | 3/3/21 | 221.6 | 9.03 | 4.47 | 2 | 13.5 | 1.5 | 15 |
| 6 | Tn7::rpsU1-3 | 3/3/21 | 181.5 | 11.02 | 2.48 | 2 | 13.5 | 1.5 | 15 |
| 7 | Tn7::rpsU2-1 | 3/3/21 | 248.7 | 8.04 | 5.46 | 2 | 13.5 | 1.5 | 15 |
| 8 | Tn7::rpsU2-2 | 3/3/21 | 154.3 | 12.96 | 0.54 | 2 | 13.5 | 1.5 | 15 |
| 9 | Tn7::rpsU2-3 | 3/3/21 | 277.8 | 7.20 | 6.30 | 2 | 13.5 | 1.5 | 15 |
| 10 | Tn7::rpsU3-1 | 3/3/21 | 185.9 | 10.76 | 2.74 | 2 | 13.5 | 1.5 | 15 |
| 11 | Tn7::rpsU3-2 | 3/3/21 | 178 | 11.24 | 2.26 | 2 | 13.5 | 1.5 | 15 |
| 12 | Tn7::rpsU3-3 | 3/3/21 | 183.1 | 10.92 | 2.58 | 2 | 13.5 | 1.5 | 15 |

Reaction 2:

Master Mix Table:

| Component | Final Concentration | Volume | x 13.5 |
|-----------|---------------------|--------|--------|
|-----------|---------------------|--------|--------|

| | | | |
|----------------------------|-----------|------|-------|
| 5X 1st strand buffer | 1x | 6 | 81 |
| RNase-free water | | 2.88 | 38.88 |
| 100 mM DTT | 10 mM | 3 | 40.5 |
| 10 mM dNTPs | 0.5 mM | 1.5 | 20.25 |
| Superscript III (200 U/ul) | 10.8 U/ul | 1.63 | 22.01 |

Aliquot 15uL to each tube from first reaction for a total volume of 30uL
Purify tomorrow -> didn't work

Wednesday, March 10, 2021

To Do:

1. Repurify cDNA
2. Make test run cDNA from new and old

| Sample # | Strain | Date Isolated | RNA conc (ng/ul) | Volume RNA (ul) | Volume H2O (ul) | Total Desired RNA (ug) | Total Volume RNA and H2O (uL) | NS5 Volume (ul) | Total Volume (ul) |
|----------|--------------|---------------|------------------|-----------------|-----------------|------------------------|-------------------------------|-----------------|-------------------|
| 1 | LVS (A-1) | 2/5/21 | 248.6 | 8.05 | 5.45 | 2 | 13.5 | 1.5 | 15 |
| 2 | LVS (B-2) | 2/16/21 | 258.1 | 7.75 | 5.75 | 2 | 13.5 | 1.5 | 15 |
| 3 | LVS-3 | 3/3/21 | 255.9 | 7.82 | 5.68 | 2 | 13.5 | 1.5 | 15 |
| 4 | Tn7::rpsU2-3 | 3/3/21 | 277.8 | 7.20 | 6.30 | 2 | 13.5 | 1.5 | 15 |

Thursday, March 11, 2021

To Do:

1. ~~Make test run cDNA from new and old~~
2. Check concentrations

Methods and Results:

| # | Sample ID | User name | Date and Time | Nucleic Acid | Unit | A260 (Abs) | A280 (Abs) | 260/280 | 260/230 | Sample Type | Factor |
|---|-----------|-----------|-----------------------|--------------|-------|------------|------------|---------|---------|-------------|--------|
| 1 | 1 | Science | 3/11/2021 11:56:52 AM | 17.1 | ng/ul | 0.517 | 0.293 | 1.76 | 1.85 | ssDNA | 33 |
| 2 | 2 | Science | 3/11/2021 11:57:29 AM | 14.8 | ng/ul | 0.448 | 0.246 | 1.82 | 1.75 | ssDNA | 33 |
| 3 | 3 | Science | 3/11/2021 11:57:57 AM | 14.6 | ng/ul | 0.443 | 0.251 | 1.77 | 1.71 | ssDNA | 33 |
| 4 | 4 | Science | 3/11/2021 11:58:26 AM | 14.4 | ng/ul | 0.435 | 0.253 | 1.72 | 1.44 | ssDNA | 33 |

Given that all of the samples have similar low concentrations and poor ratios I can conclude that my RNA samples are fine and that there is a problem with either the cDNA prep or the purification.

I'm going to purify a PCR product that is not cDNA to investigate the purification step

Friday, March 12, 2021

To Do:

1. ~~Check concentrations~~
2. Purify old PCR product

Methods and Results:

I used an old PCR product (from 9/23) of mine to check if it is the purification step that is causing the issue with cDNA. The purification worked fine.

| # | Sample ID | User name | Date and Time | Nucleic Acid | Unit | A260 (Abs) | A280 (Abs) | 260/280 | 260/230 | Sample Type | Factor |
|---|-----------------------------|-----------|----------------------|--------------|-------|------------|------------|---------|---------|-------------|--------|
| 1 | PCR prod before purificatio | Science | 3/12/2021 8:24:53 AM | 219.4 | ng/μl | 4.388 | 2.348 | 1.87 | 2.36 | DNA | 50 |
| 1 | PCR prod after purification | Science | 3/12/2021 8:44:47 AM | 21.6 | ng/μl | 0.432 | 0.228 | 1.89 | 2.53 | DNA | 50 |

Monday, March 15, 2021

To Do:

1. ~~Purify old PCR product~~
2. Streak LVS to isolation
3. Start making buffer for sucrose gradient

Tuesday, March 16, 2021

To Do:

1. ~~Streak LVS to isolation~~
2. Start making buffer for sucrose gradient
3. Re-test cDNA prep with new reagents
4. Make overnight culture for sucrose gradient

Wednesday, March 17, 2021

To Do:

1. ~~Start making buffer for sucrose gradient~~
2. ~~Re-test cDNA prep with new reagents~~
3. ~~Make overnight culture for sucrose gradient~~
4. Start gradient protocol

Methods and Results:

Overnight Culture OD:

Ribosome/subunits preparation of *T. thermophilus*

1. Streak out *T. thermophilus* onto a TEM (ATCC Thermus Enhanced Medium) plate and incubate at 65 °C.
2. Start a 10 ml TEM culture from a single colony and shake overnight at 65 °C.
3. Dilute overnight culture 1:100 in 70 ml TEM and aerate at 65 °C to mid-log to late-log phase.
4. Let cells cool to room temperature on bench. (for subunits) (For polysomes make sure you quick cool)
5. Pellet in centrifuge at 10,000 RPM for 10 mins (camberg lab)*.
6. Remove supernatant & resuspend pellet in 3 ml wash buffer** without lysozyme:

| | |
|---------------------------|------------------------------|
| 25 mM Hepes pH 7.6 | 1 ml 1M Hepes pH 7.6 |
| 0.6 mM MgCl ₂ | 24 uL 1M MgCl ₂ |
| 100 mM NH ₄ Cl | 0.8 ml 5M NH ₄ Cl |
| 20 % sucrose | 8 g |
| | H ₂ O to 40 ml |

7. Pellet in centrifuge at 10,000 rpm for 10 mins. (camberg lab)*
8. Remove supernatant and resuspend in 300 µl lysozyme solution (wash buffer plus 200 µg/ml lysozyme).
9. Transfer to 1.5 ml Eppendorf tube and freeze-thaw five times in dry-ice and warm water.
10. Add, on ice, 0.8 ml detergent cocktail, made fresh. (again, low mg for subunits be sure to adjust for other uses)

| | |
|---------------------------|------------------------------|
| 25 mM Hepes pH 7.6 | 0.25 ml 1M Hepes pH 7.6 |
| 0.6 mM MgCl ₂ | 6 uL 1M MgCl ₂ |
| 100 mM NH ₄ Cl | 0.2 ml 5M NH ₄ Cl |
| 0.6% Brij 58 | 1 ml Brij 58 (6 %) |
| 0.2% deoxycholate | 20 mg deoxycholate |
| 20 µl | Worthington DNase I 1 mg/ml |
| | H ₂ O to 10 ml |

11. Incubate 30 minutes on ice.
12. Spin at max speed for 10 minutes. Transfer supernatant to 1.5 ml microfuge tube, take A260.
 - a. Dilute sample 1:100 in water and take A260.

Solutions to prepare

1. Ribosome buffer (25mM Hepes pH 7.6, 0.6mM MgCl₂, 100mM Nh₄Cl) *** make sure at the end any dilutions are done in ribosome buffer and not wash buffer because of the sucrose***

- To a sterile 50mL conical tube, add 1 ml of 1M Hepes pH 7.6, 24µl 1M MgCl₂, 800µl 5M NH₄Cl.
 - Q.s to 40mL
1. Wash buffer (25mM Hepes pH 7.6, 10mM MgCl₂, 100mM NH₄Cl, 10% sucrose)
 - Add 20% sucrose to the wash buffer**.

*Camberb Lab is referring to a 4°C centrifuge

**Wash buffer only contains 20% sucrose for the first spin. Don't use sucrose with buffer for the rest of the protocol.

The above protocol is from the Gregory Lab and was written for *T. thermophilus*. Since *Francisella* has different growth requirements, Steps 1-3 had to be altered to accommodate it. I set up overnight cultures in 70ml MHB in baffled flasks and incubated them in the shaking incubator at 37°C

| Sample # | Measured OD600 | Dilution Factor | Actual OD600 | Desired Volume mL | Desired OD | Volume of Resuspended Cells µL | OD After Resuspension T0 1700 | OD Next Day 1000 |
|----------|----------------|-----------------|--------------|-------------------|------------|--------------------------------|-------------------------------|------------------|
| 1 | 0.553 | 10 | 5.53 | 70 | 0.005 | 63 | 0.003 | 0.346 |
| 2 | 0.504 | 10 | 5.04 | 70 | 0.005 | 69 | 0.005 | 0.473 |

cDNA:

Samples: 1. LVS A-1 2. LVS B-2 3. LVS B-1

| # | Sample ID | User name | Date and Time | Nucleic Acid | Unit | A260 (Abs) | A280 (Abs) | 260/280 | 260/230 | Sample Type | Factor |
|---|-----------|-----------|----------------------|--------------|-------|------------|------------|---------|---------|-------------|--------|
| 1 | 1 | Science | 3/18/2021 8:29:07 AM | 6.4 | ng/µl | 0.195 | 0.103 | 1.89 | 1.72 | ssDNA | 33 |
| 2 | 2 | Science | 3/18/2021 8:30:48 AM | 12.2 | ng/µl | 0.371 | 0.188 | 1.98 | 1.98 | ssDNA | 33 |
| 3 | 3 | Science | 3/18/2021 8:31:59 AM | 5.7 | ng/µl | 0.172 | 0.078 | 2.2 | 1.89 | ssDNA | 33 |

Thursday, March 18, 2021

To Do:

- ~~1. Start making buffer for sucrose gradient~~
- ~~2. Re-test cDNA prep with new reagents~~
- ~~3. Make overnight culture for sucrose gradient~~
- ~~4. Prepare cell lysates~~
5. Make sure I have all reagents necessary for cell lysate

Methods and Results:

Cell lysates:

There were some things that went wrong. I precipitated the detergent, and I forgot the DNase. I will prepare another set. Also, the cold centrifuge in INBRE was in use so the samples were incubated on ice for longer than 30 minutes.

Friday, March 19, 2021

To Do:

1. ~~Start making buffer for sucrose gradient~~
2. ~~Re test cDNA prep with new reagents~~

Saturday, March 20, 2021

To Do:

4. Patch LVS

Sunday, March 21, 2021

To Do:

1. ~~Patch LVS~~
2. Make overnight cultures

| Sample # | Measured OD600 | Dilution Factor | Actual OD600 | Desired Volume mL | Desired OD | Volume of Resuspended Cells μ L | OD After Resuspension T0 1730 |
|----------|----------------|-----------------|--------------|-------------------|------------|-------------------------------------|-------------------------------|
| 1 | 0.446 | 10 | 4.46 | 70 | 0.005 | 78 | 0.004 |
| 2 | 0.572 | 10 | 5.72 | 70 | 0.005 | 61 | 0.003 |

Monday, March 22, 2021

To Do:

1. ~~Make overnight cultures~~
2. Prepare cell lysates

I checked the OD at a little after 10am, they are a little over 0.2. I will give it another 45 minutes.

| Sample # | Measured OD600 | Dilution Factor | Actual OD600 | Desired Volume mL | Desired OD | Volume of Resuspended Cells μ L | OD After Resuspension T0 1730 | OD Next Day 1145 |
|----------|----------------|-----------------|--------------|-------------------|------------|-------------------------------------|-------------------------------|------------------|
| 1 | 0.446 | 10 | 4.46 | 70 | 0.005 | 78 | 0.004 | 0.311 |
| 2 | 0.572 | 10 | 5.72 | 70 | 0.005 | 61 | 0.003 | 0.334 |

I made wash buffer with 20% sucrose by adding 4g sucrose and filling up to 20mL with Hepes buffer. I made 200 ug/ml lysozyme buffer by making a 10 mg/ml solution in Hepes buffer and then further diluting it by adding 20ul of that solution to 1ml of buffer.

| # | Sample ID | User name | Date and Time | Nucleic Acid | Unit | A260 (Abs) | A280 (Abs) | 260/280 | 260/230 | Sample Type | Factor |
|---|------------------|-----------|----------------------|--------------|-------|------------|------------|---------|---------|-------------|--------|
| 1 | 1 3_22 undiluted | Science | 3/22/2021 2:09:49 PM | 206.9 | ng/μl | 5.172 | 2.241 | 2.31 | 2.04 | RNA | 40 |
| 2 | 2 3_22 undiluted | Science | 3/22/2021 2:11:50 PM | 437.8 | ng/μl | 10.945 | 5.328 | 2.05 | 1.36 | RNA | 40 |
| 3 | 1 3_22 1:10 | Science | 3/22/2021 2:13:08 PM | -56.5 | ng/μl | -1.412 | -1.541 | 0.92 | 0.27 | RNA | 40 |
| 4 | 2 3_22 1:10 | Science | 3/22/2021 2:14:51 PM | 94 | ng/μl | 2.349 | 1.77 | 1.33 | 0.55 | RNA | 40 |
| 5 | 1 3_17 undiluted | Science | 3/22/2021 2:15:37 PM | 230.4 | ng/μl | 5.761 | 2.459 | 2.34 | 1.47 | RNA | 40 |
| 6 | 2 3_17 undiluted | Science | 3/22/2021 2:16:10 PM | 349.8 | ng/μl | 8.745 | 3.993 | 2.19 | 1.25 | RNA | 40 |
| 7 | 1 3_17 1:10 | Science | 3/22/2021 2:16:50 PM | 75.5 | ng/μl | 1.886 | 0.752 | 2.51 | 14.48 | RNA | 40 |
| 8 | 2 3_17 1:10 | Science | 3/22/2021 2:17:25 PM | 91.4 | ng/μl | 2.285 | 1.07 | 2.14 | 2.82 | RNA | 40 |

Tuesday, March 23, 2021

To Do:

1. Prepare cell lysates
2. Run gradients

Methods and Results:

I re-diluted yesterday's sample #1 1:10 and 1:100 since I got a negative value for the A260.

| # | Sample ID | User name | Date and Time | Nucleic Acid | Unit | A260 (Abs) | A280 (Abs) | 260/280 | 260/230 | Sample Type | Factor |
|---|------------|-----------|----------------------|--------------|-------|------------|------------|---------|---------|-------------|--------|
| 1 | Undiluted | Science | 3/23/2021 7:45:11 AM | 1119.4 | ng/μl | 27.984 | 15.188 | 1.84 | 0.99 | RNA | 40 |
| 2 | one to ten | Science | 3/23/2021 7:45:57 AM | 189.8 | ng/μl | 4.744 | 4.794 | 0.99 | 0.33 | RNA | 40 |
| 3 | one to 100 | Science | 3/23/2021 7:46:39 AM | 21.8 | ng/μl | 0.544 | 0.525 | 1.04 | 0.32 | RNA | 40 |

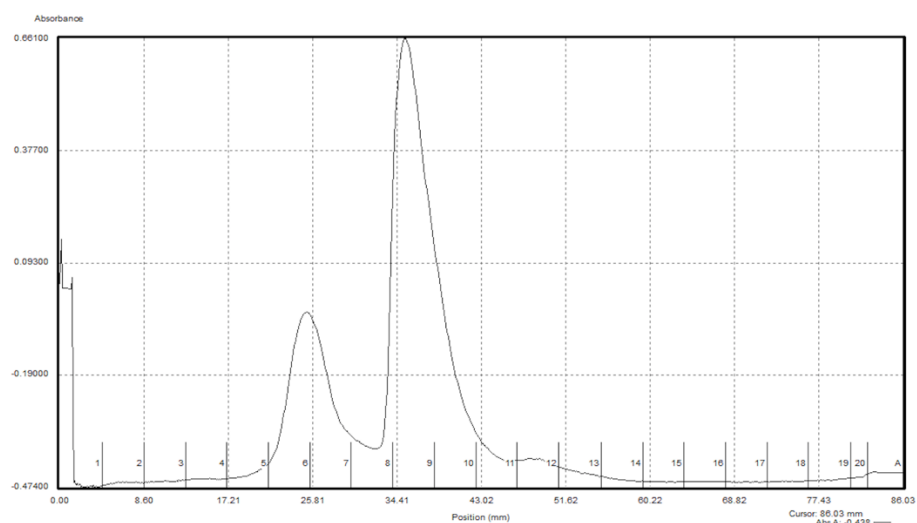
Sample Calculations for Gradient:

| Sample Nam | Tube # | A260 | DF (10) | Divided by 1 | C1 (ug/ml) | V2 (ul) | C2 (ug/ml) | V1 (ul) | Vol buffer (ul) | Total Volume |
|------------|--------|------|---------|--------------|------------|---------|------------|---------|-----------------|--------------|
| 3_22-1 | 5 | 4.74 | 47.4 | 3.27 | 3269 | 205 | 600 | 37.6 | 167.4 | 205 |
| 3_22-2 | 4 | 2.34 | 23.4 | 1.61 | 1614 | 205 | 600 | 76.2 | 128.8 | 205 |
| 3_17-1 | 6 | 1.89 | 18.9 | 1.30 | 1303 | 205 | 600 | 94.4 | 110.6 | 205 |
| 3_17-2 | 3 | 2.29 | 22.9 | 1.58 | 1579 | 205 | 600 | 77.9 | 127.1 | 205 |

Gradients:

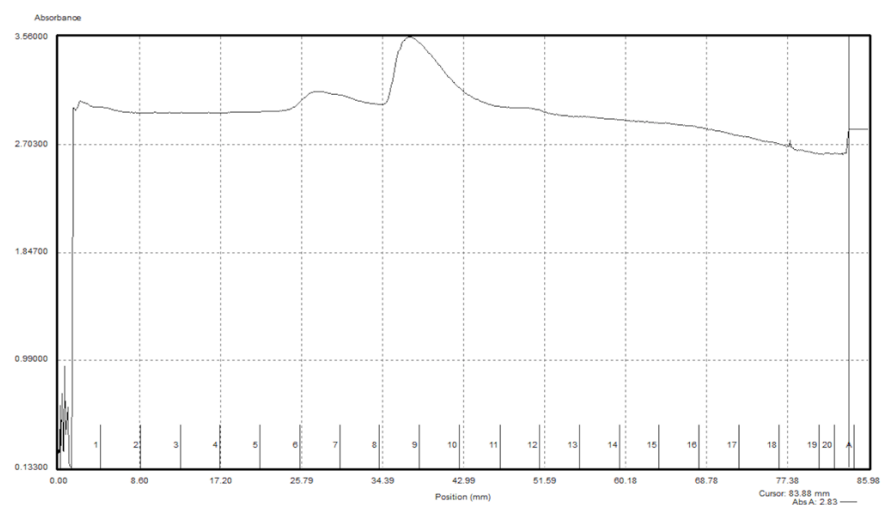
Tube 5kb

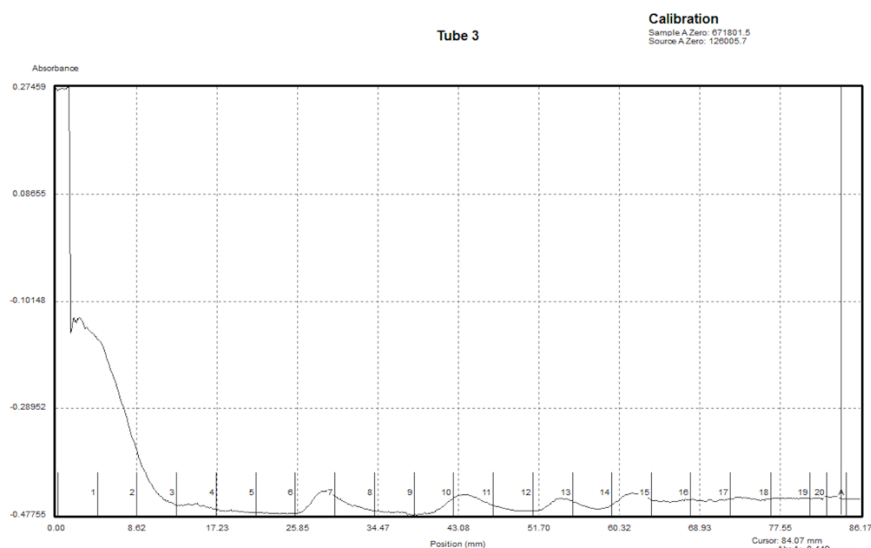
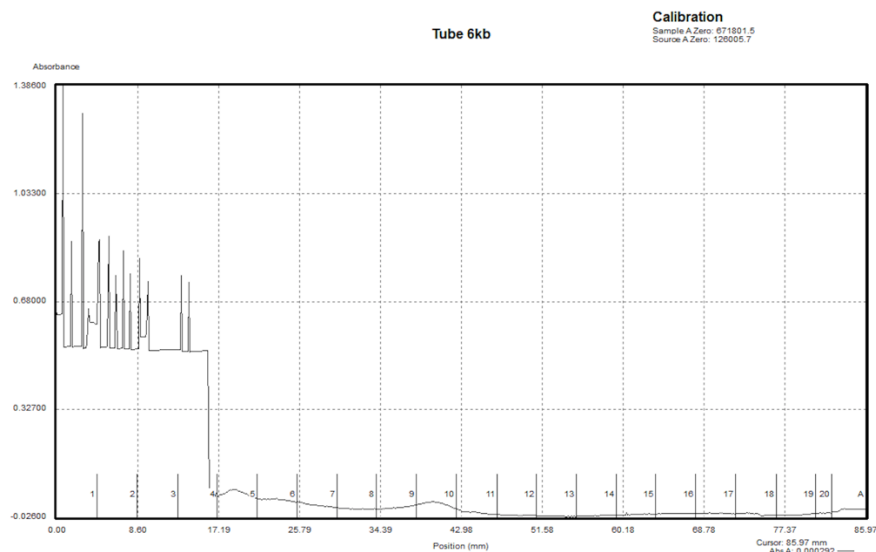
Calibration
Sample A Zero: 671851.5
Source A Zero: 128055.7



Tube 4

Calibration
Sample A Zero: 671851.5
Source A Zero: 128055.7





I did not expect tubes 3 and 6 to be successful because those were from the first cell lysates I made. I am happy to see that tube 5 worked very well, but unfortunately tube 4 doesn't look good because that was the first sample I put on the fractionator and there were some adjustments that needed to be made, such as moving the collection tubes. When I went to start the run again the dispenser didn't start at the right tube, so I'm not

Wednesday, March 24, 2021

To Do:

1. ~~Run gradients~~
2. Test qRT-PCR

Methods and Results:

The latest cDNA I made I never made into 1.5ug/ul stock concentrations:

| Sample # | Strain | cDNA stock | cDNA new | Total Desired | Volume cDNA | 0.1xE B |
|----------|---------|------------|----------|---------------|-------------|---------|
| 1 | LVS A-1 | 6.4 | 1.5 | 3 | 12.8 | 9.8 |
| 2 | LVS B-2 | 12.2 | 1.5 | 3 | 24.4 | 21.4 |
| 3 | LVS B-1 | 5.7 | 1.5 | 3 | 11.4 | 8.4 |

q-RT-PCR:

| Gene | Primers | Sample | Strain |
|-------------|---------------|--------|-------------|
| FTL_1181 | A KROL383/384 | 1 | LVS A-1(G) |
| <i>tul4</i> | B KROL63/64 | 2 | LVS B-2 (G) |
| | | 3 | LVS B-1 (G) |
| | | 4 | LVS A-1 (B) |
| | | 5 | LVS B-2 (B) |
| | | 6 | LVS B-1 (B) |

6 samples x 2 primer pairs = 12 reactions x triplicate = 36 wells

Master mix for single reaction (20uL): 10uL sybr green, 1uL primer pair (F and R are in same tube), 8uL H₂O (accounting for 1uL DNA in the strip tube)

$$6 \times 3,5 + 3,5 = 24,5 \text{ uL}$$

$$10\mu\text{L sybr green} \times 24.5 = 245 \mu\text{L}$$

$$1\mu\text{L primer pair} \times 24.5 = 24.5 \mu\text{L}$$

$$8\mu\text{L H}_2\text{O} \times 24.5 = 196 \mu\text{L}$$

Total = 465.5 uL

Put 3.5uL DNA in strip tubes and add 66.5uL of Master Mix for a total volume of 70uL.

[illegible]

Thursday, March 25, 2021**To Do:**

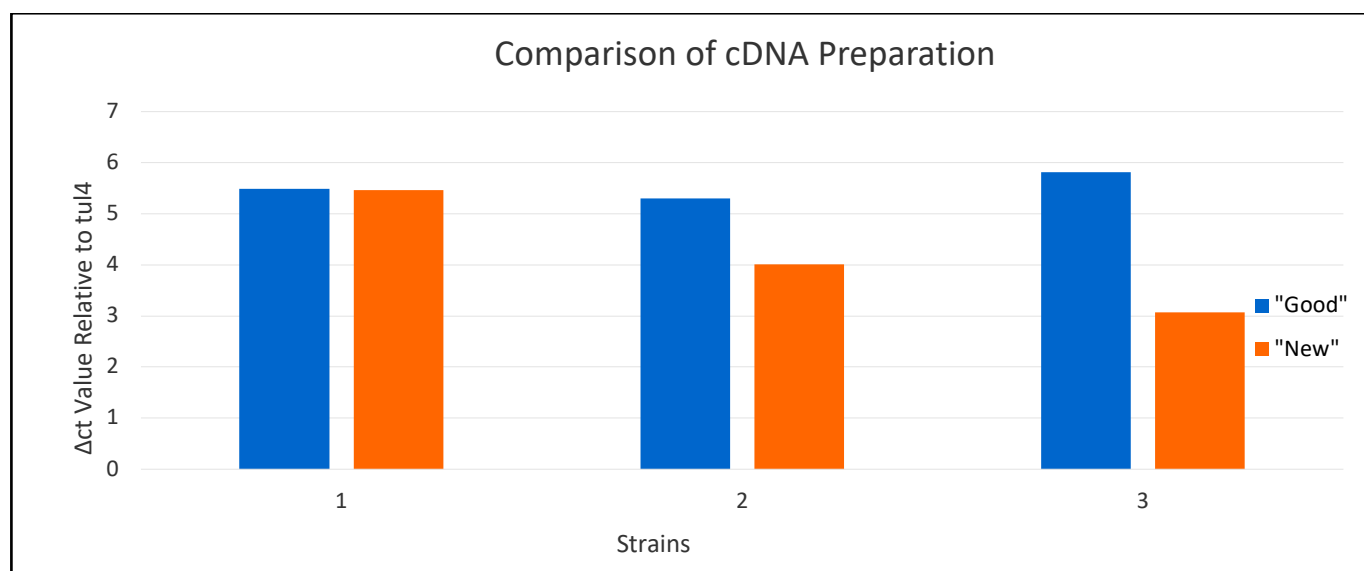
1. ~~Test qRT-PCR~~
2. Analyze qPCR

Friday, March 26, 2021**To Do:**

1. Analyze qPCR
2. Start WGS project

Sunday, March 28, 2021**To Do:**

1. Analyze qPCR
2. Start WGS project
3. Patch Tn7::rpsU2 and 3

Results:

With the exception of the first sample, there is a difference in abundance of LVS of the “Good” strain relative to *tu/4* compared to the “Bad (New)” strain. Further analysis will have to be done to determine why this is the case, but one avenue we’re looking into is reviewing the cDNA protocol. Kathryn mentioned that both she and Hannah would every now and then run into a sample that would show unexpected results, but they were considered outliers; now it seems to be approaching a systemic problem.

Monday, March 29, 2021

To Do:

1. ~~Analyze qPCR~~
2. ~~Start WGS project~~
3. ~~Patch Tn7::rpsU2 and 3~~
4. Set up overnight cultures

I’m concerned that I didn’t set up the qPCR correctly because I only set each sample up one time. Therefore, in my analysis there’s nothing to average.

I started the WGS project by setting up the genomes in SnapGene for the deletion strains.

I set up overnight cultures of Tn7::rpsU2 and Tn7::rpsU3. I will prepare the lysates tomorrow using the standard lysis method, then prepare another set of lysates using the bead-beating method to emulate what we will do when we get Bat’s samples.

| Sample # | Strain | Measured OD600 | Dilution Factor | Actual OD600 | Desired Volume mL | Desired OD | Volume of Resuspended Cells μ L | Starting OD T0 (1700) | Final OD (1100) |
|----------|------------|----------------|-----------------|--------------|-------------------|------------|-------------------------------------|-----------------------|-----------------|
| 1 | Tn7::rpsU2 | 0.515 | 10 | 5.15 | 70 | 0.005 | 68 | 0.003 | 0.337 |
| 2 | Tn7::rpsU3 | 0.374 | 10 | 3.74 | 70 | 0.005 | 94 | 0.005 | 0.307 |

| # | Sample ID | User name | Date and Time | Nucleic Acid | Unit | A260 (Abs) | A280 (Abs) | 260/280 | 260/230 | Sample Type | Factor |
|---|-----------|-----------|----------------------|--------------|-------------|------------|------------|---------|---------|-------------|--------|
| 1 | 1 | Science | 3/30/2021 1:07:07 PM | 6.6 | ng/ μ l | 0.166 | 0.077 | 2.15 | 0.82 | RNA | 40 |
| 2 | 1 u | Science | 3/30/2021 1:08:08 PM | 92.7 | ng/ μ l | 2.317 | 1.298 | 1.79 | 0.73 | RNA | 40 |
| 3 | 2 | Science | 3/30/2021 1:08:49 PM | 7.9 | ng/ μ l | 0.199 | 0.091 | 2.17 | 0.8 | RNA | 40 |
| 4 | 2 u | Science | 3/30/2021 1:09:26 PM | 79 | ng/ μ l | 1.975 | 1.064 | 1.86 | 0.74 | RNA | 40 |

