Hi, I'm Kaelynn and I will be presenting my work from the past year with Abigail and Dr. Agrawal on Apoptosis in *Crithidia fasciculata*.

This is a brief overview of what I will cover. First, I will go over background information for the project, then I will talk about the genes I studied, and then about the CRISPR-Cas9 optimization for *Crithidia*. Finally, Abby will go over the drug selection experiment.

First of all, what is *Crithidia*? It is a unicellular, eukaryotic parasite that is defined by its single specialized mitochondrion called the kinetoplast. It infects mosquitoes and not humans.

Crithidia is related to pathogenic kinetoplastids. *Leishmania* is spread by the sand fly and causes leishmaniasis. *Trypansoma brucei* is spread by the tsetse fly and causes African sleeping sickness, and *Trypanosoma cruzi* is spread by the triatomine bug and causes Chagas disease. These diseases are classified as neglected tropical diseases by the World Health Organization, which mean we need better treatments.

Specifically, I am studying Apoptosis in *Crithidia*. The classic hallmarks of apoptosis have been observed in kinetoplastids. Such as, cell fragmentation, mitochondrial depolarization, and chromatin fragmentation. However, apoptosis in kinetoplastids is understudied, because the pathways are very different than in mammalian cells. This makes sense because they serve very different purposes. In mammalian cells, apoptosis protects the integrity of the entire organism by ensuring proper development and preventing diseases like cancer. But, in a single-celled organism, committing to apoptosis would mean the entire organism dies. Which on the surface does nit seem very good, but it is hypothesized this is a form of selfish altruism that selects for the fittest parasites and prevents hyper-parasitism in the host.

Next, I will discuss the genes I chose to study.

The BAX1 Inhibitory protein gene is a hypothetical protein whose function is inferred through gene ontology. It is predicted to localize to the mitochondrion where it acts as an inhibitor of apoptosis by inhibiting the proapoptotic BAX protein.

PGAM5, or Phosphoglycerate mutase family member 5 gene is also a hypothetical protein, whose function is inferred through gene ontology that is predicted to localizes to the mitochondrion. However, it is a proapoptotic gene that promotes apoptosis by activating ASK1, which is a kinase.

Next, I will talk about the optimization of CRISPR-Cas-9 in *Crithidia*.

In case you are not familiar with CRISPR-Cas9, here is a brief overview. Single-guide RNA binds to the Cas9 complex, which will then bind to the target site on the genome. There it will initiate a double stranded break. The cell will try to repair the break, because damaged DNA is bad. It

can do so either through non-homologous end joining, where base pairs are deleted and inserted randomly. This could result in a mutation or in rare cases the correct repair. The other method is homologous end joining. In this method, the homologous chromosome is used as a template to repair the break correctly. *Crithidia* is diploid and other trypanosomes prefer homology directed repair, so it is unlikely that non-homologous end-joining will happen. Which is actually a good thing, because we can provide a homologous repair cassette that will replace the target gene with a drug resistance that can then be selected for. This way, we will know exactly what the recombinant locus looks like.

To generate the homologous repair cassette, three pieces of DNA must be amplified: Two homology arms (one on each side of the target gene) and the drug resistance cassette. Then fusion PCR will combine those three pieces into the homologous repair cassette, which can then be cloned, and finally transfected.

The fusion PCR process works like this. The light blue strands represent the homology arms, which are from the *Crithidia* genome, and the green strand represents the drug resistance cassette, which is amplified from a plasmid. The place where they overlap is complimentary, so it binds during annealing along with the nested primers. Then this will be amplified into one long strand. This is the homologous repair cassette.

I have successfully generated the homologous repair cassette through fusion PCR for BAX1 Inhibitory protein gene. As you can see in the gel electrophoresis picture, the lane labelled "blast" is lower than all the lanes labelled "Blast fusion." Because of this increase, we know the homology arms were successfully added and the homologous repair cassette has been generated.

The fusion PCR process is still being optimized for PGAM5, but I have successfully generated the three pieces necessary: "blast" is the Blasticidin resistance cassette, "puro" is the puromycin resistance cassette, and 5′ and 3′ refer to the homology arms. This shows the primers designed for this do work.

Finally, Abby will go over our drug selection experiment.

Hello, my name is Abigail Delapenha and I will be talking about the drug selection portion of this experiment.

In the drug selection protocol, we had two selection drugs that we choose. One was Blasticidin and the other was puromycin. For each of our two selection drugs, Blasticidin and puromycin, we had two replicates. Each replicate was inoculated with one milliliter of *Crithidia*. We then allowed the *Crithidia* to grow until it reached its logarithmic growth stage. Once it reached its logarithmic growth stage, we were then ready to add drugs. For our puromycin drug selection, we added one microgram per milliliter of puromycin to one of our treatment groups and to our other treatment group we added 50 micrograms per milliliter of puromycin. We also had a control group which had no drugs. After the addition of drugs, we counted *Crithidia* for the next

seven days using a hemocytometer. These are the results of our drug selection count for *Crithidia* over seven days and this is all from puromycin. The blue line on this graph represents our control. This is our group with no addition of drugs. The orange line on this graph is our p1 group or our one microgram per milliliter concentration of puromycin. The gray line represents our p50 or our 50 microgram per milliliter concentration of puromycin. As you can see by comparing each of our treatment groups to the control. You can see our p50, our 50 microgram per milliliter concentration of puromycin inhibited the growth of *Crithidia*. While in our addition of one microgram per milliliter or puromycin led to an increased growth of crithidia. We are not sure what led to this increased growth of *Crithidia*, but since this increased growth was seen in both of our replicates. We are assuming that since one microgram per microliter is a very small concentration of drug, it is not enough to affect the growth of *Crithidi*a. Based on the results of our counts of *Crithidia* puromycin drug selection, we would proceed with using our p5o concentration, our 50 microgram per milliliter of puromycin because it led to an inhibition of growth of our *Crithidia*.

We are still in the process of doing our Blasticidin drug selection.

Thank you for listening!