Lydia Dupuis, Luke Domanico: Biological Science (Montana Tech)
Mentor: Joel Graff -- Biological Sciences (Montana Tech)
Targeted Deletion of IFNγ- and GM-CSF-Activated STAT Proteins

Various CRISPR-Cas systems act as adaptive immune system in the archaeal and bacterial domains. These systems utilize captured fragments of foreign genetic sequences to enable the prokaryote to defend against specific threats such as viral genomes. The CRISPR associated proteins (Cas), when expressed along with short segment of guide RNA (gRNA), are able to be used as tools for editing genomes with exquisite precision across all domains of life. Here, we created tools designed to employ CRISPR-Cas technology to target genes that code for STAT1 and STAT5A/B proteins and hypothesize that the resulting STAT knockout cells will be unable to adequately respond to transgenic leishmanial parasites expressing recombinant human IFNγ and GM-CSF, respectively. STAT1 and STAT5A oligonucleotide duplexes were successfully cloned into the pSpCas9(BB)-2A-EGFP plasmid at the tandem BbsI restriction sites. HEK293 cells were successfully transfected with the pSpCas9(STAT1)-2A-EGFP and pSpCas9(STAT5A)-2A-EGFP plasmids as demonstrated by EGFP expression in these cells. Monoclonal strains of HEK293 cells are being screened for unresponsiveness to STAT pathway-activating stimuli. Upon confirmation of successful gRNA-directed Cas9 mutations in STAT genes, lentiviral vectors containing these gRNA-encoding sequences will be used to similarly mutate human monocytic cell lines as an important tool for characterizing human IFNγ- and GM-CSF-expressing leishmanial parasite-mediated monocytic cell activation.