End of Summer Report

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**Research title**: A study of T7 and E. coli RNAP – Template interaction utilizing CRISPR/dCas9 protein

I proposed to research a process called transcriptional pausing in this summer. Transcription is the main cellular activity that copies DNA into RNA. This step is critical for further protein productions and their functioning. In Transcription, a protein called RNA polymerase binds and unwinds DNA and copies into RNA. I am working with T7 RNA polymerase, which comes from virus/bacteriophage/ called T7. Purpose of this project is to understand what happens, in other words what kind of physical, chemical interactions between these components of transcription at molecular level when transcription is paused.

I started amplifying and cleaning my U5 DNA template for a week. In order to verify my work, I used a technique called gel electrophoresis. From the third week, I started working on transcription. After confirming that my transcription reaction worked, I need to optimize certain conditions. Because this project is studying transcription in sterile tube, which also means that out of the cell, optimizing and finding the best conditions that work for desired transcription activity is necessary. I tried many possible ways and verified all of them in gel. All of them are worked currently. We started pausing at different time points from 30 sec to two hours. Even at 30 second transcription, I saw RNA material in the gel. T7 RNA polymerase is incredibly fast for copying. After several time-course transcription, we have chosen 30 min as good transcriptional pausing time and started working on modifying template and other component’s concentration. So far, we verified all our work in gel. We plan to insert guide RNA and dCas9 protein in this fall. Before we insert dCas9 to pause transcription, we need to determine where in the DNA I am going to pause. Currently, I have couple of plans to do that from the papers I read.

While I was working on this project, I have learnt many things, gained much lab experiences and read many interesting papers. I encountered plenty of challenging problems. Reading papers that has similar experiment and different protocols helped me to troubleshooting my mistakes and improve my laboratory skills. My samples are all 0.25-20 micro litter in 0.5 ml sterile tubes. Working with such a small concentration requires me to be highly focused and cautious.