Enhancer Verification in Populations of *D. melanogaster*

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The purpose of the project I worked on this summer was to determine whether transcriptional regulatory variation plays a significant role in adaptive evolution in fruit flies. If transcriptional regulatory variation influences evolution, there should be a large number of active enhancers in some fly populations but not in others, referred to as polymorphic enhancers.

Previous students in the Palolopi lab analyzed millions of DNA sequences. For every fly population sampled, brain tissue from young adult flies was assessed for chromatin conformation and noncoding stretches of DNA compared. Roughly 25,000 active enhancers were detected. The identification of these enhancers were working off the assumption that the noncoding DNA sequences in an open chromatin conformation found are functioning enhancers. This is because for certain important proteins to bind to enhancers and drive gene expression, chromatin must be in an open configuration.

This assumption is where my work this summer intersected with previous work done in the Palopoli lab. Previous work determined whether or not the putative enhancers are open or closed using chromatin conformation but did not show that these sequences actually function in transcription. During this eight-week summer fellowship, I, along with three other lab mates, created transgenic reporter constructs to provide definitive evidence that these sequences are truly enhancers.

The transgenic reporter construct we used consisted of the putative enhancer sequence, a basal promoter, and the coding sequence for green fluorescent protein (GFP). GFP is a protein that glows green when exposed to UV light. By placing the stretch of DNA we think is an enhancer sequence in front of the GFP sequence, we can check if that stretch of DNA triggers transcription by verifying that cells in the fly glow green.

First we used the previous data to locate two stretches of non-coding DNA with open chromatin conformation that are polymorphic between species to be our enhancer candidates. We then grew up, transformed, plated, and cultured bacteria with a known plasmid. We mini-prepped the bacteria to isolate the plasmid. Then through a process called restriction digest we “cut” out the original enhancer in the plasmid using restriction enzymes and isolated the plasmid backbone through gel purification. We used polymerase chain reaction to amplify our enhancer candidates. Finally, we combined the two fragments (plasmid backbone and enhancer candidate sequence) using Gibson Assembly and sent the final construct for sequencing to ensure our results were what we expected.

Sequencing results showed that our lab work yielded the expected constructs! We did not have time to insert the reporter construct we made into the fly genome and check if the cells glow (this will be completed by students in the future), but of the constructs we made (each for a different species of fruit fly) we expect to see cells glow in the species that showed this enhancer active according to the chromatin conformation data and vice versa.

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