**DELIVERABLE ONE PAGE RESEARCH SUMMARY**

Jan Louis Bilbao Del Valle, Undergraduate Student

The microbiome is an unseen "organ" consisting of all the microorganisms that naturally reside on an organism and how they interact with each other. It encompasses bacteria, fungi, viruses, archaea, and eukaryotes, with some being beneficial and others harmful. This intricate microbiome performs crucial functions, such as maintaining homeostasis, stimulating the immune system, synthesizing essential amino acids like vitamin B12 and vitamin K, and providing protection against pathogens. However, factors like age, diet, infectious illnesses, and antibiotics can disrupt the microbiome, leading to an imbalance and dysplasia. Alcohol is another factor that can disrupt the microbiome. Specifically, alcohol consumption alters the composition of the gut microbiota, which plays a crucial role in the gut-brain axis, a bidirectional communication system. Altering the gut microbiota-brain axis has been implicated in many chronic diseases including neurodegenerative diseases.

To study the changes in the abundance and composition of the microbiome, genomic techniques such as whole genome shotgun sequencing (WGS) and 16s rRNA sequencing (16s rRNAseq) are employed. When analyzing the bacterial composition and abundance in complex samples, WGS has the advantage that because you are sequencing the whole genome you can detect many bacteria at the species level. Whereas in traditional 16s rRNAseq, scientists will sequence only a portion of the 16s rRNA gene usually of around 300-500 bp leading to the identification of bacteria only at the genus level and thus missing information about species. To circumvent this problem, scientists have turned to sequence the whole 16 s rRNA gene (~1500 bp) to get species-level information. This combined with being quick and cost-effective provides an additional advantage of using 16s rRNAseq vs metagenomics as an initial approach to study differences in abundance and composition of the gut microbiome. For my proposed project I will sequence the whole 16s rRNA gene of flies exposed or not to ethanol using Nanopore sequencing technology.

Nanopore sequencing utilizes nanopores, which are small openings found in natural membranes, are incorporated into a synthetic membrane, and immersed in an electrophysiological solution. By applying an ionic current through these nanopores, the movement of molecules like DNA and RNA can be detected, causing changes in the current. This real-time signal analysis allows for the determination of the base sequence in the passing DNA or RNA strands. Nanopore sequencing has many advantages, such as being the only sequencing technology that offers real-time analysis, meaning that it allows users to run their experiment until a predetermined parameter is met. Also, a longer sequencing run permits more DNA/RNA molecules to be sequenced, thereby enhancing the probability of identifying low-abundance sequences within a sample, leading to increased result reliability and a broader spectrum of analysis. Also, it produces incredibly long-read sequence data far cheaper and faster than other techniques.

In this research, I will collaborate with graduate student Patricia Pujols who is focusing on investigating how alcohol affects the gut microbiome and its impact on the tolerance and sensitivity in young and old *Drosophila melanogaster*. The objective of this research is to sequence the gene encoding the ribosomal subunit 16s of the bacteria in the microbiome of the gut in the fruit fly exposed or not to alcohol using Nanopore sequencing. The experiment begins by aging wild-type (Canton-S) *Drosophila melanogaster* fruit flies for 7 days or 50 days to collect young and old flies, respectively. At the desired age, we divide the flies into three groups named: 1) Control, 2) one exposure and 3) two exposures. The protocol runs for three days where ethanol or water is given to the flies on day 1 or day 3. For *‘Group 1, Control’* no intervention is performed on the flies but are placed in the same incubators as the other groups for three days. *‘Group 2, 1 exposure’* are exposed on day 1 to water for 20 minutes, on day 2 placed on regular food, and on day 3 exposed to ethanol for 20 minutes. *‘Group 3, 2 exposures’* are exposed to ethanol on day 1 and day 3. On day 3, flies are dissected and 10 guts per sample are collected 500 µL sterile PBS. For each sample, three replicates are collected. Importantly, when collecting the samples we follow strict aseptic techniques by sterilizing the flies in 70% alcohol and washing them in sterile PBS to wash away the alcohol. Proper sterilization is crucial to prevent the contamination of the gut sample with surface bacteria that could significantly impact the 16s rRNAseq outcome. Then, the guts are flash-frozen in dry ice. The guts are homogenized in PBS and the gDNA will be extracted using a gDNA extraction kit and subsequently sequence the samples in the Nanopore sequencing machine. Currently, we are working on the protocol and are in communication with Prof. Miguel Urdaneta who is helping us with Nanopore sequencing.