Generating Transgenic Reporter Constructs to Characterize *cis*-Regulatory Enhancer Candidates
Claire Nguyen, 2025

Phenotypic differences—differences in observable traits—drive the process of evolution. If an organism has an advantageous trait, it is more likely to survive and reproduce, passing on this advantageous trait. This phenotypic diversity can be caused by either structural or regulatory variation. Structural variations affect coding regions of DNA, in turn affecting the structure of expressed proteins. In contrast, regulatory variation affects non-coding DNA regions, affecting the degree, location, and timing of protein expression. Structural variations tend to have a high degree of pleiotropy; a small change in a region of coding DNA can produce many phenotypic changes in an organism. Thus, structural variations typically have many effects, some increasing fitness and others decreasing fitness. In contrast, regulatory variation tends to have a low degree of pleiotropy, producing specific and precise phenotypic effects. This precision makes regulatory variation a better candidate than structural variation for being the raw material for most phenotypic variation.

Our research focused on enhancers—short stretches of non-coding DNA where the RNA polymerase binds to initiate the process of transcription. Active enhancers tend to have an open-chromatin conformation (i.e. their DNA is loosely wound around the histone proteins). This allows for transcription factors to physically access and bind to the enhancer, initiating transcription.

Previous research in the Palopoli lab performed ATAC-sequencing to measure chromatin conformation across different populations of *D. melanogaster* and between closely related species (*D. simulans* and *D. mauritiana*). This work supported the existence of chromatin conformation variation between these species and populations, accounting for their phenotypic variations. However, their research operated under the assumption that short stretches of non-coding DNA with open chromatin conformations were functional enhancers. The purpose of the research project this past summer was to support this assumption. To do so, we created reporter constructs containing enhancer candidates and a protein that codes for green fluorescence protein. In the future, the lab will insert these constructs into flies and observe for GFP, confirming the functionality of the enhancer candidates.

To begin, data generated through ATAC-sequencing and processed through our pipeline was analyzed. Polymorphic enhancer candidates were selected: one that had an open conformation in *D. melanogaster* only and one that had an open conformation in *D. simulans* and D. *mauritiana* only.

Cell transformation was performed in order to insert the plasmid (pJFRC28), containing a gene that codes for green fluorescence protein, into bacteria. The bacteria was plated and cultured, and a mini-prep procedure was performed to isolate the plasmid from the bacterial cultures. The isolated plasmid contained a UAS enhancer that had to be replaced with the enhancer candidate. A restriction digest was performed in order to remove the UAS enhancer. A gel purification was run in order to isolate the plasmid backbone from the UAS enhancer. A polymerase chain reaction was then performed to amplify the selected enhancer candidates. Finally, Gibson assembly was conducted in order to insert the enhancer candidate into the plasmid backbone, generating a transgenic reporter construct. The construct was sent to an independent laboratory for sequencing, and results showed that the enhancer candidate was successfully inserted into the plasmid backbone.

Thus, our research lab successfully constructed transgenic reporter constructs containing an enhancer candidate. In the future, these constructs will be injected into *Drosophila* larvae. The brains of these *Drosophila* will then be examined to confirm the functionality of the enhancer candidates. If green fluorescence protein is observed in their brains, then the enhancer candidates are indeed functional. This would also be strong evidence supporting the notion that variation in enhancer function is common between species.

Faculty Mentor: Michael Palopoli
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