microRNAs (miRNAs) are small RNA molecules that widely regulate gene expression and are important in animal development. let-7 is a miRNA that has specialized function in developmental timing. In the nematode worm *C. elegans*, a genetic model organism, *let-7* mutations cause developmental timing defects in several tissues. Specifically, *let-7* mutations delay skin development, when some skin cells repeat cell divisions associated with earlier developmental fates. In short, these cells act "younger" than they are and continue to proliferate. let-7 sequence and its role in inhibiting these inappropriate cell divisions are conserved between *C. elegans* and humans. In humans, let-7 acts as a tumor suppressor, where defects in let-7 activity promote tumorigenesis. In *C. elegans*, let-7 defects result in animal death during the transition from the L4 larval stage to adulthood. let-7 miRNA is most abundant and active at the L4 larval stage. The goal of my project is to improve understanding of how the let-7 miRNA functions to repress genes and regulate cell fates in development. Specifically, I plan to pulldown the let-7 miRNA using a 2'-O-methylated oligo and identify what associates with let-7 through mass spectrometry. To conduct this experiment, I first needed a supply of L4 larval *C. elegans*. I also needed to learn how to lyse worms, make protein preparations, and perform miRNA pulldowns.

The progress I made on my research project was modifying my protocol of worm collection, collecting the necessary sample, and beginning to learn how to make protein preparations and carry out miRNA pulldowns. I have improved the animal collection protocol, which is a five-day process and I averaged two worm collections a week. As I optimized the protocol for staged animal collection, I have also increased my collections to ~40,000 worms per single collection. This is a crucial part of my experiment because nine milliliters of worms (nearly a million) are needed to conduct the let-7 microRNA pulldowns per strain. Three strains of different genotypes carrying mutations in different let-7-related miRNAs will be used for the pulldown experiments. Performing let-7 pulldown from multiple strains will allow me to determine the proteins that specifically associate with the let-7 miRNA. Currently, I have 6mls of one strain, 6.5mls of another strain, and 5mls of my third strain of *C. elegans*. I will continue to collect L4 worms until I have reached my 9ml goal. At the same time, I have begun learning how to make worm lysates and how to do miRNA pulldowns. I plan to continue this project in the Fall 2018. Identification of let-7 binding proteins will allow us to further study how let-7 regulates gene expression. This, with further research, can have important implications for understanding how let-7 contributes to diseases such as cancer.