BIT 161B: Experiment 4
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# Validating the Presence of Chimeric Protein in Tobacco

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## Abstract

RNA is translated into protein at rates that are affected by many systems of regulation. To determine the role of these regulatory systems in the expression of a target gene, today's lab will require us to follow a methodology that has been widely used to quantify protein in a laboratory setting, the western blot. We will isolate the protein from a sample, quantify it, separate the proteins by molecular weight, treat with protein-specific antibodies, and observe the results of the following reaction. This experiment produced incomplete results that prevented a proper analysis of the regulatory mechanisms taking place around translation but the results for trials that contained only GFP and GFP with y-gliadin seemed to support our results in previous experiments on mRNA transcripts that showed evidence for improved GFP accumulation with the presence of y-gliadin.

## Introduction and Objectives

The concentration of protein in the tobacco leaves should correlate to the abundance of mRNA observed in Experiment 2. The systems of regulation in the cell that would cause a discrepancy in this prediction are translational and post-translational. Our goal in this experiment is to observe the concentration of protein in the leaves to determine whether the protein effectively accumulating in the cells. This will involve extracting, isolating, and identifying the chimeric proteins in a leaf sample. With this data, a comparison to the final results of our mRNA quantification is in order.

## Methods

The first day of lab work consisted of extracting the protein from the sample and quantifying the protein abundance. In the acidic environment of a reagent we introduce the Coomassie dye which binds to the protein as it changes in color. The dyed sample is analyzed in a Bradford

colorimetric assay with a spectrophotometer for the difference in wavelength between samples of varying protein concentrations. The second day of lab work required the calculated concentrations from the spectrophotometer results and preparation of the protein samples (measured to be equivalent in total protein mass) for gel electrophoresis. The samples were run in SDS and polyacrylamide gel. The relative migration distance for a polypeptide in the gel is negatively proportional to the logarithm of its mass. This way we find the masses for our unknown samples. On Day 3 we treated the protein samples with primary and secondary antibodies that bind to our proteins of interest and induce luminescence. Exposure of light from the reaction is captured on photographic film as an image.

# Results

Our developed photographic film did not capture luminescence in all of the treated protein samples. The image is included below as Figure 1.

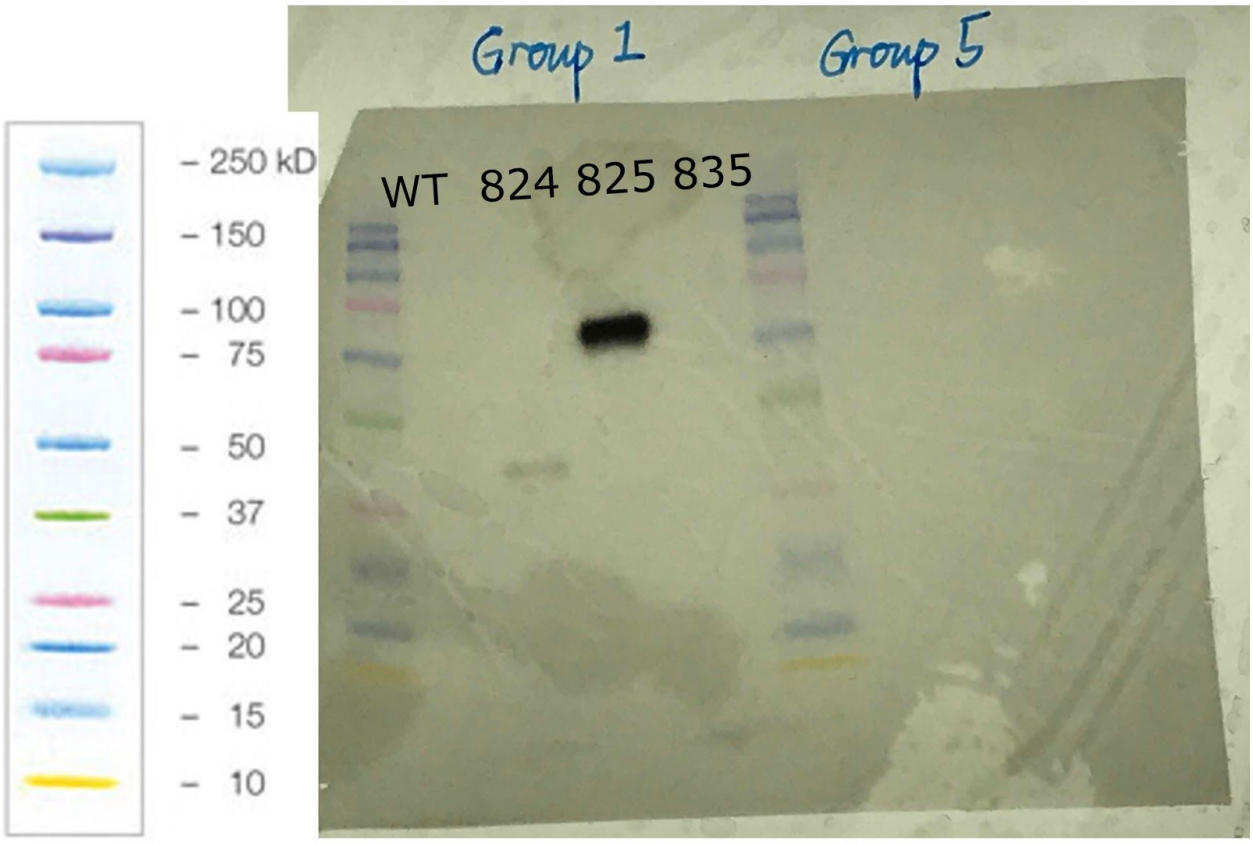


*Figure 1. The results of antibody detection on photographic film*

The most visible exposures belonged to the 824 sample and the adjacent 825 sample. The 824 exhibited a mass somewhere between 25 and 37 kD. The 824 proteins were between 50 and 75 kD. In experiment 2, we used a spectrophotometer and gel electrophoresis to observe differences in expression of our chimeric construct across the different treatments. Analysis of

our isolated samples for the chimeric mRNA of pPIPRA824 and pPIPRA825 revealed that 825 displayed more relative expression in both total RNA abundance and GFP transcript abundance. The actual values of mRNA molecular weight in 824 versus 825 were not obtained due to experimental error so a proper comparison between the results of these experiments cannot be made.

# Conclusion

Our results in this lab confirmed the presence of GFP proteins expressed by our chimeric constructs, further indicating that the transformation was successful. The 825 sample displayed a higher amount of GFP accumulation demonstrating the intended function of the y-gliadin insertion. This result is in line with our results from experiment 2, where, of the two sample 824 and 825, we showed that the sample with the y-gliadin sequence presented the higher levels of mRNA transcription for GFP-containing RNA products. The 835 sample did not successfully produce results in this experiment so we cannot assess the functionality or expression of the zein sequence. The wild type did not express any GFP presence in the western blot which is as intended, however we found evidence that might have indicated chimeric transcripts present in the wild type in experiment 2. We can now say that the reason for that error would likely be contamination of the sample. The reason for why we did not obtain results for 835 could lie in a failure of the preparation of standards for the gel electrophoresis, the antibody treatment, or the development of the film.

# References

Bennett, Alan “EXPERIMENT 2: Gene Expression - RNA: Detection of chimeric mRNA transcripts in transgenic tobacco.” Lab Write-up. UC Davis. May 21, 2018

Bennett, Alan “EXPERIMENT 4: Gene Expression - Protein: Detection of chimeric proteins in transgenic tobacco.” Lab Write-up. UC Davis. May 21, 2018