# Undergraduate Research Symposium May 18, 2018 Mary Gates Hall

# Online Proceedings

## POSTER SESSION 1

**MGH 258, Easel 179**

*11:00 AM to 1:00 PM*

**Novel Red Fluorescent Protein (RFP) Reporter for Quantifying Intracellular Invasion of *Shigellia flexneri***

*Shareef Shaheen, Junior, Extended Pre-Engineering*
*Mentor: Samuel Arnold, Medicine*
*Mentor: Molly McCloskey*

Utilizing the Type III Secretion System (T3SS), *Shigella spp.* uses a cascade of proteins to manipulate, penetrate, and colonize host eukaryotic cells. Inducing epithelial necrosis, *Shigella spp.* infection is responsible for moderate to severe diarrhea in millions of children and immunocompromised individuals — the majority from under-developed communities. Previous translational research on *Shigella spp.* has been limited due to the lack of proper in vitro and in vivo models. Understanding infectivity of *Shigella spp*. heavily relies on imprecise estimations of intracellular *Shigella spp*., which ultimately impacts vaccine and antibiotic efforts. This project aims to address this problem by developing a novel red intracellular reporter to quantify successful invasion of *Shigella flexneri*. By using a series of polymerase chain reaction (PCR) assemblies, we aim to construct a plasmid with a RFP reporter to be expressed during successful invasion of *Shigella spp*. The IpaH9.8 MxiE promoter, which has been shown to be expressed upon cell entry, has been integrated within the pUltra RFP plasmid through a Gibson-Reaction assembly and cloned using PCR. The new DNA replicate was electroporated into a streptomycin-resistant *S. flexneri* strain. We initially tested each strain in vitro by inoculating HCT-8 cells with the newly engineered *S. flexneri* and monitored for selective RFP expression by intracellular *S. flexneri*. The outcomes of this project will provide an accurate and efficient method of quantifying invasive *S. flexneri.* in vitro and in vivo, as well as quantifying efficacy of new antibiotic treatments. The implications of this project are crucial to the advancement of shigellosis research and in furthering the efforts of the international community to abate the rates of disease mortality and burden.

## POSTER SESSION 4

**Balcony, Easel 97**

*4:00 PM to 6:00 PM*

**Characterizing the Role of Chlamydia Inclusion Protein CT147 in Inclusion Formation and Pathogenesis**

*Forrest Michael Kwong, Junior, Biology (Molecular, Cellular & Developmental)*
*Mary Gates Scholar, UW Honors Program*
*Mentor: Kevin Hybiske, Medicine*

*Chlamydia trachomatis* is a human urogenital pathogen that is the leading cause of sexually transmitted infection worldwide. A major aim of the Hybiske lab is to develop a functional genetic understanding for *Chlamydia*, with a particular emphasis on known and predicted secreted type II and type III effector proteins that are injected into a host cell by the bacterium and predicted to play important roles in pathogenesis. I am studying a set of newly generated *C. trachomatis* chimeric mutants that were generated from interspecies lateral gene transfer between *C. trachomatis* and the mouse adapted species *C. muridarum*. This series of recombinant strains contain a differing extent of genetic exchange surrounding the predicted inclusion membrane protein (Inc) CT147. CT147 is predicted to be secreted into the *Chlamydia*-containing vacuole (inclusion) membrane by type III secretion, and subsequently mediate molecular interactions with host proteins. Interestingly, in cultured cells, this strain prematurely ruptures its resident vacuole at 24 hours post infection (hpi), in stark contrast to wildtype *C. trachomatis* or control recombinant strains that grow normally inside host cells and do not rupture vacuoles at any stage of infection. We therefore hypothesize that the *C. muridarum* ortholog of Inc-CT147 (which is significantly divergent from the *C. trachomatis* gene) is incompatible with the series of ˜30 Inc proteins normally secreted by *C. trachomatis*, in such a way that inclusion integrity is not properly maintained during this strain's developmental growth. We anticipate that a detailed molecular characterization of the function of this Inc protein will reveal important new insight into the mechanisms by which *Chlamydia* manipulate host cell function to facilitate their infection. My immediate focus is on characterizing Inc-CT147's role in inclusion formation through qRT-PCR to determine gene expression, immunofluorescence microscopy to characterize subcellular localization, and co-immunoprecipitation and mass spectrometry to identify CT147 interaction targets.