Fall 2023

Examining the Role of CtEG using Drosophila

JaDaya V. Davis
Ja889449@ucf.edu

Om Patel
ompatel@ucf.edu

Sebastian Velez
sebastianvelez@ucf.edu

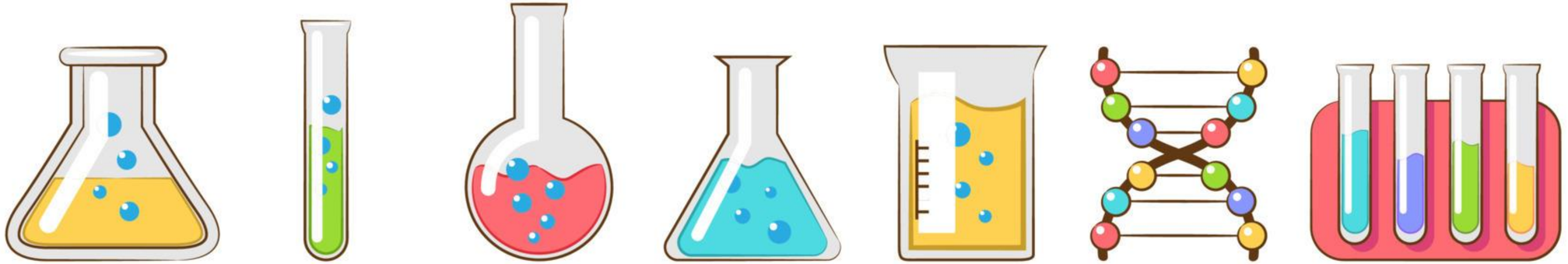
Fall 2023

Examining the Role of CtEG using Drosophila

JaDaya V. Davis

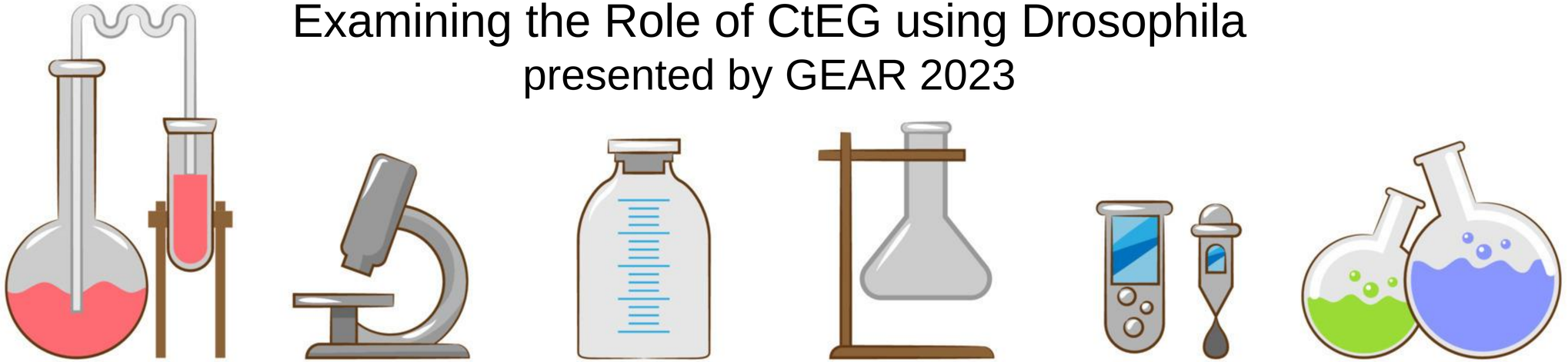
Om Patel

Sebastian Velez



Examining the Role of CtEG using Drosophila

presented by GEAR 2023



Project Summary

Within our GEAR course the project was centered around setting up viable models using Drosophila to evaluate the relevance of the N terminus portion of translocating actin recruiting phosphoprotein (N-tarp), in the effector genes of *Chlamydia trachomatis (C. trachomatis)*. One of the leading inclinations that encouraged interest in this portion of the gene is that it is found to be conserved among multiple, if not all, pathogenic chlamydial species. The significance in conservation is well known in the world of science and an indication of possible importance to a species life cycle and molecular maintenance.

There are multiple effector genes involved in the molecular functions of *C. trachomatis* with prominent studies primarily focusing on the C terminal end however there isn't remotely any regarding the molecular value of the N terminus of tarp. So, to address the unknown surrounding N-tarp our lab began with developing the plasmid containing an assigned gene of interest and vector; this was done through series of plate isolations, PCR's, gel electrophoresis chromatography's, and quantifications using nano-drop technique.

The gene of interest in our case was the effector gene ctEG that was inserted into *E. coli* in order to be cloned via polymerase chain reactions, then confirmed using gel electrophoresis and quantified using nano-drop technique. Similar process was done to isolate vector pUAST; pUAST is a vector system that is well studied and effective in generating transgenic flies and controlling transgene expression. After ctEG and pUAST were properly isolated and confirmed, DNA clean ups were performed using mini-prep protocol and then each component underwent a double digestion using NotI and KpnI. By double digesting with unique restriction enzymes, it ensured that pUAST and ctEG was ligated together in correct orientation; From there a confirmational screening was run on *E. coli* transformants, by doing serial dilutions that allowed for isolated colonies to be sampled. Transformants of at least 8 colonies were separated into samples that were amplified using PCR, then ran on an agarose gel to confirm transformants with ctEG (gene of interest/effector gene) aptly within the pUAST vector.

The identified E. coli transformants were then sent to a laboratory where they will be inserted into Drosophila allowing for N-tarp to be observed through physical characteristics and molecular evaluations. From this research, future studies regarding the relevance and effects that the N-tarp portion of the effector genes has on *C. trachomatis* bacterial cycle, survival, proliferation, and ability to alter its host cell are now possible.

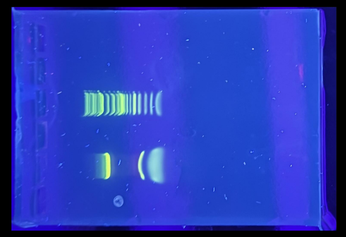
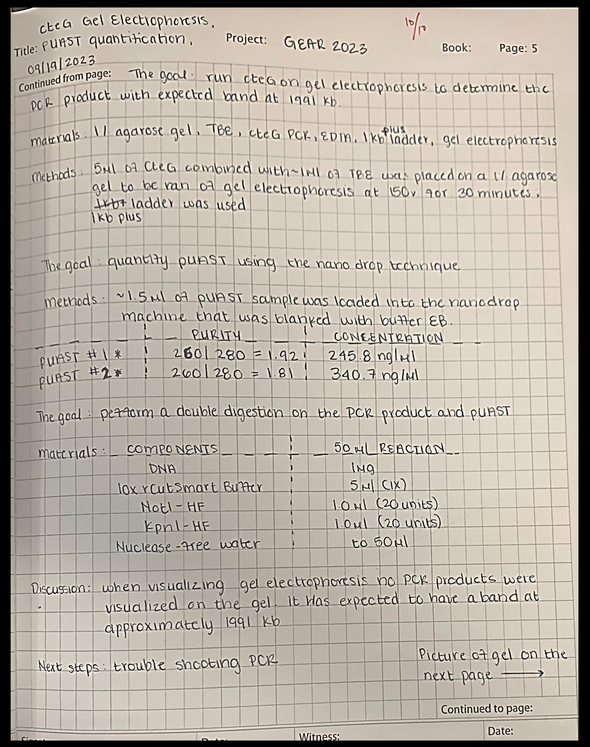


Image 1: Desired DNA fragment purified from gel electrophoresis in UV box



ctEG Gel Electrophoresis,
Title: pUAST quantification, Project: GEAR 2023 Book: Page: 5
09/19/2023
Continued from page: The goal: run ctEG on gel electrophoresis to determine the PCR product with expected band at 1991 kb.

Materials: 1% agarose gel, TBE, ctEG PCR, EDTA, 1kb plus ladder, gel electrophoresis

Methods: 5ul of ctEG combined with ~1ul of TBE was placed on a 1% agarose gel to be ran on gel electrophoresis at 150v for 30 minutes. ~~1kb~~ ladder was used
1kb plus

The goal: quantify pUAST using the nano drop technique

Methods: ~1.5ul of pUAST sample was loaded into the nanodrop machine that was blanked with buffer EB

	PURITY	CONCENTRATION
pUAST #1*	260/280 = 1.92	245.8 ng/ul
pUAST #2*	260/280 = 1.81	340.7 ng/ul

The goal: perform a double digestion on the PCR product and pUAST

Materials:

COMPONENTS	50 ul REACTION
DNA	1ug
10x rCutSmart Buffer	5ul (1X)
NotI-HF	1.0ul (20 units)
KpnI-HF	1.0ul (20 units)
Nuclease-free water	to 50ul

Discussion: when visualizing gel electrophoresis no PCR products were visualized on the gel. It was expected to have a band at approximately 1991 kb

Next steps: trouble shooting PCR

Picture of gel on the next page →

Continued to page:

Witness: Date:

Image 2: Laboratory notebook on CtEG gel electrophoresis and pUAST quantification

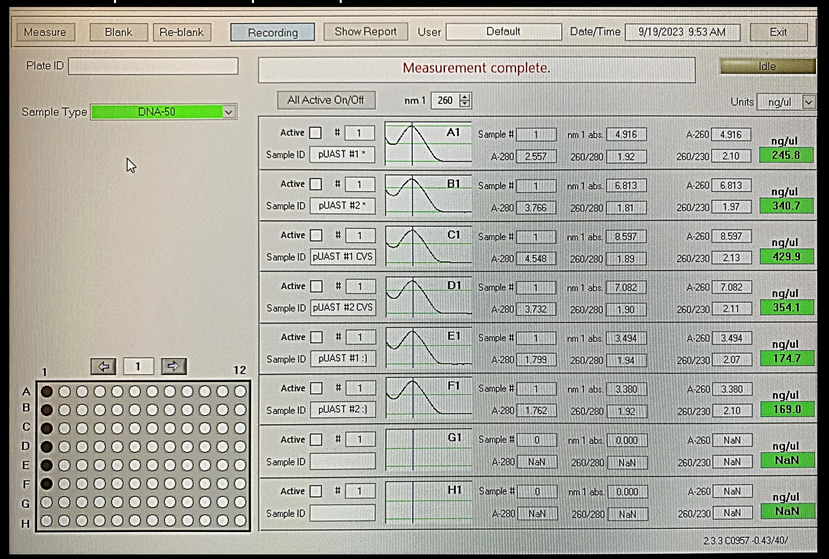
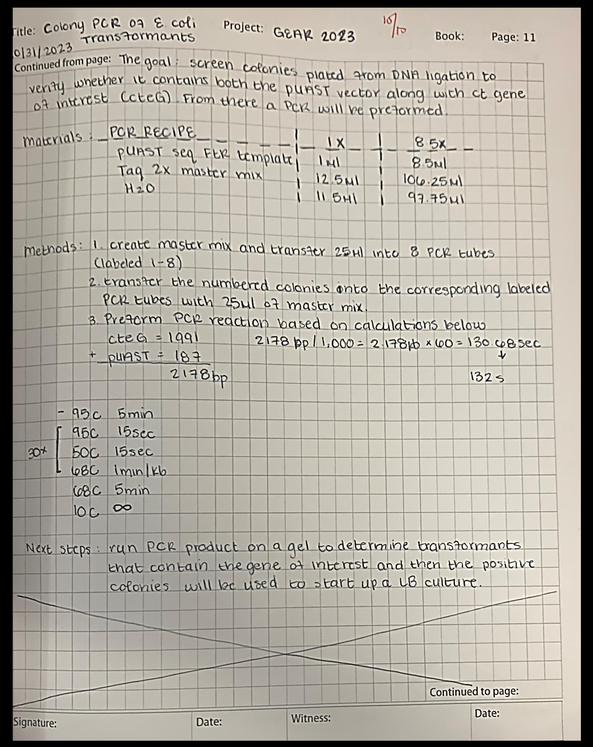


Image 4: Results from nano-drop quantification of pUAST vector



Title: Colony PCR of E coli transformants Project: GEAR 2023 Book: Page: 11
01/31/2023
Continued from page: The goal: screen colonies plated from DNA ligation to verify whether it contains both the pUAST vector along with ct gene of interest (ctEG). From there a PCR will be preformed.

Materials:

PCR RECIPE	1X	8.5X
pUAST seq PCR template	1ul	8.5ul
Taq 2x master mix	12.5ul	106.25ul
H2O	11.5ul	97.75ul

Methods:
1. create master mix and transfer 25ul into 8 PCR tubes (labeled 1-8)
2. transfer the numbered colonies onto the corresponding labeled PCR tubes with 25ul of master mix
3. Preform PCR reaction based on calculations below
ctEG = 1991 2178 bp / 1,000 = 2.178kb × 60 = 130.68 sec
+ pUAST = 187 ↓
2178bp 132s

- 95C 5min
30× 95C 15sec
50C 15sec
68C 1min/kb
68C 5min
10C ∞

Next steps: run PCR product on a gel to determine transformants that contain the gene of interest and then the positive colonies will be used to start up a LB culture.

Continued to page:

Signature: Date: Witness: Date:

Image 3: Laboratory notebook colony PCR of *E. coli* transformants

Image 5: Drosophila melanogaster (N.d.). photograph. Retrieved from https://www.pghr.org/post/the-impact-of-inhaled-pollution-on-health-outcomes-in-drosophila-melanogaster.

N.d.-a). photograph. Retrieved from https://economictimes.indiatimes.com/magazines/panache/pills-or-injections-pharmaceutical-scientist-explains-science-behind-medication-intake/articleshow/92303954.cms?from=mdr.

Community Impact

This research on the N terminus of the translocating actin recruiting phosphoprotein (N-tarp) within *Chlamydia trachomatis* offers pivotal insights with profound community implications. As this gene segment is conserved across various pathogenic chlamydial species, exploring its molecular relevance could revolutionize disease management strategies. *Chlamydia trachomatis* infections pose significant public health challenges globally, making the investigation into its effector genes, particularly the overlooked N terminus of tarp, a vital endeavor.

By unraveling the molecular significance of this gene segment, this research might pave the way for innovative therapeutic interventions or preventative measures against *Chlamydia trachomatis* (Pais). The project's meticulous methodology, involving plasmid creation and genetic manipulation in Drosophila, sets a precedent for rigorous scientific exploration. This not only advances our understanding within the scientific community but also showcases a template for precision and methodology that can inspire future research endeavors, potentially reshaping approaches to combatting chlamydial infections and impacting broader scientific domains.

Course Connection

Research on the N terminus of the translocating actin recruiting phosphoprotein (N-tarp) within Chlamydia trachomatis perfectly mirrors the ethos of UCF's GEAR course. GEAR, known for its collaborative, interdisciplinary approach, finds resonance in this study's fusion of molecular biology, genetics, and microbiology. This project, nestled within the faculty instructor's program, embodies GEAR's focus on hands-on, team-oriented projects aligned with concept-driven lectures.

The research methodology, from plasmid creation to utilizing the Drosophila model, echoes the structured and practical approach advocated by GEAR. Exploring uncharted territories within Chlamydia trachomatis effector genes, the project embodies the curiosity, collaboration, and interdisciplinary spirit central to GEAR. It provides students with real-world research experiences while emphasizing teamwork and diverse skill integration, core tenets of the GEAR curriculum.

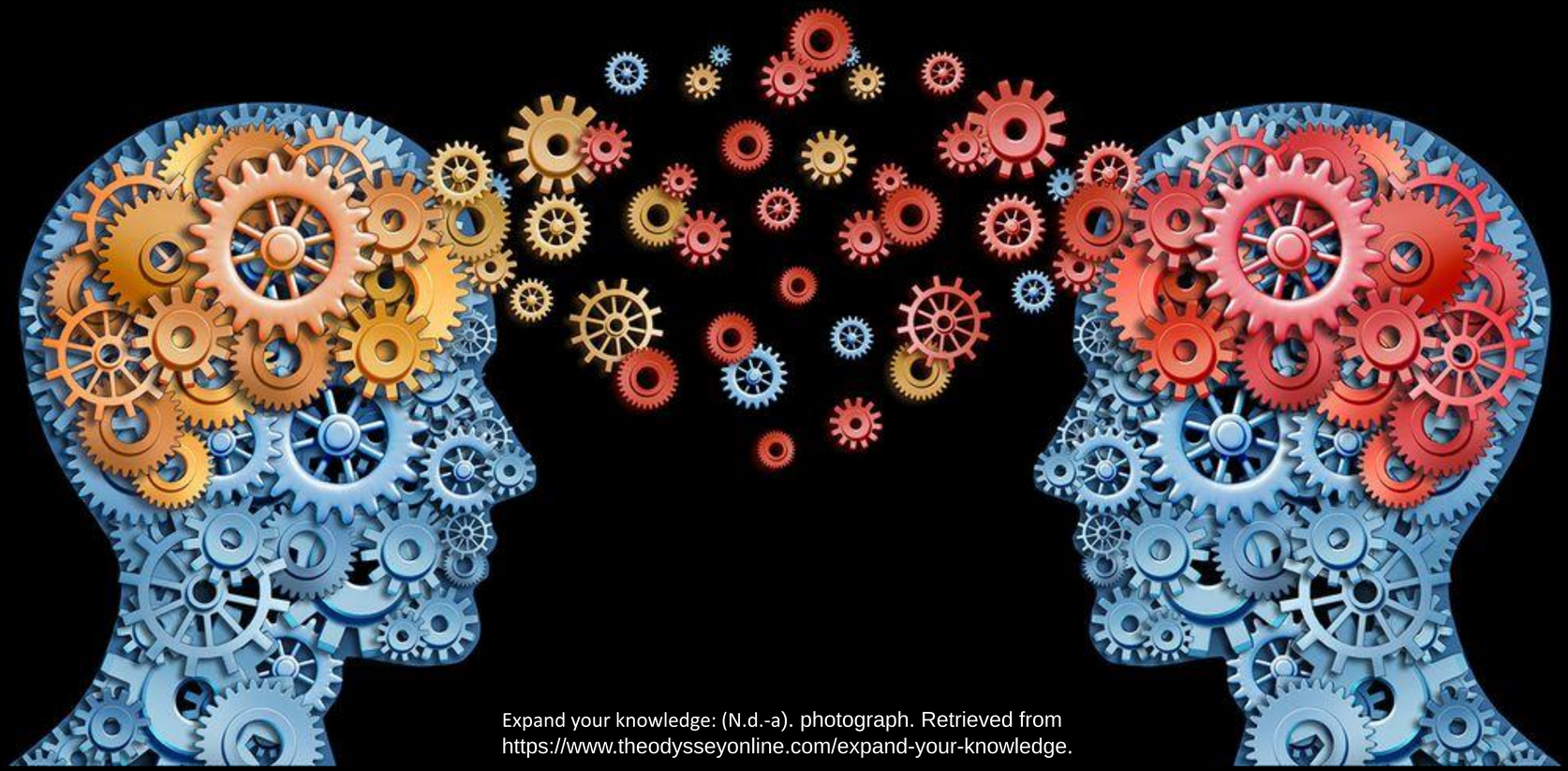
(N.d.-a). photograph. Retrieved from https://www.freepik.com/premium-vector/graph-with-decrease-report-diagram-with-recession-bankruptcy-progress-business-finance-vector-illustration_21721517.htm

Civic Responsibility

The project in this course allowed for greater knowledge of *Chlamydia trachomatis*, as well as learning how important effector genes of *C. trachomatis* work. This can help to expand our knowledge of the disease, and furthermore pave the way for discovery into its more complex mechanisms and physiology. Studying *C. trachomatis* helped us learn many important biomedical laboratory techniques that allowed for purification and amplification of the effector gene DNA. It allowed us to become more informed members of our community to spread greater awareness on how this disease works. In this way, patients and scientists alike will be able to use this work as a framework from which to branch off to study a variety of different illnesses.

Reflection

This project showcases the approach to biomedical research. Proper methodology is critical when working through the various experiments that were completed with the effector genes. We learned important problem-solving skills that allowed us to think of how to proceed with our experiments in the midst of setbacks. For example, when our PCR product did not work as anticipated, we troubleshooted our problem by modifying the annealing temperature to allow for the expression of our desired PCR product. Overall, these skills allowed us to become better-prepared scientists in an environment that constantly requires change and modification to ensure success.



Expand your knowledge: (N.d.-a). photograph. Retrieved from https://www.theodysseyonline.com/expand-your-knowledge.

Project Summary

Within our GEAR course the project was centered around setting up viable models using Drosophila to evaluate the relevance of the N terminus portion of translocating actin recruiting phosphoprotein (N-tarp), in the effector genes of *Chlamydia trachomatis (C. trachomatis)* (Pais et al). One of the leading inclinations that encouraged interest in this portion of the gene is that it is found to be conserved among multiple, if not all, pathogenic chlamydial species. The significance in conservation is well known in the world of science and an indication of possible importance to a species life cycle and molecular maintenance.

There are multiple effector genes involved in the molecular functions of *C. trachomatis* with prominent studies primarily focusing on the C terminal end however there isn't remotely any regarding the molecular value of the N terminus of tarp. So, to address the unknown surrounding N-tarp our lab began with developing the plasmid containing an assigned gene of interest and vector; this was done through series of plate isolations, PCR's, gel electrophoresis chromatography's, and quantifications using nano-drop technique.

The gene of interest in our case was the effector gene ctEG that was inserted into *E. coli* in order to be cloned via polymerase chain reactions, then confirmed using gel electrophoresis and quantified using nano-drop technique. Similar process was done to isolate vector pUAST; pUAST is a vector system that is well studied and effective in generating transgenic flies and controlling transgene expression. After ctEG and pUAST were properly isolated and confirmed, DNA clean ups were performed using mini-prep protocol and then each component underwent a double digestion using NotI and KpnI. By double digesting with unique restriction enzymes, it ensured that pUAST and ctEG was ligated together in correct orientation; From there a confirmational screening was run on *E. coli* transformants, by doing serial dilutions that allowed for isolated colonies to be sampled. Transformants of at least 8 colonies were separated into samples that were amplified using PCR, then ran on an agarose gel to confirm transformants with ctEG (gene of interest/effector gene) aptly within the pUAST vector.

The identified E. coli transformants were then sent to a laboratory where they will be inserted into Drosophila allowing for N-tarp to be observed through physical characteristics and molecular evaluations. From this research, future studies regarding the relevance and effects that the N-tarp portion of the effector genes has on *C. trachomatis* bacterial cycle, survival, proliferation, and ability to alter its host cell are now possible.

Image 1: Desired DNA fragment purified from gel electrophoresis in UV box

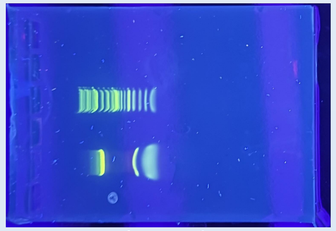


Image 1

Community Impact

This research on the N terminus of the translocating actin recruiting phosphoprotein (N-tarp) within *Chlamydia trachomatis* offers pivotal insights with profound community implications. As this gene segment is conserved across various pathogenic chlamydial species, exploring its molecular relevance could revolutionize disease management strategies. *Chlamydia trachomatis* infections pose significant public health challenges globally, making the investigation into its effector genes, particularly the overlooked N terminus of tarp, a vital endeavor.

By unraveling the molecular significance of this gene segment, this research might pave the way for innovative therapeutic interventions or preventative measures against *Chlamydia trachomatis* (Pais et al.). The project's meticulous methodology, involving plasmid creation and genetic manipulation in Drosophila, sets a precedent for rigorous scientific exploration. This not only advances our understanding within the scientific community but also showcases a template for precision and methodology that can inspire future research endeavors, potentially reshaping approaches to combatting chlamydial infections and impacting broader scientific domains.

Course Connection

Research on the N terminus of the translocating actin recruiting phosphoprotein (N-tarp) within Chlamydia trachomatis perfectly mirrors the ethos of UCF's GEAR course. GEAR, known for its collaborative, interdisciplinary approach, finds resonance in this study's fusion of molecular biology, genetics, and microbiology. This project, nestled within the faculty instructor's program, embodies GEAR's focus on hands-on, team-oriented projects aligned with concept-driven lectures.

The research methodology, from plasmid creation to utilizing the Drosophila model, echoes the structured and practical approach advocated by GEAR. Exploring uncharted territories within Chlamydia trachomatis effector genes, the project embodies the curiosity, collaboration, and interdisciplinary spirit central to GEAR. It provides students with real-world research experiences while emphasizing teamwork and diverse skill integration, core tenets of the GEAR curriculum.

Civic Responsibility

The project in this course allowed for greater knowledge of *Chlamydia trachomatis*, as well as learning how important effector genes of *C. trachomatis* work. This can help to expand our knowledge of the disease, and furthermore pave the way for discovery into its more complex mechanisms and physiology. Studying *C. trachomatis* helped us learn many important biomedical laboratory techniques that allowed for purification and amplification of the effector gene DNA. It allowed us to become more informed members of our community to spread greater awareness on how this disease works. In this way, patients and scientists alike will be able to use this work as a framework from which to branch off to study a variety of different illnesses.

Reflection

This project showcases the approach to biomedical research. Proper methodology is critical when working through the various experiments that were completed with the effector genes. We learned important problem-solving skills that allowed us to think of how to proceed with our experiments in the midst of setbacks. For example, when our PCR product did not work as anticipated, we troubleshooted our problem by modifying the annealing temperature to allow for the expression of our desired PCR product. Overall, these skills allowed us to become better-prepared scientists in an environment that constantly requires change and modification to ensure success.

References

Pais, Sara V et al. "CteG is a Chlamydia trachomatis effector protein that associates with the Golgi complex of infected host cells." Scientific reports vol. 9,1 6133. 16 Apr. 2019, doi:10.1038/s41598-019-42647-3

Acknowledgements

Special thanks to Dr. George Aranjuez, instructor of the course, for his effort and dedication in ensuring each concept and experiment was understood by all while sharing his passion and dedication for his work.