Date: Week 1 - June 3-7
Brief Summary of What I did this time period: Transformation of BL21 and homemade DH5alpha cells. Comparison expression of MtDalaDala using IPTG and Autoinduction media.
Pellet weight:
Autoinduction (0.5L) - **5.68g**
IPTG (1L)- **4.83g**

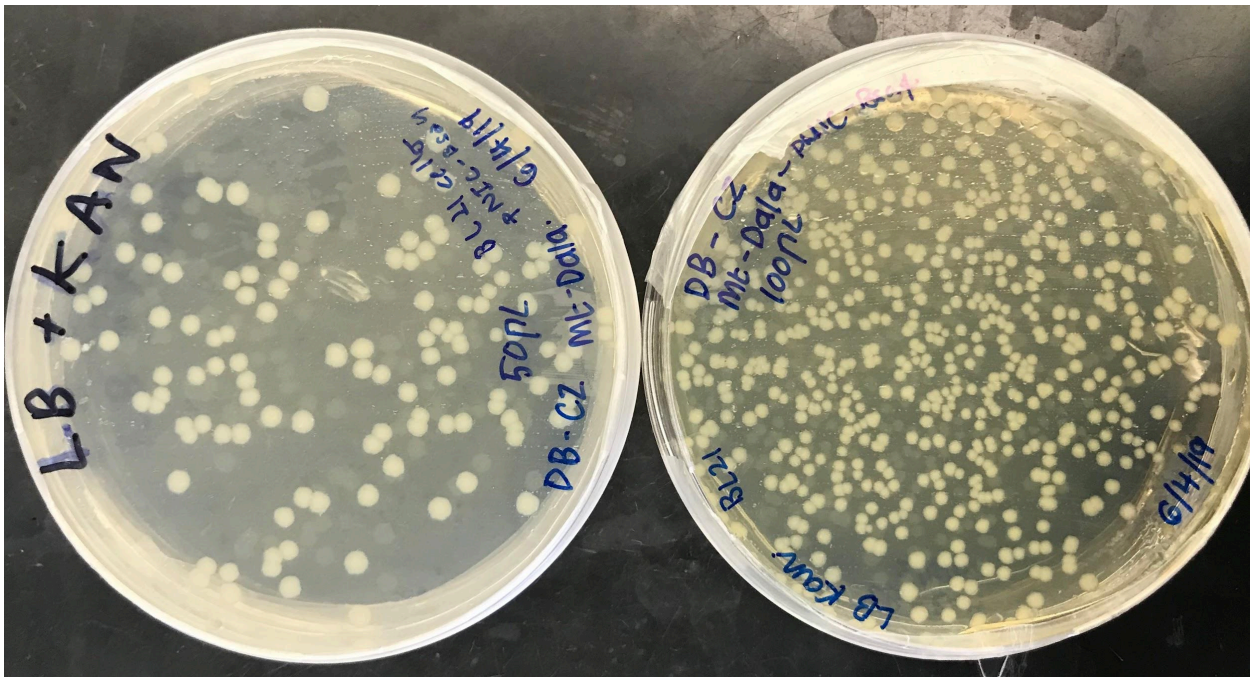

Figure 1: Transformed E.Coli BL21 cells using pNIC-Bsa4 plasmid-MtDala (left is 50uL, right is 100uL)

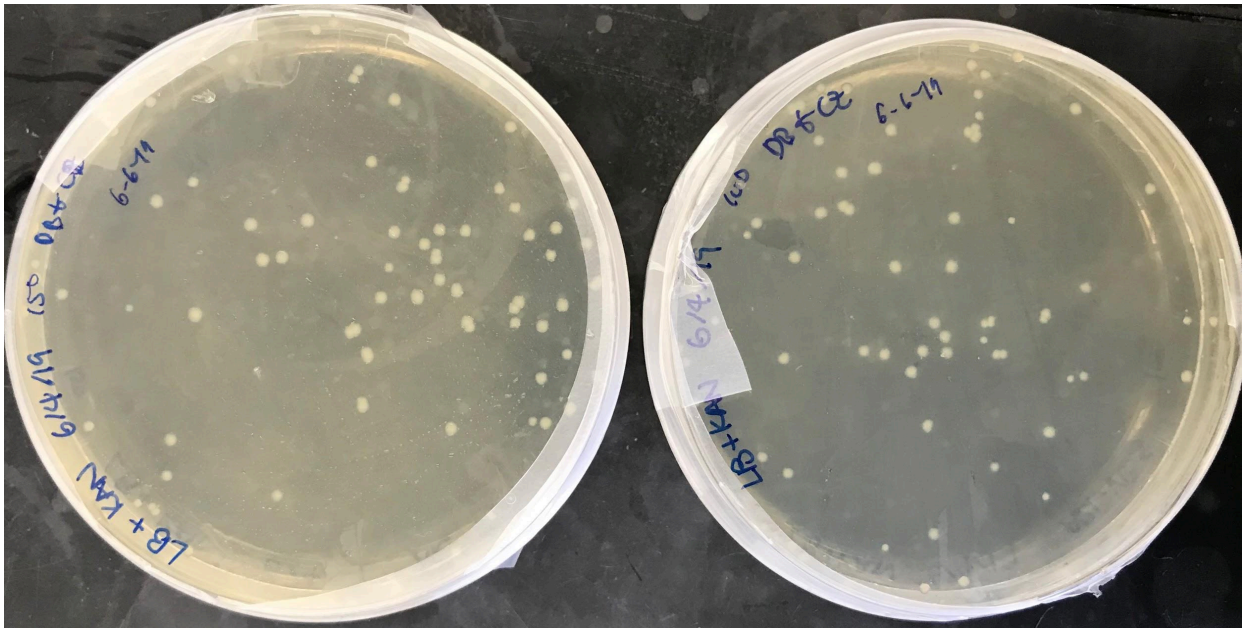

Figure 2: 2nd Attempt- Transformed E.Coli DH5alpha cells using pNIC-Bsa4 plasmid-MtDala (left is 150uL, right is 100uL)

Analysis:
Transformation with homemade DH5alpha was not very successful the first time. The procedure was modified by using 50uL of the bacteria and heat shocking for 30 seconds instead of 45 seconds.

Pellet weight for autoinduction was higher than for IPTG induced expression. However, the contents of these cells will be determined next week during purification and characterization. It was observed that the pellet for autoinduction appeared 'green' when compared to the IPTG pellet.

Date: Week 2 (June 10-14)
Brief Summary of What I did this time period: Sonicated, spun down, and syringe filtered the pellets (IPTG and autoinduction) before purification. Found the absorbance of Elution 1 using the nanodrop and ran samples from Elution 1 and 2 on SDS-Page gel. DSF was conducted using mastermixes for autoinduction and IPTG.

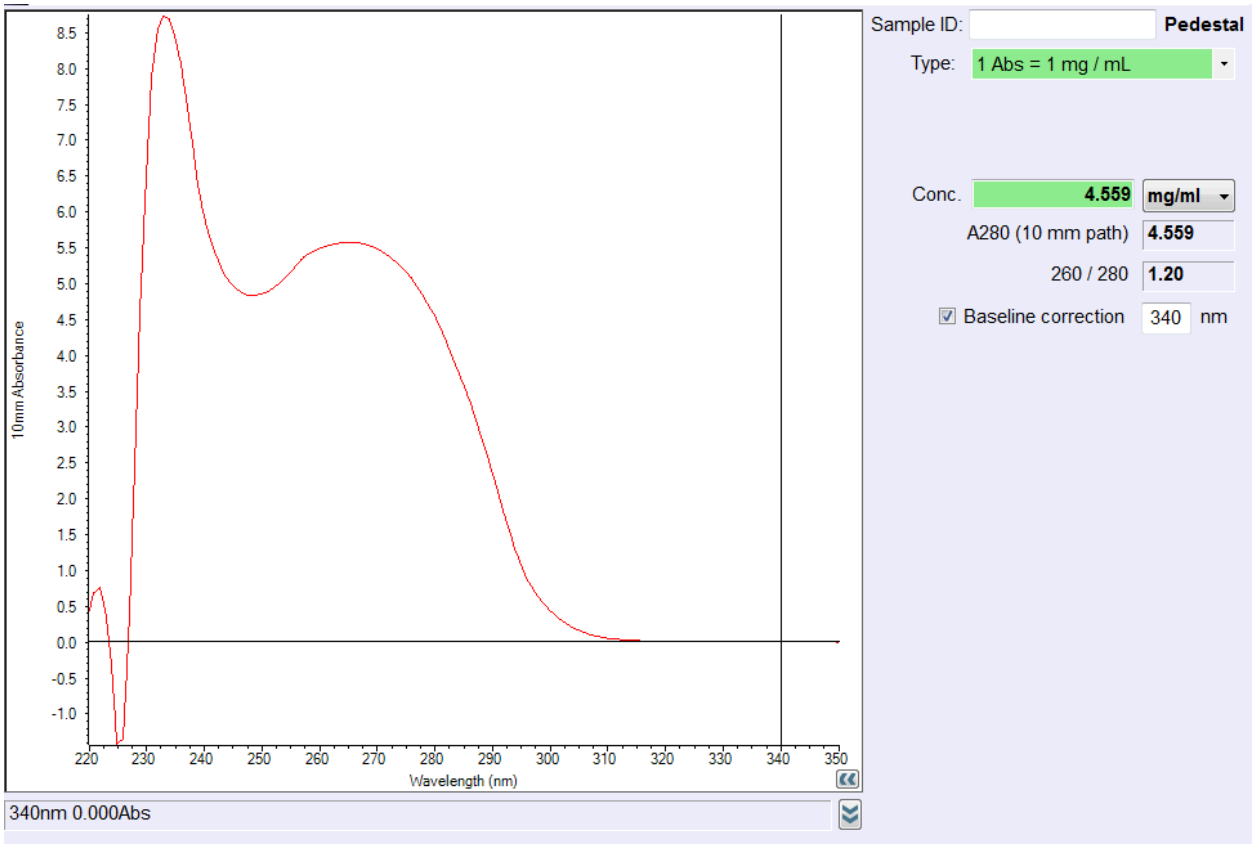

Figure 1. Absorbance graph for MtDalaDala (IPTG). Peak at 280nm (protein) and 230nm (contamination).

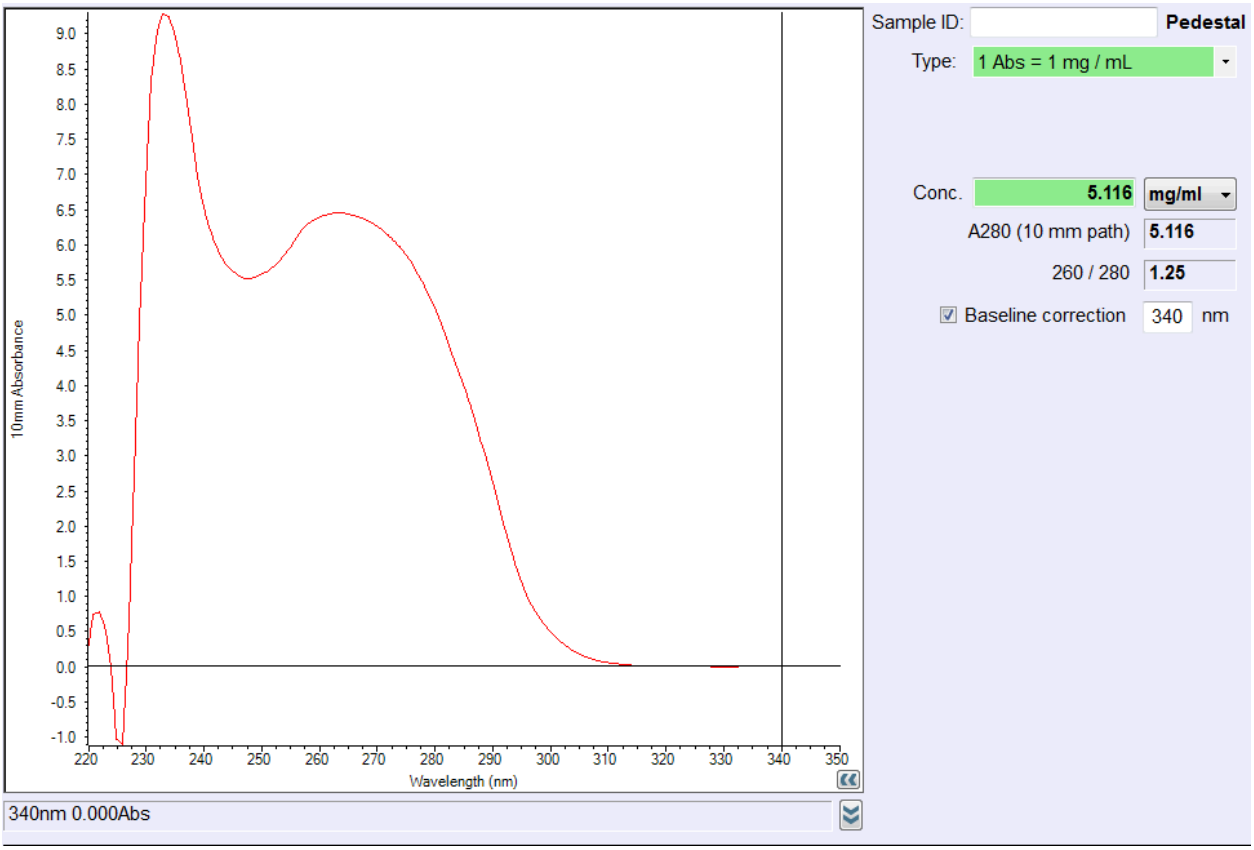

Figure 2. Absorbance graph for MtDalaDala (Autoinduction). Peak at 280nm (protein) and 230nm (contamination).

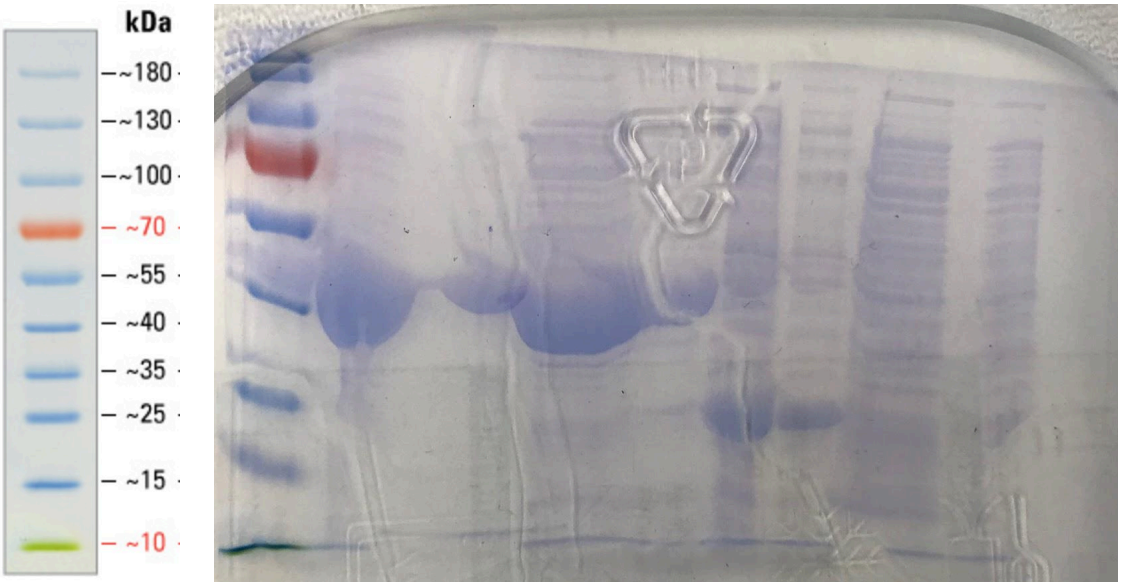

Figure 3. Characterization SDS-Page Gel for MtDala. From left to right: protein ladder, IPTG Elution 1, IPTG Elution 2, Autoinduction Elution 1, Autoinduction Elution 2. Thick band observed around 40kDa (MtDalaDala= 39.7kDa)

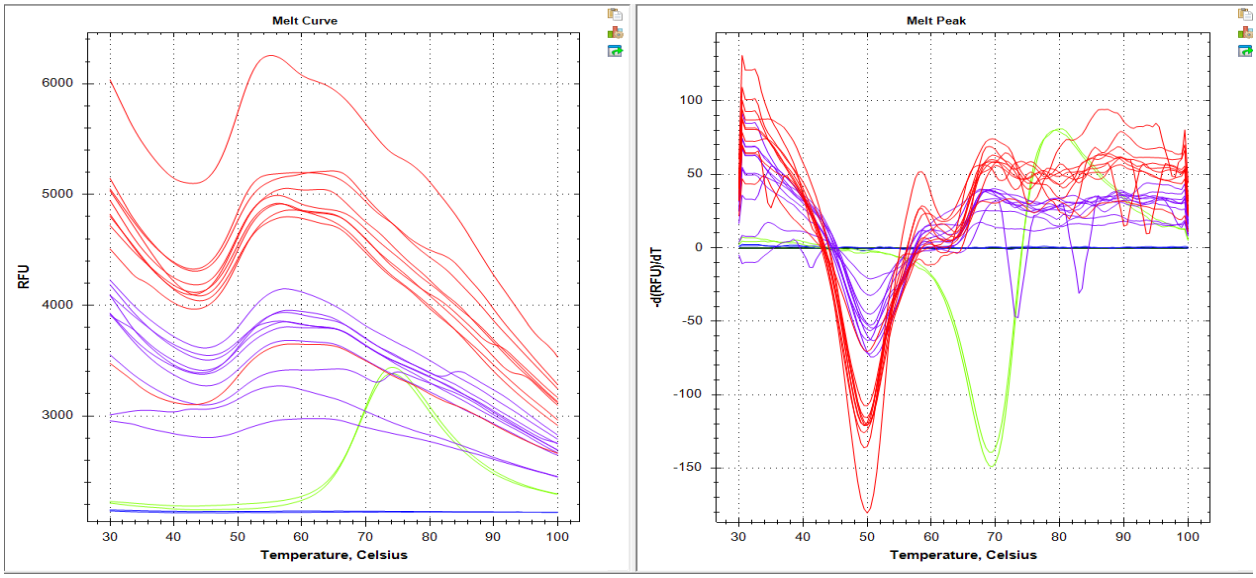

Figure 4. DSF Melt Curve for MtDala: Autoinduction Elution 1 (Red), IPTG Elution 1 (Purple), Lysozyme (Green), Water (Blue).

Analysis: From purification, characterization, and DSF, autoinduction appears to have produced more of the target protein than IPTG induced expression. The absorbance (A280) for autoinduction was higher than for IPTG. For characterization, a thick band was observed at around 40kDa which should represent the target protein (MtDala is 39.7kDa). However, there are some additional bands (contamination).

For DSF, the melting curves show relatively good precision. The melting temperature of MtDala was measured to be around 50° C which is consistent with previous DSFs. The curves could be further improved by using 15mM of protein and re-nanodroping the elution 1 samples since it appears that there was a higher concentration of the autoinduction protein than IPTG protein despite '10mM' added for both.

Date: Week 3 (June 17-21)
Brief Summary of What I did this time period: Midi-prep for MtDala and agarose gel (gel electrophoresis). Autoinduction expression on MtDala using lactose instead of arabinose and purification (Ni-NTA affinity chromatography).

Autoinduction Pellet weight- **2.92g**

Images:

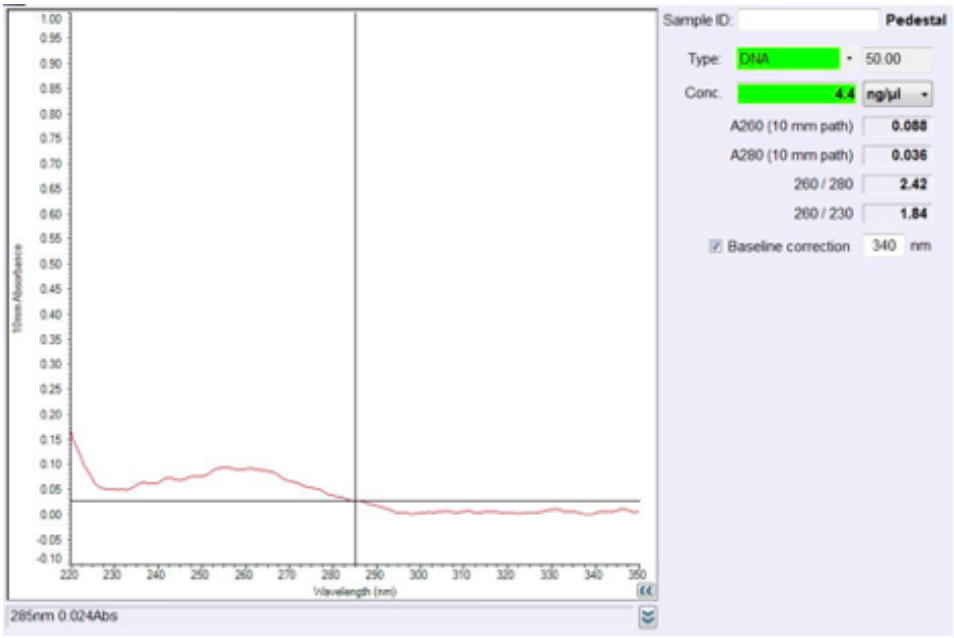

Figure 1. Nanodrop spectrophotometer absorbance graph for Midiprep of MtDala. Peak at 260nm (DNA).

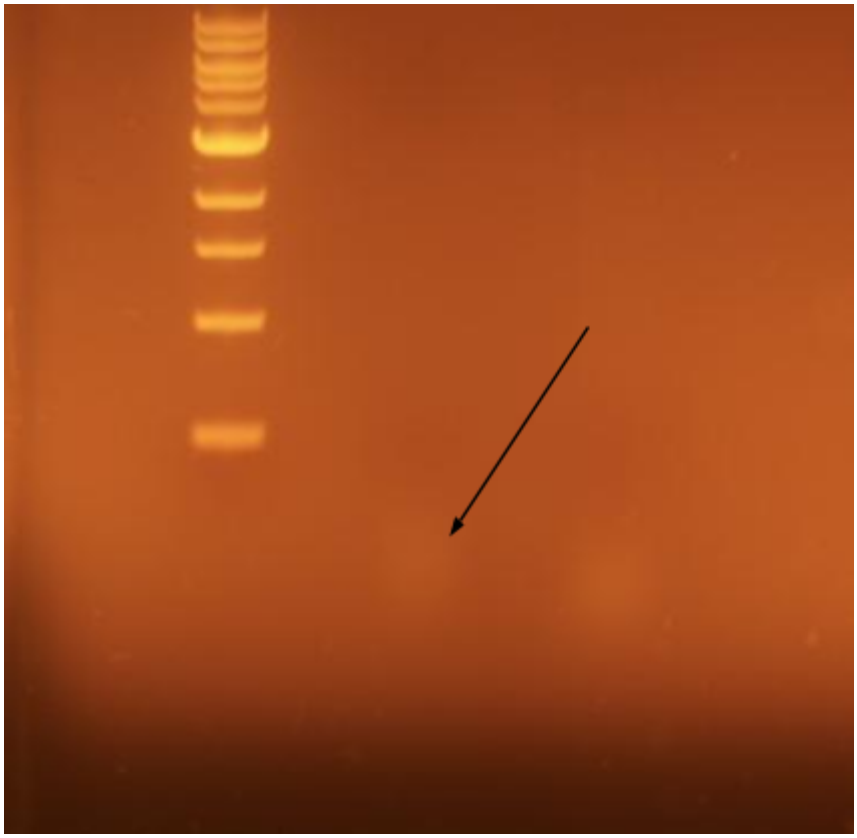

Figure 2. Agarose gel for Midiprep of MtDala. 1kb ladder shown on the left. Arrow indicates ***very*** faint band for Midiprep MtDala plasmid.

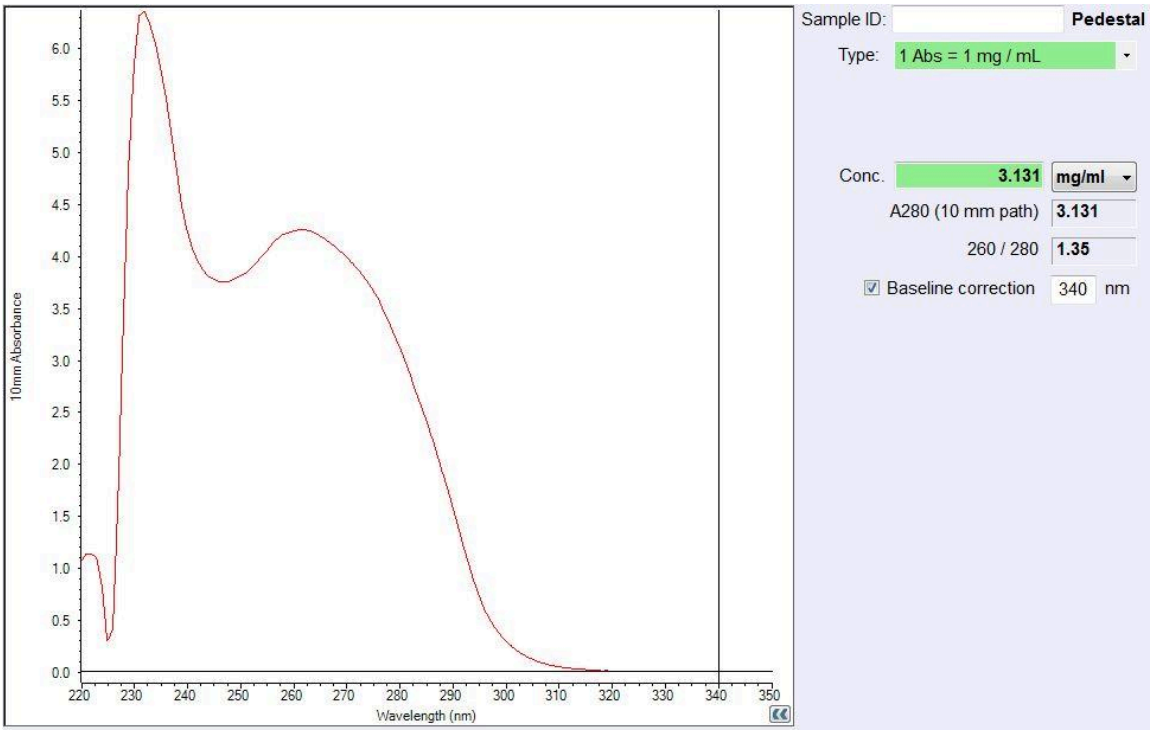

Figure 3. Nandrop spectrophotometer absorbance graph for autoinduction (MtDala) Elution 1. Peak at 280nm (protein) and 230nm (contamination).

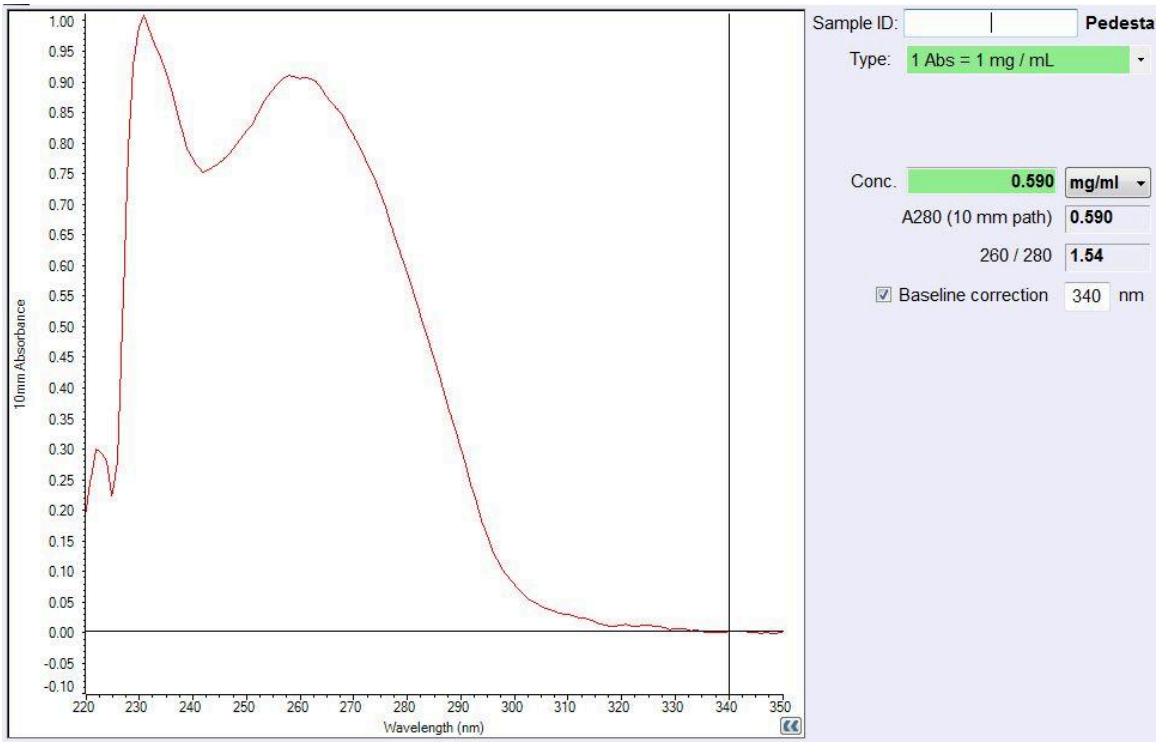

Figure 4. Nandrop spectrophotometer absorbance graph for autoinduction (MtDala) Elution 2. Peak at 280nm (protein) and 230nm (contamination).

Analysis: Gel electrophoresis on the Midi-prep sample suggested that there was none of the target DNA in the sample despite the nanodrop spectrophotometer recording positive values. The very faint band that is visible on the agarose gel travelled past the 1kb DNA ladder which suggests that the sample is very small in size/molecular weight which contradicts the target plasmid (pNIC-Bsa4 is around 7284bp).

For autoinduction of MtDala, the pellet was smaller than the first attempt. Additionally, the A280 absorbance values were also lower (E1- 3.131, E2- 0.590) than the first attempt at autoinduction. This could be the result of: the bacteria/plasmid having a more efficient relationship with arabinose than lactose or a consequence of the bacteria being grown at room temperature for a few hours before being placed in the 37°C shaker (was too full initially) which may have inhibited the growth. The identity and quantity of the target protein (MtDala) will be determined next week using characterization and DSF.

Date: Week 4 (June 24-28)
Brief Summary of What I did this time period: Ran characterization gel for autoinduction samples with lactose. DSF was conducted using mastermixes to compare two storage methods: glycerol and snap freeze. Started procedure for making competent E.Coli BL21 cells (made 0.1M CaCl2 and glycerol solutions, streaked plates, small culture+large culture, centrifuge at 3000g).

Images

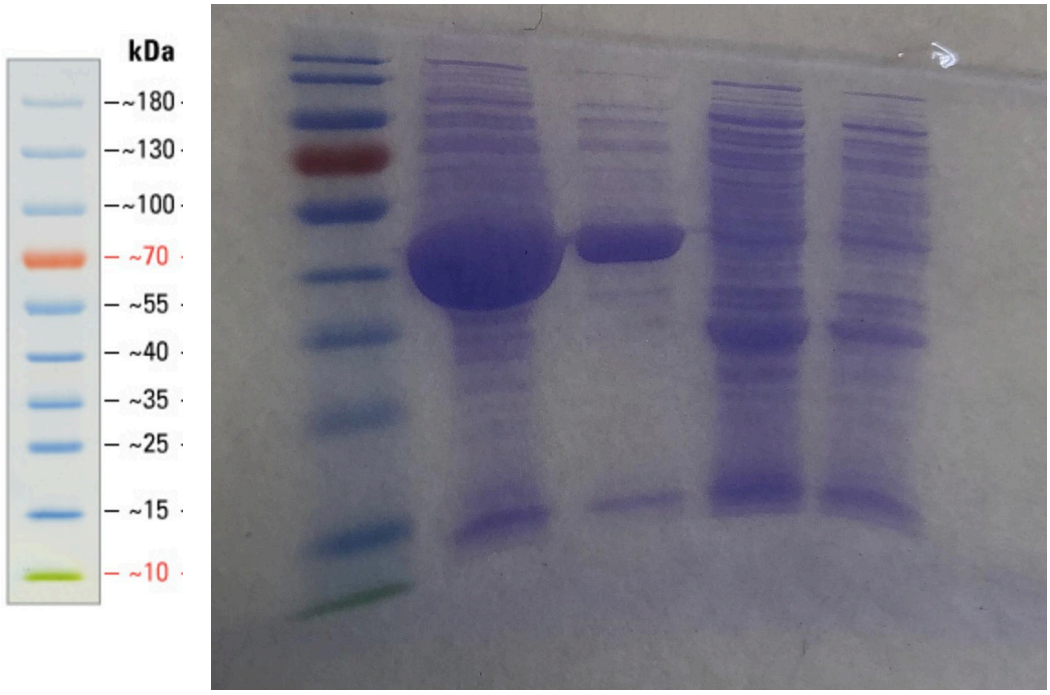

Figure 1. Characterization SDS-Page gel for MtDala (Autoinduction with lactose). From left to right: protein ladder, Elution 1, and Elution 2. Thick band observed around 40kDa (MtDalaDala= 39.7kDa)

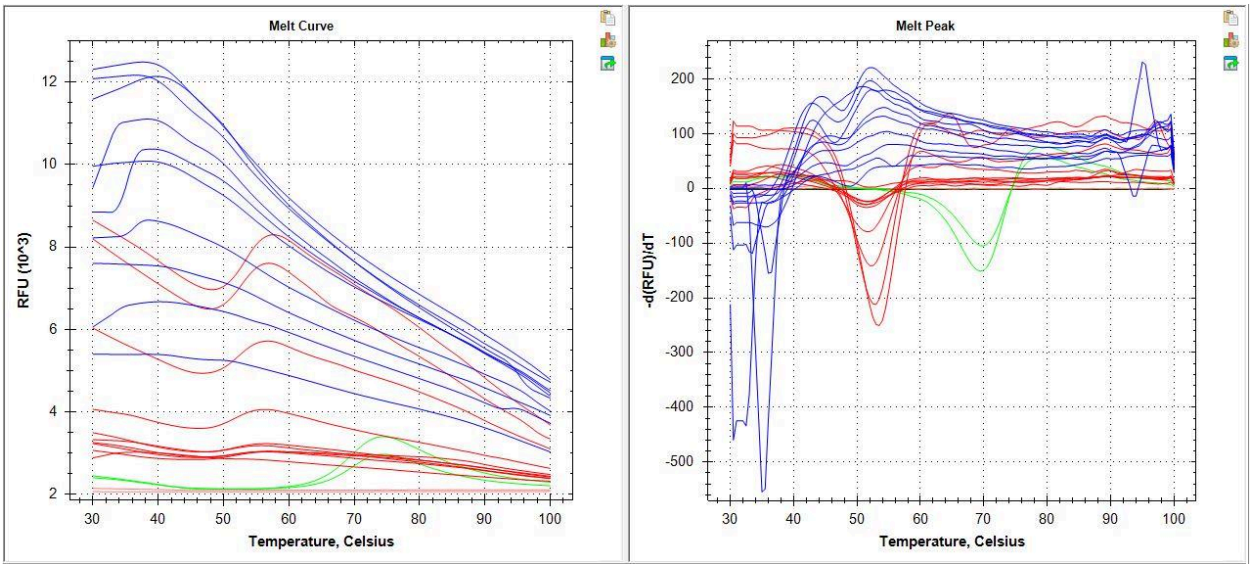

Figure 2. DSF Melt Curve for MtDala (Autoinduction with lactose): Snap freeze (blue), glycerol (red), lysozyme (green), water (pink)

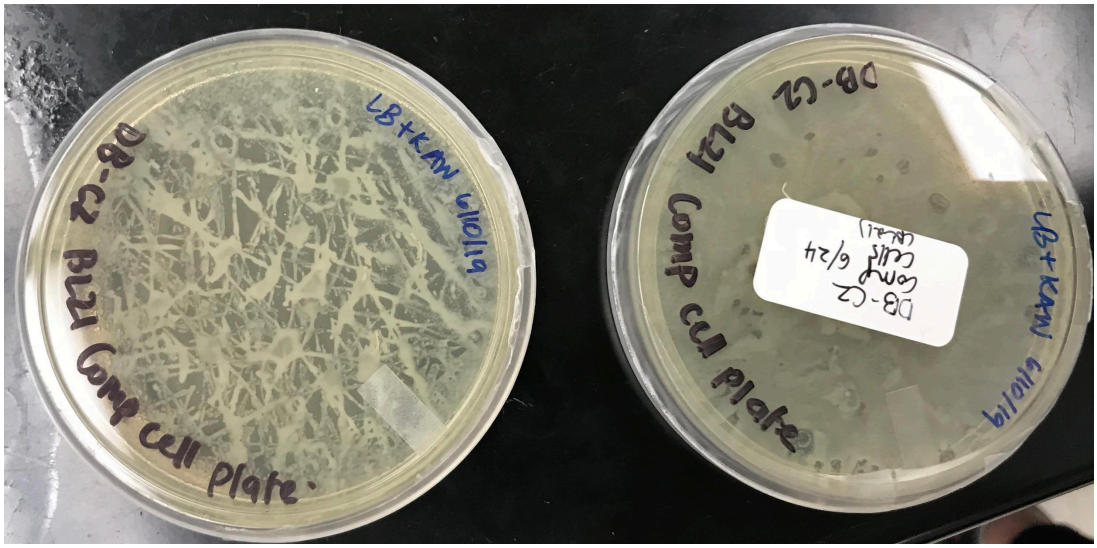

Figure 3. 1st attempt at streaking BL21 cells for competent cells.

Analysis: The characterization gel shows that the target protein is likely present since there is a thick band around 40kDa (MtDalaDala= 39.7kDa). However, there are additional bands (contamination). The DSF melt curves show that the snap frozen protein was already denatured (no peak) while the protein stored using glycerol still had a melting temperature/peak. This suggests that MtDala is not compatible with snap freezing and should instead be stored using glycerol in the future.

For competent cells, the first attempt at streaking the cells was unsuccessful as seen in above Figure 3. For the second attempt, only plates with LB+agar (no KAN) were used and 25uL was added to each plate. However, this attempt was also not very ideal (formed a “lawn” not individual colonies). Despite this,

a small culture was made and incubated overnight. A total of 25mL of small culture was added to 500mL of LB for large culture for an OD of 0.135 (grew to 0.5) and harvested. A very small pellet was formed and frozen at -20°C (the rest of the procedure will be continued next week).