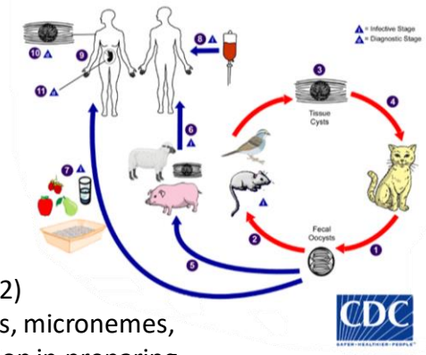
# *Toxoplasma gondii* SUB4 Gene Deletion Mutants and Their Viability in Host Cell Invasion

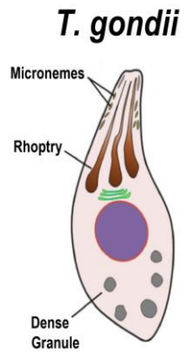
John Asmus and Dr. Swati Agrawal

University of Mary Washington

## Background & Significance

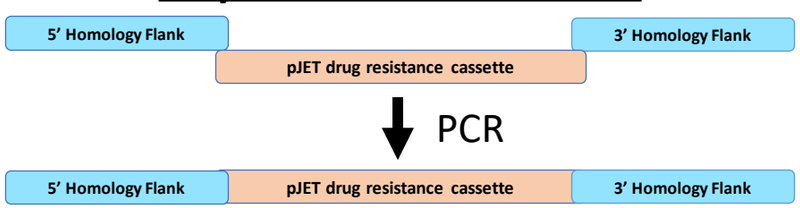
- *Toxoplasma gondii* is a single-celled parasitic protist that causes toxoplasmosis. It is a good model organism for studying Acomplexans, a phylum with pathogens that cause potentially deadly diseases such as cryptosporidiosis and malaria.
- Toxoplasmosis is not a major health threat for most people, but immunocompromised individuals and the babies of pregnant women who are infected can have serious health problems including blindness.
- Subtilisin like proteins (SUB1-12) are secreted from the rhoptries, micronemes, and dense granules, and function in preparing other proteins for adhesion and invasion.
- SUB1 processes microneme adhesion proteins.
- SUB2 processes Rhoptry Protein 1 (ROP1).
- Question: Is the SUB4 gene vital for *T. gondii* host cell invasion?
- Objective: Create gene deletion mutants in ΔKU80 *T. gondii* tachyzoites by targeting the SUB4 gene locus with 3' and 5' sgRNA primers and replace it with a homologous repair template. Then examine the mutants' ability to invade and replicate inside cultured human foreskin fibroblast cells.




## Techniques

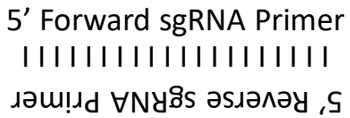
### Polymerase Chain Reaction



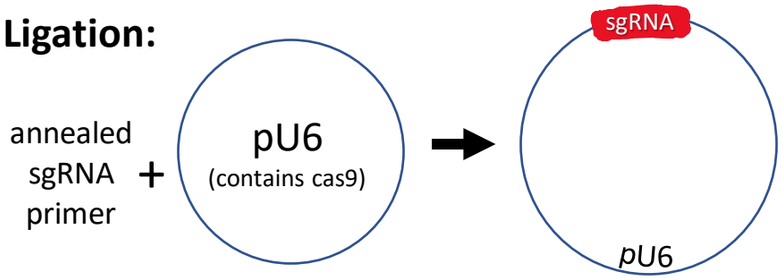

Homologous Repair Template

### SUB4 sgRNA / pU6 Plasmid Construction

**Annealing:**



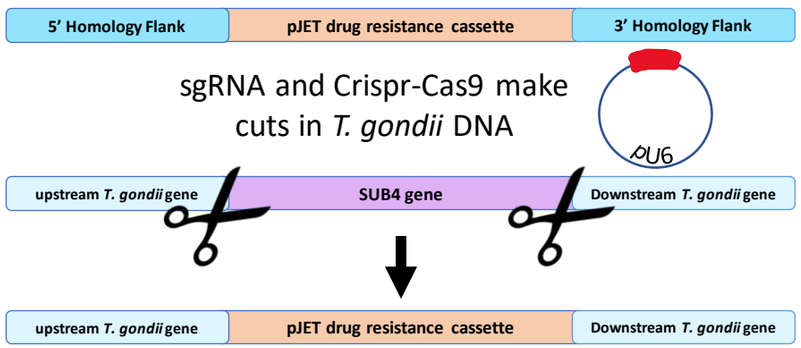

**Ligation:**




**Bacterial Transformation:**

### Transfection

Homologous Repair Template matches *T. gondii* genome



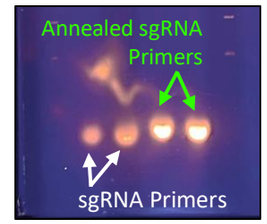

Drug resistance cassette replaces SUB4 gene

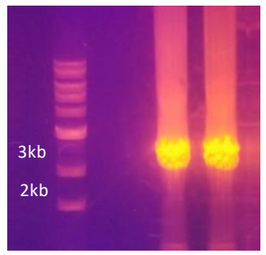
## References

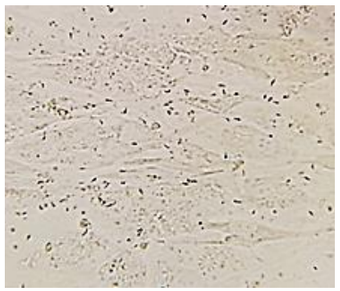
• CDC • English, Adomako-Ankomah, & Boyle • Fox, Et. Al
•ThermoFisher Scientific

## Results

- sgRNA and homology flank primers designed for SUB4
- Gel Electrophoresis (top image) shows sgRNA primers. The right lanes ran higher because the successfully annealed double stranded sgRNA primers are twice the size of the single stranded sgRNA primers.
- Homologous repair template successfully created for SAG1 and SUB4.
- Gel Electrophoresis (middle image) shows homologous repair template. The leftmost lane shows a DNA ladder. The right two lanes show the homologous repair template after construction with PCR at about 2.6 kb.
- Currently working towards successful ligation of sgRNAs in pU6.
- HFF cells grown to create stock for later use in invasion and egress studies.
- *T. gondii* ΔKU80 cells grown in HFF cells (bottom image) to create stock for later use in transfection and invasion and egress studies.



## Future Work

- I plan to return as an alum this summer to continue this research. After successful ligation of sgRNAs in pU6 I will transfect the ΔKU80 *T. gondii* cells. This will be followed by drug selection to verify whether the SUB4 gene was successfully knocked out. Finally, I will conduct invasion and egress studies to determine SUB4's vitality in host cell invasion.

## Acknowledgements

- Thank you to Dr. Swati Agrawal, the greatest research mentor I could ever ask for, to the UMW Biology Department, and to all the donors who make this research possible!