



Experimental Evolution of Mutation Rates

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This summer I had the pleasure of working in the biology department as a summer research assistant under the mentorship of Dr. Paul Sniegowski. Dr. Sniegowski's team focuses on experimental evolution – specifically the evolution of mutations over generations of competing strains of *E. coli*. Recently, Dr. Sniegowski's team has become particularly interested in finding ways to increase the precision and the accuracy of competition experiments. I spent my ten weeks learning how to use a flow cytometer to implement a new competition protocol in order to increase the accuracy and replicability of our current experiments.

The first few weeks of my research internship were dedicated to learning the traditional, sterile, methods and techniques that are used to compete two strains of *E. coli* and also calculate the fitness costs of potential beneficial mutations. While the traditional five-day protocol is able to provide the amount of increase in fitness after a competition, we are only able to plate 100 total microliters which is a mixture of two diluted strains (50 microliters each) to be competed. With the dilution, we are only able to accurately count 300-400 total colonies of bacteria, reproducing our competition five times. This may seem like quite a large number, but is rather small when talking about the amount of the bacteria that are present in 100 microliters of a competition solution. While keeping the limitations of the traditional methods in mind, the last seven weeks of my internship were dedicated to working with Ben Sprung and Brooks Martino to develop a competition protocol that utilizes flow cytometry to increase our sample size, the amount of replicates, and eliminate the process of physically counting and plating cultures, which are often prone to contamination.

Using the Guava easyCye flow cytometer, we faced several challenges detecting two competing strains of fluorescent bacteria because we continually saw an overlap between the GFP and the YFP detection spectrum. Our team realized that inoculating our strains with filtered media and also filtering our solutions before running them in the Guava flow cytometer can increase our ability to differentiate the strains from the continually present noise. We finally discovered that there is a level of instrumental noise that is always present when running a sample, and we are continuing to research methods that can lead to the ease of distinction between different fluorescent strains of bacteria.

While I did some research in high school, the opportunity that the Penn Undergraduate Research Mentorship program provided for me was like no other. Although I had the opportunity to learn about evolution and mutations from Professor Sniegowski in Biology 102, PURM allowed me to learn from him firsthand and gain the experience that is necessary to become a successful researcher. I am fortunate to have had the opportunity to work with Dr. Sniegowski's team, and I am looking forward to joining the lab and continuing my research during my time at Penn.