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Trenton Dawson
Washington University in St. Louis

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Using the CRISPR/Cas9 System on Murine Cytomegalovirus

Trenton Dawson

Mentor: Wayne Yokoyama

The CRISPR/Cas9 system is a recently developed tool to quickly edit genomes. While most CRISPR research examines eukaryotic genomes due to their DNA repair processes, this project examines the use of CRISPR on murine cytomegalovirus (MCMV). The goal of this work was to familiarize ourselves with using the system on a virus. Since the CRISPR/Cas9 system cuts the DNA in a precise location but NHEJ is an imprecise method, the method of inactivation is largely unknown. Genes can be inactivated but their exact inactivating mutation can be quite unpredictable. We hope to use our findings to better characterize the molecular events that are occurring. Gene inactivation using the CRISPR/Cas9 system has extensive applications in the field of health and genetic research. Studies such as those described above examining the method of gene inactivation will help us to determine exactly how this complex functions.

Since the herpes viral family is non-zoonotic, trial and manipulation of the human herpes virus is difficult. Murine cytomegalovirus falls in the herpes cluster and provides a convenient and accessible analogue due to its remarkable similarity to human herpes virus and mouse host. After optimizing MCMV knockout methods, we explored the virus's immunoevasion capabilities through its ability to downregulate MHC-I receptors and elude NK cells. Using the CRISPR system on non-eukaryotic genomes, specifically those that evade the immune system with unknown molecular pathways, provides insight into viral-host interactions and using the CRISPR tool in novel ways.