End of Summer Report

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**Introduction**

The interaction between *E. coli* RNA polymerase (RNAP), U5 DNA template, and the CRISPR dCas9 system was the focus of the project this summer. The overall goal of the project is to use the dCas9 system to stop the transcription process *in vitro* and explore how *E. coli* RNAP behaves on a molecular level with the dCas9 system. It has been observed in previous studies that the *E. coli* RNAP stays bound to the DNA template at the pause site, but I wanted to know why. In order to answer this question, I created an experimental workflow that involved several steps. First, I needed to amplify U5 template DNA using a technique called polymerase chain reaction (PCR). The next steps involved running a transcription reaction without the dCas9 protein, running the transcription reaction with the dCas9 protein, optimizing these transcription reactions, and finally, inspecting the interaction between the U5 template, the RNA polymerase, and the dCas9 system using chemical cross linking.

**Summary**

This summer I was able to successfully amplify the U5 template DNA using PCR. However, I ran into some issues when running the transcription reaction. The picture below was captured using a technique called gel electrophoresis. In biochemistry, this is the main visualization tool that we use. In this specific gel, there are 8 wells with wells 2 through 7 containing product. Wells 2 and 5 had a marker in them, while wells 3, 4, 7, and 8 had the transcribed DNA in them. The only difference between wells 3 and 4 and 7 and 8 was that wells 7 and 8 had been treated with DNAse. DNAse is an enzyme that chews up any DNA so that we can visualize if we had made RNA or not. Unfortunately, wells 7 and 8 did not show a band indicating that I had not successfully transcribed the DNA into RNA. This result means that I now need to focus on figuring out a more effective way of transcribing the U5 DNA into RNA so that I can then start to introduce the dCas9 system.

**Conclusions**

Overall, this work will reveal a deeper understanding of how the dCas9 system, and the U5 DNA template interact with one another. This work is important because it will eventually explain what is going on between the dCas9 system, the DNA template, and the *E. coli* RNA polymerase on a molecular level, which will allow for a better understanding of how the CRISPR/Cas9 system works and how it can be applied to other areas of study. I plan on continuing this work that I have started, and I also plan on presenting the work I have thus far at the Elkin Isaac Research symposium this upcoming spring. Furthermore, this research has had an incredible impact on my life because it has allowed me to explore a question that is intriguing to me. In addition, this research has taught me so much about how to apply different techniques that I have learned in my classes to my own questions. Finally, this research has allowed me to have a meaningful and memorable summer job that I am very grateful for. I would like to thank the JBruce A., ’53 and Peggy Kresge, ’53 Endowed Science Fellows for their hard work and support in making my FURSCA experience possible.