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End of Summer Report
FURSCA Summer 2025

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A. Introduction

This summer, I continued working on our ongoing research into RNA replication in flaviviruses—specifically focusing on the NS5 RNA-dependent RNA polymerase protein. These viruses, like dengue, are transmitted through arthropods such as mosquitoes and ticks and can cause millions of infections every year. The NS5 protein plays a major role in helping the virus replicate, and since it's conserved across many flaviviruses, it's a really strong target for studying how these viruses function and evolve.

The goal of the project was to purify the full-length NS5 protein and test its ability to replicate an RNA template accurately. Once that's confirmed, the big idea is to introduce RNA modifications into the template and then measure how those modifications impact the speed and accuracy of replication. Basically, we're trying to figure out how changes to the RNA affect how well NS5 can do its job.

B. Results/Summary

This summer, we focused on optimizing the overexpression of NS5 using *E. coli* BL21(DE3) cells. These cells are great for protein expression since they contain the T7 polymerase system, which helps crank out the protein of interest. We transformed them with our NS5-containing plasmid and plated them on LB-AMP plates (Lysogeny Broth plates containing the antibiotic Ampicillin) to select for successful colonies (**Figure A**). The ampicillin-resistant tag ensures only the bacteria carrying our plasmid grow. We did several rounds of streaking and incubation to build up a large working stock of NS5-expressing cells that we can freeze and use for future purifications.

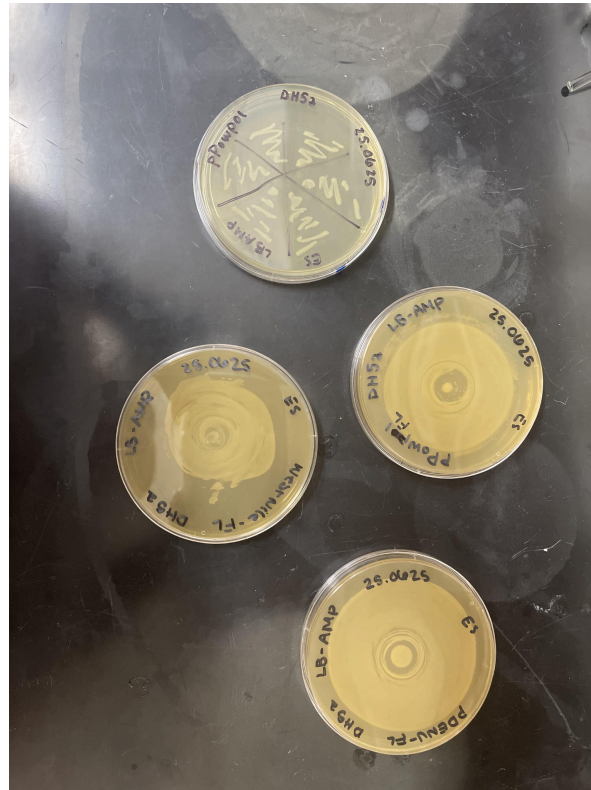


Figure A

To check if our NS5 protein was being expressed at usable levels, we used gel electrophoresis to view protein concentration (**Figure B**). We ran SDS-PAGE gels with samples from our expression experiments. The gel separates proteins by size using an electric field, and we stained the gel to visualize bands representing different proteins. By comparing our bands to a known protein ladder and checking the intensity, we were able to estimate how much NS5 protein was present. A strong, clear band at the expected molecular weight confirmed successful expression. These gels help us decide which samples to proceed with for purification.

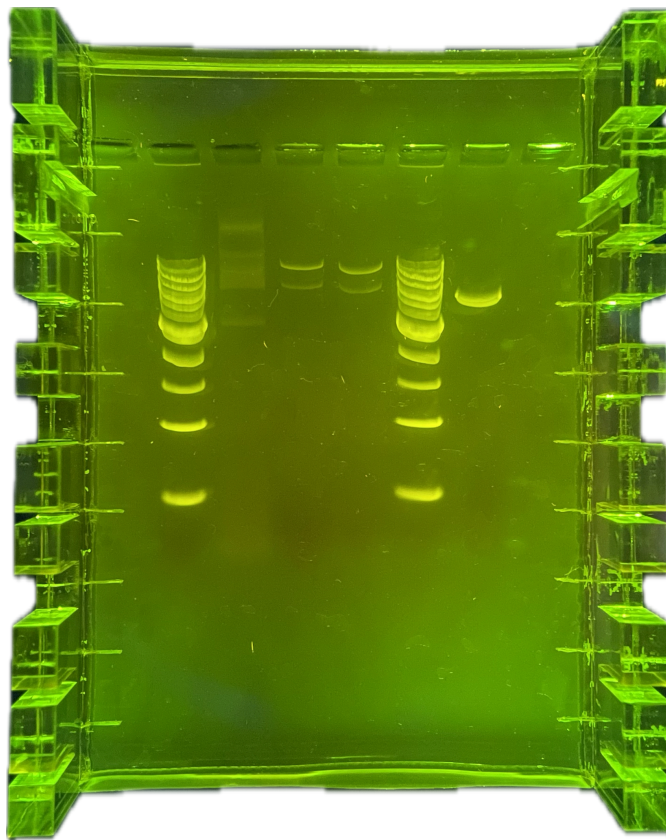


Figure B

While we haven't yet run the RNA template assays, we've laid the groundwork for what's coming next. In the fall, we plan to purify the NS5 protein from these stocks and begin validating its structure and function using biochemical assays.

C. Conclusions

This research experience has had a big impact on my development as a scientist. It's one thing to learn about gene expression and protein purification in class, but it hits differently when you're actually doing the work—making buffers, prepping media, troubleshooting protocols. Being part of this project has taught me a lot about RNA biology and what goes into real-world research.

I plan to continue this project through the school year and present my work at Albion's Elkin Isaac Symposium and hopefully at other conferences too. I'm really thankful for the

chance to work on such meaningful research with Dr. Rohlman and for the support that made this summer possible.

Thank you to the donors whose generosity allowed me to take part in this opportunity whilst alleviating the financial burden. Your support means a lot—not just to me, but to the future of science and the students behind it.