

# V CMB

## UNDERGRADUATE

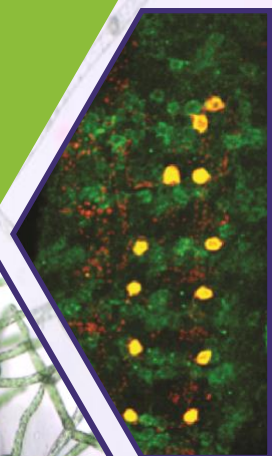
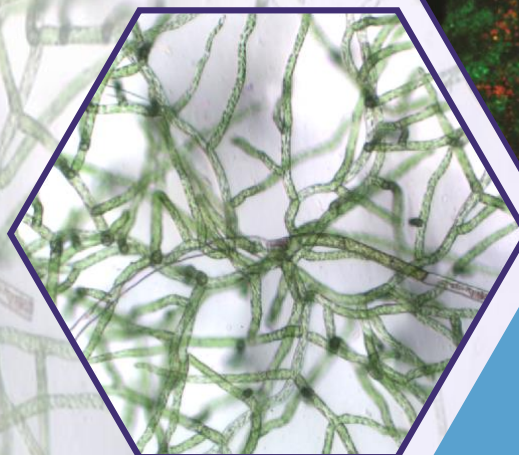
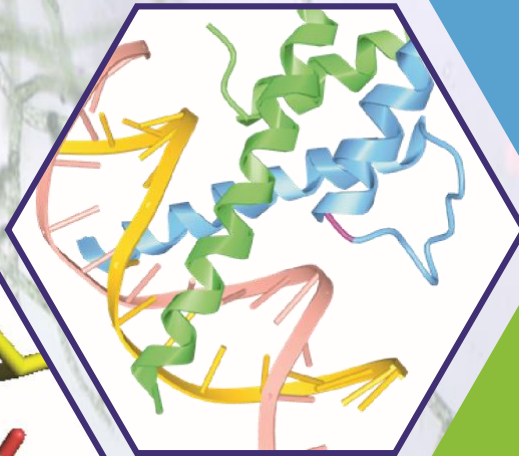
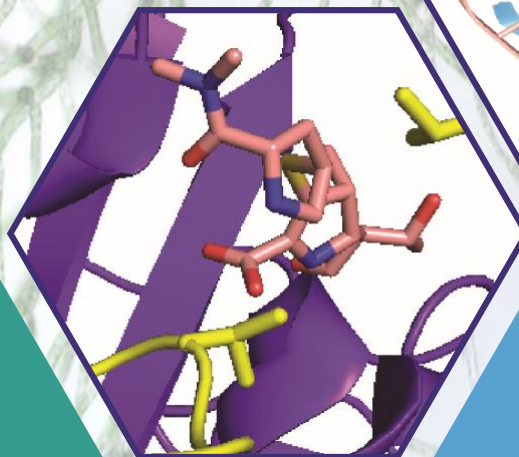
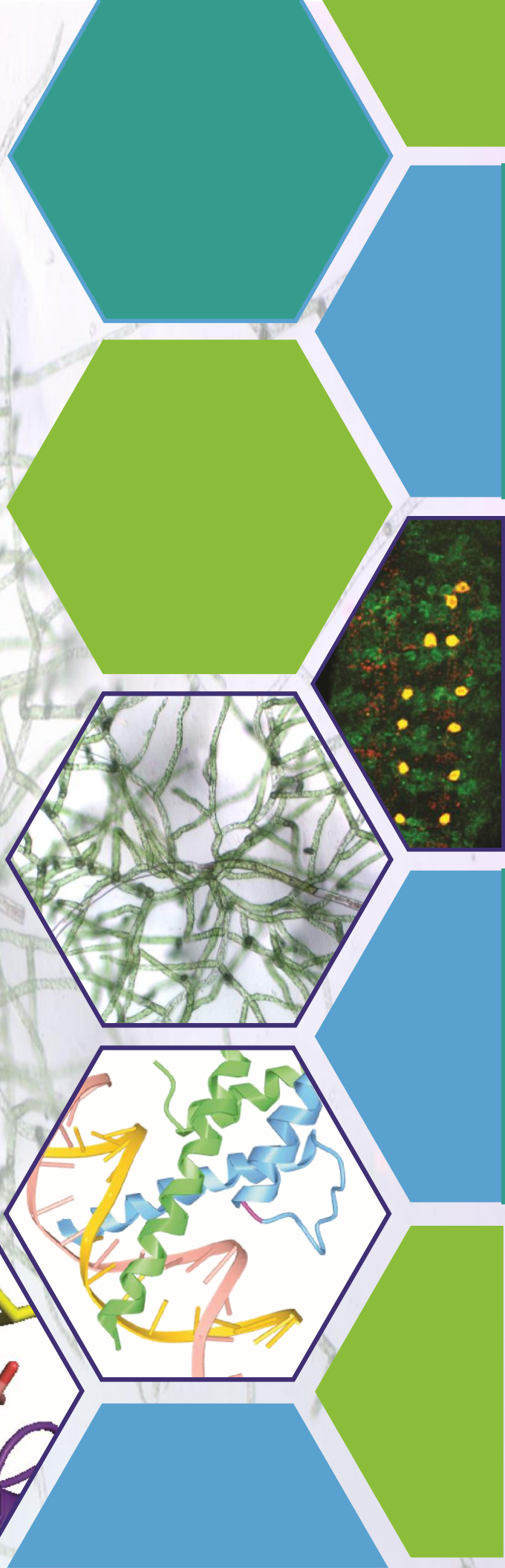
# RESEARCH

# SYMPOSIUM

Saturday, April 13th

Grand Valley

State University



## Schedule

### Morning Session (9am-12pm):

### Studies of Mutation Effects: Antibiotics, Proteins, and Crop Producing Plants

<b>Opening and Introduction</b>		<b>9-9:15</b>
<b>Emma Krueger</b>	<b>Clinical Mutations in Penicillin Binding Protein 3 from <i>A. baumannii</i> Linked to Lower Binding Affinity for <math>\beta</math>-Lactam Antibiotics</b>	<b>9:15-9:40</b>
<b>Carolina Reis</b>	<b>Analyzing the Effect of Degraded Proteins on the Control of <i>Candida Albicans</i> Filamentation</b>	<b>9:40-10:05</b>
<b>Coffee break</b>		
<b>Lauren Chunn</b>	<b>An Automated Approach to Biomarker Curation Reconciles Inconsistency Among Commercial NGS Panels</b>	<b>10:20-10:45</b>
<b>Christina Wheeler</b>	<b>Investigating Intraindividual Variation and Mutation in an Apple Tree (<i>Malus domestica</i>)</b>	<b>10:45-11:10</b>
<b>Andrew Freiman</b>	<b>Differential Gene Expression Underlying Abnormal Tip Growth of a <i>P. patens</i> Retrotransposon-Disrupted Mutant</b>	<b>11:10-11:35</b>
<b>Lunch break</b>	<b>Popular vote for the Best Speaker in the Morning Session</b>	

**Afternoon Session (12:30-3pm):**  
**Drosophila and Dopamine: Histamine in Model Organisms and Parkinson's Disease**

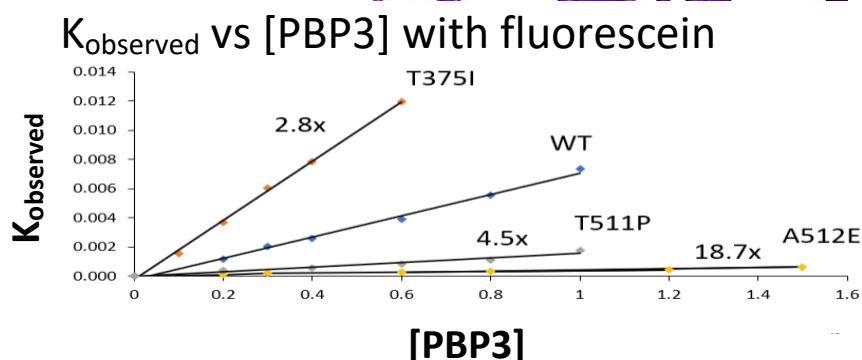
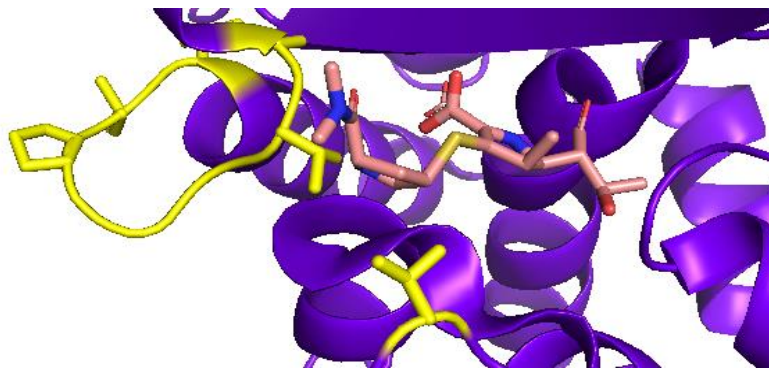
<b>Kelly Tekiela</b>	<b>Functional Characterization of Histamine in <i>D. melanogaster</i> Enteric Tissue</b>	<b>12:35-1</b>
<b>Brittany Vandenberg</b>	<b>Comparative localization of histidine decarboxylase and histamine in peripheral and central neural tissues of <i>Drosophila melanogaster</i></b>	<b>1-1:25</b>
<b>Coffee break</b>		
<b>Katelyn Anthony</b>	<b>MicroRNAs in Urine as Detection Biomarkers for Parkinson's Disease</b>	<b>1:40-2:05</b>
<b>Melina Frantzeskakis</b>	<b>The Influence of <i>Nato3</i> on Genes Involved in Dopamine Neurogenesis and Neuroprotection</b>	<b>2:05-2:30</b>
<b>Awards</b>	<b>Popular vote for the Best Speaker in the Afternoon Session Best Speaker in a Session / Faculty Award for Best Seminar</b>	

# Clinical Mutations in Penicillin Binding Protein 3 from *A. baumannii* Linked to Lower Binding Affinity for $\beta$ -Lactam Antibiotics

E.J. Krueger<sup>1</sup>, C. June, D. Leonard<sup>1</sup>

Mentor: Dr. D. L. Leonard<sup>1</sup>

<sup>1</sup>Department of Chemistry, Grand Valley State University, Allendale, MI



The gram-negative bacteria *Acinetobacter baumannii* lead to serious infections in hospital settings. Infections caused by these bacteria are usually treated with carbapenems, but resistance to these drugs has become a critical problem. We investigated six clinical variants in the penicillin-binding protein 3 (PBP3), T375I, T506I, P508L, G510A, T511P, and A512E, from *A. baumannii* and tested their ability to bind  $\beta$ -lactam antibiotics. We hypothesized that these substitutions, located in or near the active site, might affect the binding of antibiotics such as penicillin or meropenem. The six mutants were created by overlap extension PCR and transformed into BL21 cells where IPTG was used to induce expression to further purify the protein by cobalt column. Fluorescence anisotropy was used to monitor the binding affinity of the fluorescent drugs, bocillin and fluorescein meropenem, at different concentrations. We found that mutant T375I was 2.1- and 2.8-times faster binding than the WT PBP3 binding bocillin and fluorescein meropenem, respectively. The mutant T511P was 6.4- and 4.5-times slower binding than the WT PBP3 and mutant A512E was 14.1- and 18.7-times slower binding than the WT PBP3 binding bocillin and fluorescein meropenem, respectively. The information gained by this study will be valuable in the future work of synthesizing more effective antibiotics.



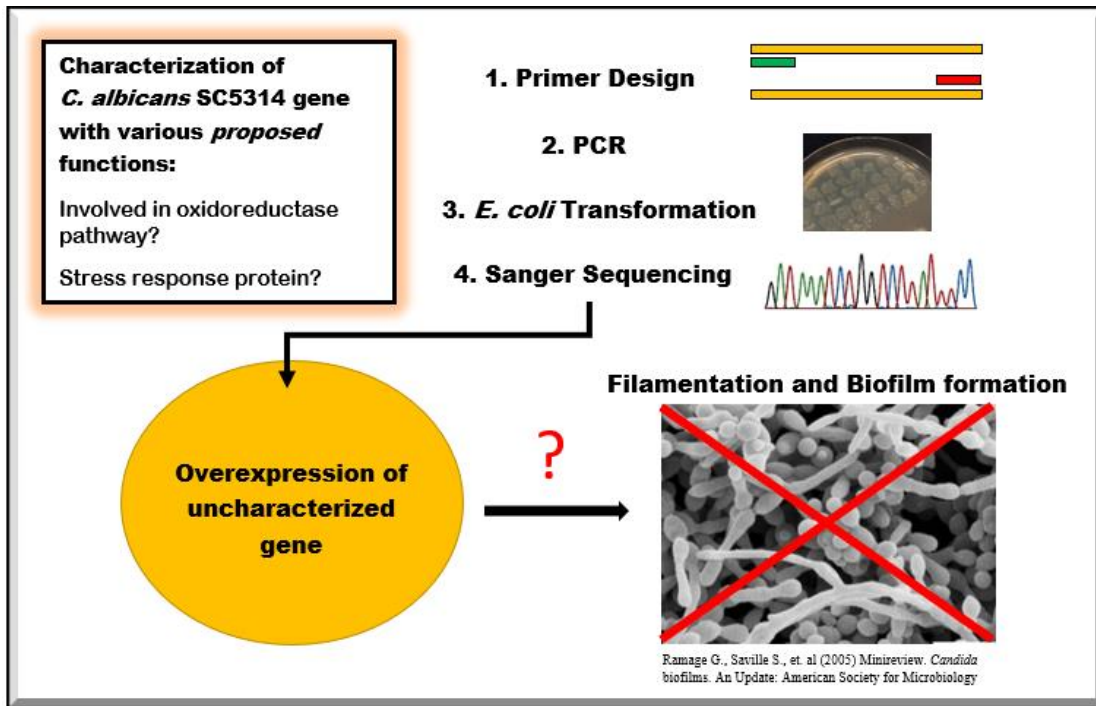
# Analyzing the Effect of Degraded Proteins on the Control of *Candida Albicans* Filamentation

Carolina Reis<sup>1,2</sup>

Mentor: Derek Thomas<sup>1</sup>

<sup>1</sup> Department of Biomedical Science, Grand Valley State University, Allendale MI

<sup>2</sup> Department of Cell and Molecular Biology, Grand Valley State University, Allendale MI



*Candida albicans* is a fungal opportunistic pathogen that naturally colonizes humans at multiple locations in and on the body. It is a leading cause of nosocomial infections and has high mortality rates, particularly for immunocompromised patients. *C. albicans* can develop into yeast or filamentous forms, the latter of which have been linked to the organism's pathogenicity and ability to infect hosts. Previous studies in our lab have identified certain proteins whose presence may negatively influence the transition to filamentation. Our current understanding of the function of these proteins is limited and additional investigation is needed to fully understand the relationship between their expression levels and the suppression or development of the filamentous forms. To further explore this, we have taken the approach of overexpressing some of the recently identified proteins under conditions that promote filamentous growth of *C. albicans* and found varied effects on morphology.

# An Automated Approach to Biomarker Curation Reconciles Inconsistency Among Commercial NGS Panels

Lauren Chunn<sup>1,5</sup>, Mark J. Kiel<sup>1</sup>, Ryan Tarpey<sup>1,2</sup>, Diane Nefcy<sup>1</sup>, Steven Schwartz<sup>1</sup>, Megan S. Lim<sup>3</sup>, Kojo S.J. Elenitoba-Johnson<sup>4</sup>

Mentor: Mark J. Kiel

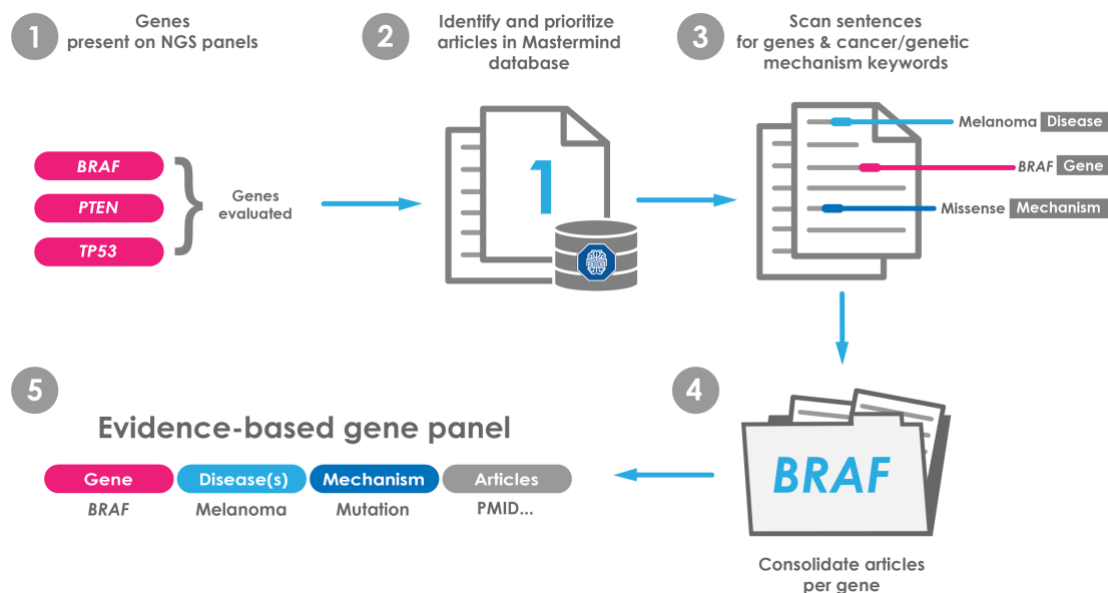
<sup>1</sup>Genomenon, Inc., Ann Arbor, MI

<sup>2</sup>University of Michigan, Department of Pharmacology, Ann Arbor, MI

<sup>3</sup>University of Pennsylvania, Department of Hematopathology, Philadelphia, PA

<sup>4</sup>University of Pennsylvania, Center for Personalized Diagnostics, Philadelphia, PA

<sup>5</sup>Grand Valley State University, Department of Cell and Molecular Biology, Allendale, MI



Gene panels are frequently used in clinical practice to reveal or confirm diagnoses, to inform prognostic determinations, and to tailor therapy to a particular molecular etiology. The selection of which genes to include as biomarkers on these panels is often highly subjective owing to variable inclusion criteria and the difficulty of manually curating biomarkers from the millions of available genetic articles. This results in the potential for substantial disagreement between panels. We aimed to investigate the consistency among commercially available gene panels marketed as comprehensive for somatic or hereditary cancers, and to assess whether an automated approach to biomarker curation could provide a more reliable framework for gene panel design. Our detailed analysis of a subset of 8 hereditary cancer panels revealed substantial discordance with levels of disagreement increasing proportionally to the size of the panel. Utilizing an automated and systematic approach to biomarker curation, we also assessed the validity of each of the genes included on these 8 panels. This technique proved to be useful to quickly provide sufficient evidence for inclusion or exclusion of biomarkers, and can provide a means to reconcile the inconsistency among commercially available NGS panels.

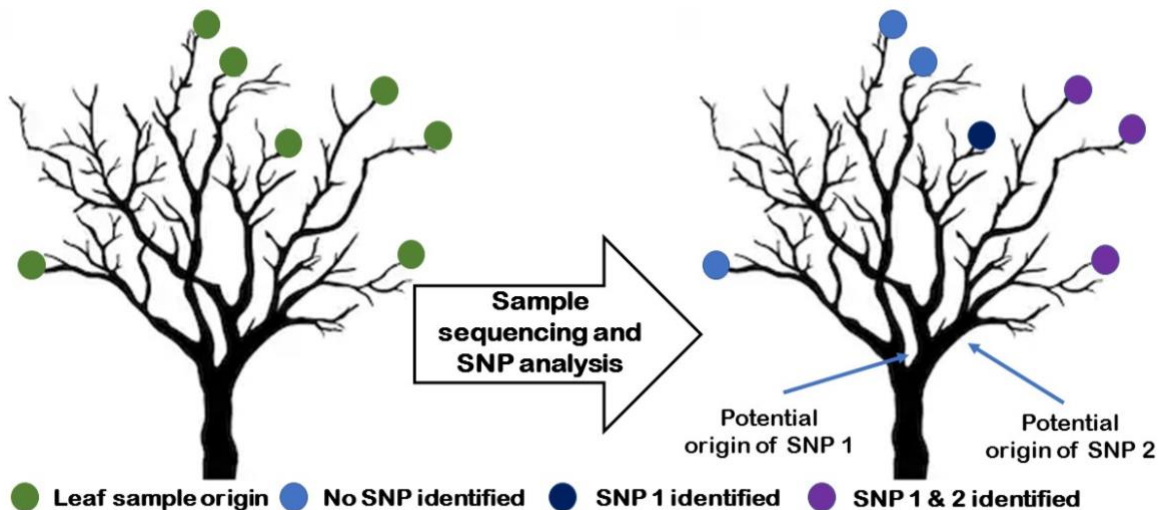
# Investigating Intra-individual Variation and Mutation in an Apple Tree (*Malus domestica*)

Christina Wheeler<sup>1</sup>, Gillian L. Ryan<sup>3</sup> & Salomon Turgman-Cohen<sup>4</sup>

Mentor: Jim Cohen<sup>2\*</sup>

<sup>1</sup>Department of Cell and Molecular Biology, Grand Valley State University, Allendale, MI

<sup>2</sup>Applied Biology, <sup>3</sup>Physics, <sup>4</sup>Chemical Engineering, Kettering University, Flint MI



In plants, somatic and germline cells are not distinct from one another, meaning any somatic mutations could potentially be carried through to new segments of the plant and to gametes. Specifically, within trees, modular growth patterns allow these somatic mutations to be passed to unique segments within a single tree, which can lead to genetic variation within a single individual. In apples, genetic variation within a single individual, can result in diversity in apple size, shape, taste, and seed viability, all of which are important factors in the uniformity of the products of apple farming which have not been investigated thoroughly. To better understand the potential for genetic variation within one apple tree, we examined variation among eight samples from across one individual apple tree were examined using genome resequencing and single nucleotide polymorphism (SNP) identification with FreeBayes, which was implemented on the web-based platform Galaxy. Results suggest the strictness of SNP calling criteria greatly affect the number of SNPs identified. In Freebayes, the number of SNPs ranges from 2,286,568, with lenient criteria, to 127,401, with strict criteria, which accounts for 0.3% to 0.01% of the genome varying across one apple tree. In addition to the data collected from Freebays, we also examined the samples using GATK on Galaxy however, we determined that GATK did not have as high of a SNP calling coverage as Freebays, making the Freebays data more accurate. Time constraints prevented project completion, however the project is being continued at Kettering University through their NSF REU program. We hope to be able to compare the percentages of intraindividual and interindividual variation to assess how much of an impact these somatic mutations have.

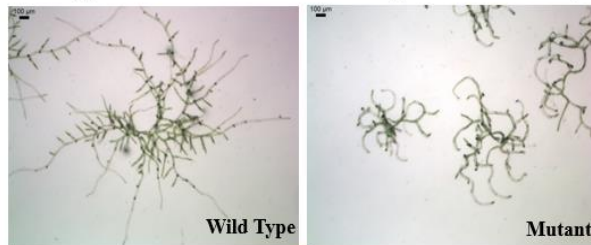
# Differential Gene Expression Underlying Abnormal Tip Growth of a *P. patens* Retrotransposon-Disrupted Mutant

Andrew Freiman<sup>1</sup>

Mentor: Dr. Margaret Dietrich<sup>1</sup>

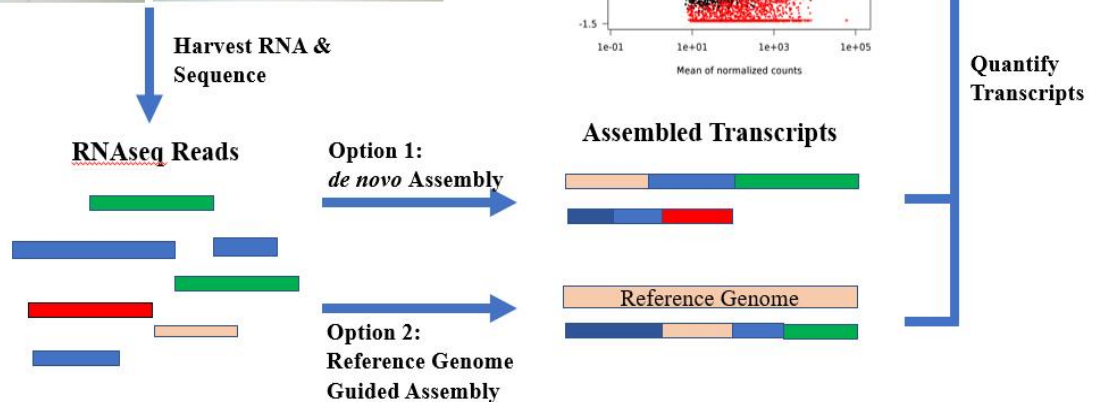
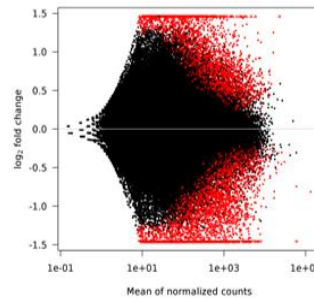
<sup>1</sup>Department of Cell and Molecular Biology, Grand Valley State University, Allendale, MI

Phenotypic differences between Wild Type and Mutant *P. patens*



Differential Gene Expression Analysis

Red = Differentially Expressed Genes



The moss, *Physcomitrella patens*, is a model species for studying tip growth due to its reliance on polarized tip cell growth. In higher plants, tip growth is crucial for nutrient and water uptake as well as plant reproduction. Gaining a better understanding of the mechanisms related to tip growth in *P. patens* will, therefore, contribute to our knowledge of tip growth in vascular plants, such as crop-producing plants. In this project, we characterize genes and gene networks in an insertional mutant in which the disrupted site is flanked by several retrotransposon sequences. This mutant shows several aberrant developmental phenotypes such as a severe lag in caulonema development and abnormal tip growth patterns. Furthermore, the wild type establishes new tip growth on the second subapical cell whereas, in our mutant, such growth initiates on the first subapical cell. The mechanisms responsible for these abnormalities have yet to be characterized in our mutant. With gene expression data derived from transcriptome sequencing of total RNA, we have begun to characterize the genes disrupted by the insertional mutant through differential gene expression analysis methods. Identifying the genes and gene networks that control the mutant phenotype will provide insight into these growth regulatory mechanisms in *P. patens* and, therefore, vascular plant species as well.



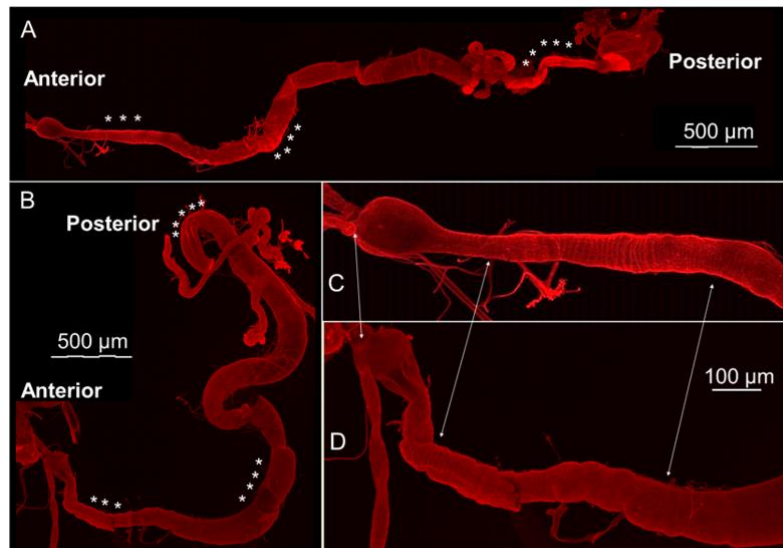
# Functional Characterization of Histamine in *D. melanogaster* Enteric Tissue

Kelly Tekiela<sup>1</sup>, Daniel Beachnau<sup>2</sup>, Sam Plaska<sup>2</sup>, Caroline Poirier<sup>2</sup>

Mentor: Martin Burg<sup>1,2</sup>

<sup>1</sup>Department of Cell and Molecular Biology, Grand Valley State University, Allendale, MI

<sup>2</sup>Department of Biomedical Science, Grand Valley State University, Allendale, MI



(A) Wild type adult gut; (B) *Hdc<sup>JK910</sup>* adult gut; (C) Enlargement of anterior wild type gut; (D) Enlargement of anterior *Hdc<sup>JK910</sup>* gut

The *Drosophila melanogaster* model system offers an effective means for studying biological mechanisms. For example, the *D. melanogaster* nervous system has been used to study the neurological functions of the neurotransmitter histamine. However, to date, there are very few studies of histamine's function elsewhere in the fly, likely due to limited knowledge of histamine in other areas. This study reports on histamine localization in enteric tissue, and preliminary investigations of its function. By using immunostaining, four distinct regions of the larval gut have been found to contain histamine: two in the anterior gut, and one each in the middle and posterior gut. Of these regions, the copper cell region, located in the anterior compartment of the gut, is of particular interest; copper cells are analogous to the acid-secreting parietal cells of the vertebral stomach. *Hdc<sup>JK910</sup>* histamine-deficient larvae were identified to have a more basic pH in the copper cell region than wild type larvae. To determine whether histamine plays a role in acid regulation, *Ortp<sup>306</sup>* larvae with mutated histamine receptors were compared to the *Hdc<sup>JK910</sup>* flies. *Ortp<sup>306</sup>* and *Hdc<sup>JK910</sup>* larvae display a similar basic phenotype. This implies that histamine plays a vital role in the regulation of acid secretion in the copper cell region. These results implicate *D. melanogaster* as a representative model organism for the study of human gut pH regulation.

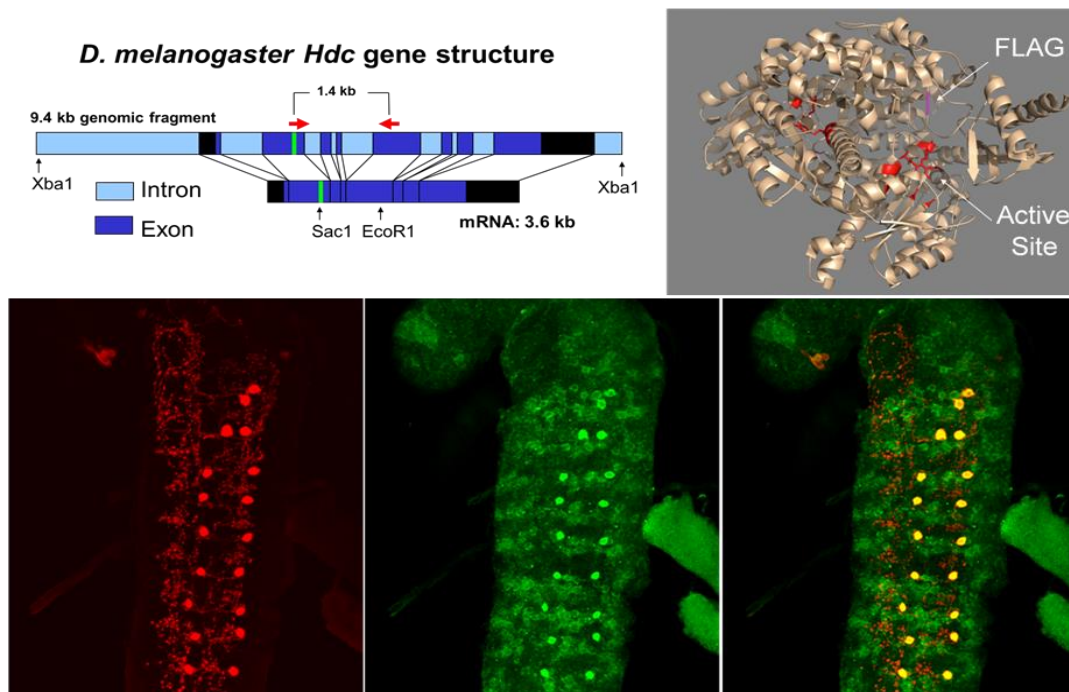
# Comparative localization of histidine decarboxylase and histamine in peripheral and central neural tissues of *Drosophila melanogaster*

Brittany Vandenberg<sup>1</sup>, Lauren Robb<sup>2</sup>, Jacob Howe<sup>2</sup>

Mentor: Martin Burg<sup>1,2</sup>

<sup>1</sup>Department of Cell and Molecular Biology, Grand Valley State University, Allendale, MI

<sup>2</sup>Department of Biomedical Science, Grand Valley State University, Allendale, MI



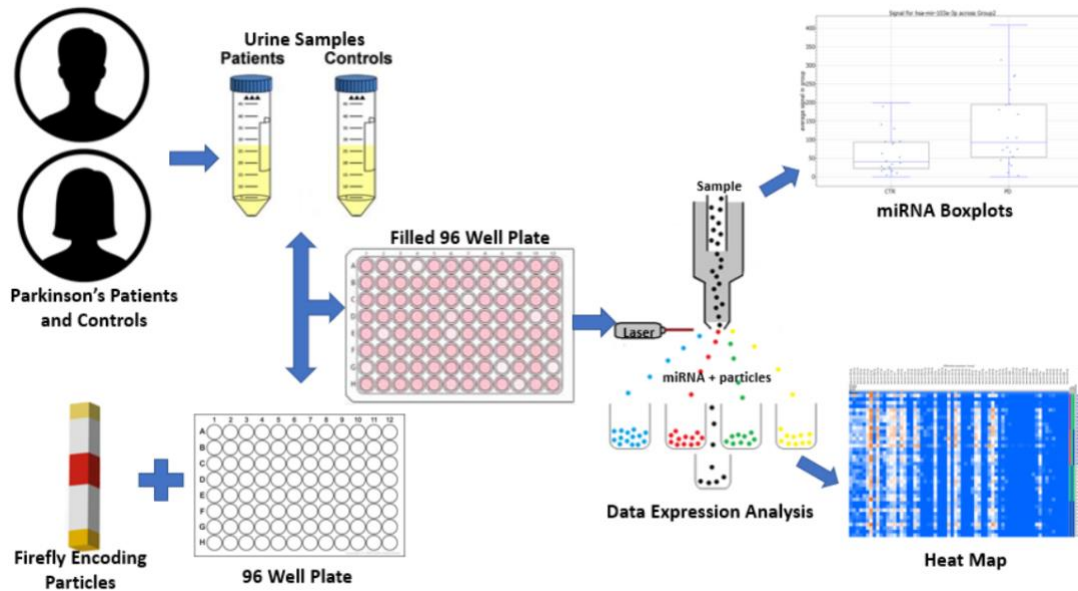
Histamine is known to be critical for a variety of functions in humans including immune response and sleep cycle regulation even though little is known about histamine regulation as a neurotransmitter. To elucidate the mechanism by which histamine synthesis may be regulated, histidine decarboxylase (HDC) localization was compared to histamine. Prior to this work, HDC detection has not been possible due to a lack of a *Drosophila*-specific HDC antiserum. An internal epitope labeling approach, using the FLAG epitope, was taken and an HDC-FLAG transgene was generated. This transgene was placed into the *Hdc<sup>JK910</sup>* mutant that lacks histamine, and the generated transgenic flies were analyzed by staining with FLAG and histamine antibodies which revealed that the HDC-FLAG transgene was functional. This enabled the localization of HDC-FLAG and histamine in various tissues. In most cases, both HDC-FLAG and histamine colocalize, with the exception of photoreceptor cells in which HDC-FLAG and histamine appear to be separately located. This subcellular localization difference suggests that it is possible that HDC may be regulated in central brain neurons differently from that in photoreceptor cells.

# MicroRNAs in Urine as Detection Biomarkers for Parkinson's Disease

Katelyn Anthony<sup>1</sup>, Mark Cunningham<sup>1</sup>

Mentor: Dr. Sok Kean Khoo<sup>1</sup>

<sup>1</sup> Department of Cell and Molecular Biology, Grand Valley State University, Allendale, MI



The current diagnosis for Parkinson's disease (PD) is based on subjective observation of motor symptoms which occur after 50-70% of a patient's dopaminergic neurons are lost. To fill this gap, it is important to identify and test a wide range of biological biomarkers to develop quantitative tools for diagnosing PD. Biomarkers are measurable substances found naturally or unnaturally within an organism that can diagnose, differentiate, predict outcome, or monitor the progression of a disease. In this study, we assessed microRNAs (miRNAs) that are small, endogenous RNAs that regulate gene expression by binding to the 3'-UTR of the messenger RNAs. miRNAs are very stable in biofluids and have been known to alter their expression in human diseases including cancers and neurodegenerative disorders. Here, we evaluated miRNA expression in urine as a noninvasive and inexpensive approach to detect PD. We tested a custom panel of 60 neurological-related miRNAs on crude urine of 20 PD and 20 health controls. miRNA expression was obtained using the Abcam FirePlex microparticle technology which allowed simultaneous measurement of up to 68 target miRNAs in a single assay. We found nine urinary miRNAs that can differentiate PD from healthy controls. Future direction is to validate our data in a larger sample size to move forward to the clinical diagnosis for PD.

Funding: Department of Cell and Molecular Biology, Grand Valley State University, Allendale, MI

The Graduate School, Grand Valley State University, Grand Rapids, MI

Center for Scholarly and Creative Excellence Catalyst Grant for Research and Creativity, Grand Valley State University, Allendale, MI

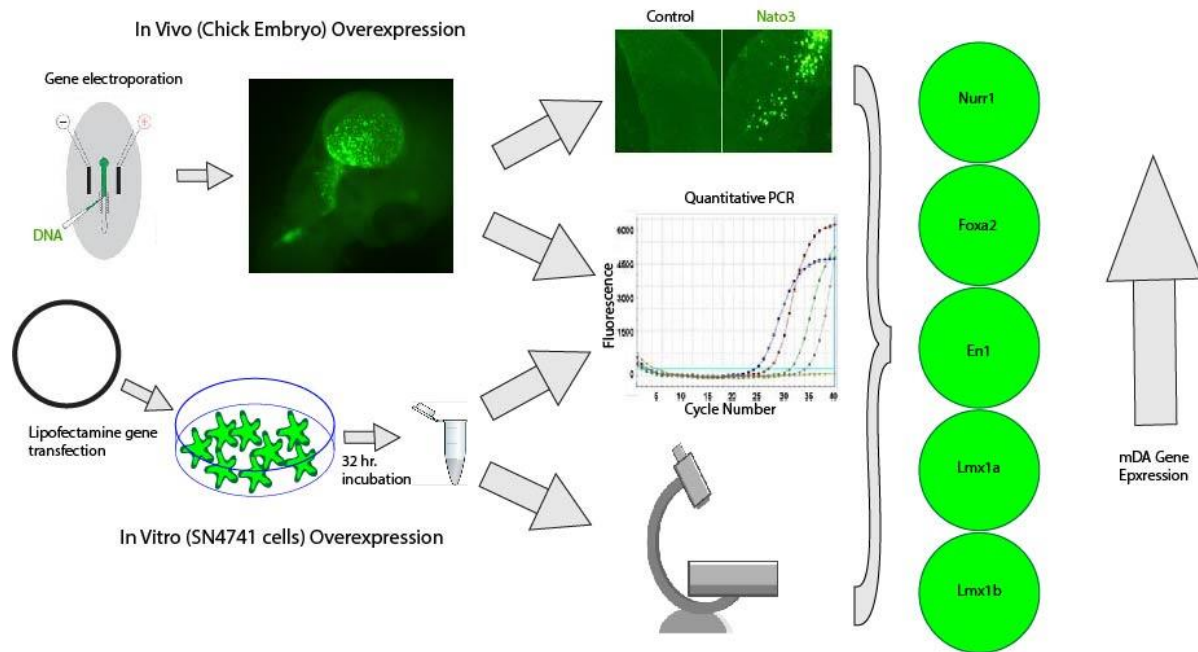
# The Influence of Nato3 on Genes Involved in Dopamine Neurogenesis and Neuroprotection

Melina Frantzeskakis<sup>1</sup>, Dayne Martinez<sup>2</sup>, Jordan Straight<sup>2</sup>, Nick Huisingh<sup>2</sup>, Daniel Doyle<sup>2</sup>

Mentor: Merritt Delano-Taylor<sup>1,2</sup>

<sup>1</sup>Department of Biomedical Sciences, Grand Valley State University, Allendale, MI

<sup>2</sup>Department of Cell & Molecular Biology, Grand Valley State University, Allendale, MI



Dopamine neurons arise from the floor plate of the midbrain and are responsible for the development of Parkinson's disease (PD) when they cease to function. Genes that promote differentiation of midbrain dopamine neurons (mDA) have potential use in clinical therapy development. Neurogenesis/protection of mDA is influenced by genes such as: En1, Nurr1, and Foxa2. Nato3 (N3), a basic helix-loop-helix (bHLH) transcription factor, contributes to the development of mDA, however, the mechanism of action is unknown. We hypothesized that N3 upregulates genes involved in mDA neurogenesis/protection *in vivo* (chick) and in a mouse midbrain cell line (SN4741). Our previous data generated through qPCR and immunohistochemistry indicated that overexpression of N3 upregulated genes involved in mDA neurogenesis/protection *in vivo*. This project focused on the role of N3 in mDA neurogenesis/protection through overexpression in SN4741 analyzed with qPCR. Upregulation of these genes indicates that N3 influences genes involved in mDA neurogenesis. We identified conserved putative phosphorylation sites by comparison to other bHLH proteins. We generated Phosphomimetic Nato3 (PM-N3) mutants to determine the effect of phosphorylation on Nato3. PM-N3 was shown to increase upregulation of these genes compared to N3. These data suggest N3 may be regulated in mDA through phosphorylation and influences expression of genes involved in neurogenesis/protection, which could have clinical implications for PD.