

Microbiology, Genetics, & Immunology

**Undergraduate ​​**

**Research Showcase​​**

**2025**

Abstract Booklet

**Table of Contents:**

|  |  |  |  |
| --- | --- | --- | --- |
| **#** | **Presenter** | **Poster Title** | **Page** |
| 1 | Baldwin, Timothy | Analysis of cardiolipin synthases’ role in *Bacteroides fragilis* resistance to increased osmolarity. | 1 |
| 2 | Feys, Nicholas & Cutinho, Veona | Characterizing Epigenetic Modifications at the Histone 3 Lys 9 Site in Zebrafish Cell Culture and 24-hpf Whole Embryos. | 1 |
| 3 | Finkel, Jared | Discovering the Function of Human Genes Using Yeast | 2 |
| 4 | Frazier, Myah | Assessing Antagonistic Potential of *Metarhizium anisopliae* against Phytopathogens | 2 |
| 5 | Giblin, Ruth | Exploring Biofilm Development and Sanitizer Efficacy in Reducing *Salmonella enterica* Transfer from Stainless Steel to Cucumbers | 3 |
| 6 | Grieve, Victoria | Demethylation of Zebrafish Embryos Using a Viral Vector | 4 |
| 7 | Lemek, Anne | Analysis of *Daphnia* Microbiomes in Search of *Spirobacillus cienkowskii* | 4 |
| 8 | McGrath, Katie | Characterization of ventral tegmental area neuromedin S expressing neurons | 5 |
| 9 | Mishina, Sofya | Evolution of VGLL3 in *Eublepharis macularius* | 5 |
| 10 | O’Brien, Jade | Can Machine Learning be Used to Effectively Quantify Infectious Pathogens for Individual Hosts? | 6 |
| 11 | Patel, Krishen | Metallophore Production in Poplar Rhizosphere Bacteria | 6 |
| 12 | Shadowens, Alyssa | Angiotensin-II Modulates Adiponectin Secretion in the different anatomical locations of Thoracic Perivascular Adipose tissue. | 7 |
| 13 | Wilson, Lucille | Exploring seasonal variation in genetic population structure of lake trout in Lake Superior | 7 |
| 14 | Yedla, Jayadeep | Molecular Cloning of NanoLuc and eGFP into CD63 Expression Vectors via SLiCE Recombination Technology | 8 |

**1) Analysis of cardiolipin synthases’ role in *Bacteroides fragilis* resistance to increased osmolarity.**

Timothy Baldwin, Matthew Schnizlein, Aretha Fiebig, Sean Crosson

Department of Microbiology, Genetics, & Immunology. Michigan State University, East Lansing, USA

Cardiolipins play a major role in the regulation of inner membrane function in the bacteria *Bacteroides fragilis*. *B. fragilis* is a Gram-negative, obligate anaerobe, that colonizes the human large intestine. The synthesis of cardiolipins, catalyzed by cardiolipin synthases, in the inner membrane, contributes to cell membrane fluidity, and is vital in minimizing ion leakage across gut bacterial membranes. I hypothesize that increases in osmolarity will increase the level of cardiolipin synthase activity to resist osmotic stress. Growth curve analysis was performed using *B.fragilis* wildtype(WT), *ΔclsA, ΔclsB*, and *ΔclsAΔclsB*. Growth of each strain was compared across conditions with four sodium concentrations (i.e.,1.7%,1.3%,0.9%,0.5%) and a growth medium control. Optical density at 600 nm was measured every 10 minutes over a 24-hour period using a microplate reader. *cls* gene expression was measured using RT- qPCR in WT, *ΔclsA*, and *ΔclsB* strains exposed to 1.3% sodium for 20 minutes. Growth curve analysis showed OD600 values of 0.35A for the *ΔclsB* strainat 10 hours in late log phase, compared to WT measured at 0.48A in 1.3% sodium. *ΔclsA* strain phase measured at 0.25A compared to WT *ΔclsAΔclsB* levels measured at 0.17A and was in an early log phase compared to WT. qPCR analysis showed increased expression of *ΔclsA* by 2-fold in WT sodium treated samples compared to untreated. Increasing osmolarity negatively affects *B. fragilis* growth. When exposed to high solute concentrations in the environment, internal turgor pressure increases. This puts pressure on the membrane lipids and pushes the cell to the point of lysis. Cardiolipin synthase activity increases to produce more cardiolipins to stabilize the membrane and prevent lysis. This increased activity utilizes more ATP for production of cardiolipin synthases and cardiolipins. This increase in activity would slow cell division and lead to longer lag phases and decreased concentrations of growth. Further qPCR analysis may show how important cardiolipin synthase activity is to the survival of *B. fragilis*, and cardiolipin contribution to membrane function.

# 2) Characterizing Epigenetic Modifications at the Histone 3 Lys 9 Site in Zebrafish Cell Culture and 24-hpf Whole Embryos.

Nicholas Feys1,2, Veona Cutinho1,3, Jose Cibelli1,4,5

1Cellular Reprogramming Laboratory, 2Lyman Briggs College, Departments of 3Microbiology, Genetics, & immunology, 4Animal Science, 5Large Animal Clinical Science, Michigan State University, East Lansing, MI 48824

Somatic cell nuclear transfer (SCNT) refers to the process of transferring a donor nucleus of a somatic cell into an enucleated recipient egg cell to generate a clone. However, this process is still largely inefficient partly due to epigenetic barriers that need to be overcome. Epigenetic modifications on histones, like methylations and acetylations are key transcriptional regulators. Modifications to histone H3 Lysine 9 (H3K9) are of particular importance due to its role in regulating chromatin structure, epigenetic memory, and the overall stability of the genome. Acetylation of H3K9 is commonly associated with transcriptionally accessible DNA, while methylation of H3K9 is generally associated with gene repression or silencing. In this experiment, the aim was to characterize H3K9 methylation and acetylation throughout an entire 24-hours post fertilization (24-hpf) zebrafish embryo and in zebrafish (ZF) cell culture. Immunofluorescence was utilized to observe methylation and acetylation marks in the ZF cell culture and 24-hpf embryos. Fluorescent signals were then quantified using ImageJ software. Results provided key insights into physiologically relevant methylation marks and differential methylation patterns in the 24-hpf embryos, ideal donor nuclei can be derived. This study also offers a future direction in the testing of compounds such as methyltransferase inhibitors and deacetylase inhibitors to aid in "reprogramming" the cell and generating ideal donor nuclei.

# 3) Discovering the Function of Human Genes Using Yeast

Jared Finkel and Tommy Vo

Department of Biochemistry & Molecular Biology

Genetics is important because it defines who we can be. The major problem we face in the study of human genetics is that direct testing of gene functions in humans is technically and ethically challenging. My project seeks to address this problem by testing the possibility of using yeast models that express human genes (humanized yeast) to discover what these genes can do. As a test case, I started with Schizosaccharomyces pombe yeast cells in which the yeast *rpb9* gene was fully replaced with the human homolog called *POLR2I*. The *rpb9*/*POLR2I* genes are well conserved and have been associated with the fundamental process of transcription. However, the details of what these genes do, especially for human *POLR2I*, remains unclear. By forcing our yeast to use human PORL2I in place of its native *rpb9* gene, we anticipate identifying functions that are shared by these two genes. I have measured the impact of yeast cells with native *rpb9*, without *rpb9*, or without *rpb9* but expressing *POLR2I* in the context of cellular growth across multiple environmental conditions. The most significant finding was that *POLR2I* could be able to complement *rpb9* in condition of NaCl-induced osmotic stress. However, *POLR2I* was not able to complement yeast sensitivity to high temperatures or to 6-azauracil drug. We conclude that yeast *rpb9* has at least two distinct functions and that human *POLR2I* can perform one of them. Future investigations into the shared function(s) promise to reveal new functional information on the well-conserved, poorly studied *POLR2I* gene in humans. 

# 4) Assessing Antagonistic Potential of *Metarhizium anisopliae* against Phytopathogens

Soumya Moonjely, Myah Frazier, Dr. Frances Trail

Plant Biology

*Metarhizium anisopliae* is an entomopathogenic fungal endophyte and symbiont of plants, used as a biocontrol agent in some agricultural systems. It colonizes plants, infecting harmful insects and returning nitrogen to the host plant. Previous research has demonstrated anti-fungal properties of *M. anisopliae* against phytopathogens such as *Fusarium graminearum*, the causal agent of Head Blight in wheat. The objective of this research is to assess the potential antagonistic properties of *M. anisopliae* against other phytopathogens: *F. virguliforme, Colletotrichum fiorinae, Magnaporthe oryzae, C. spaethianum, Alternaria alternata, Cercospora beticola*, and *Cochliobolus heterostrophus*. Antagonistic potential of *M. anisopliae* against phytopathogens was evaluated by quantifying reductions in mycelial growth, conidial germination, and symptom mitigation in diseased plants. To observe mycelial growth inhibition, dual-culture Petri dish assays were performed by co-inoculating phytopathogens with *M. anisopliae* and comparing radial growth to individual phytopathogen cultures. To analyze conidial germination rate reduction, the normal germination rate was quantified and compared to germination rate when incubated in *M. anisopliae* culture filtrates. To evaluate the mitigation of *F.* virguliforme-induced Sudden Death Syndrome (SDS) symptoms in *Glycine Max*, the soil of infected plants was inoculated with *M. anisopliae* spores, and disease symptoms were compared to untreated diseased plants. Dual culture assays have demonstrated significant mycelial growth inhibition of phytopathogens in the presence of *M. anisopliae*. In addition, spore-germination of *C. fioriniae, F. virguliforme,* and *M. oryzae* show significant susceptibility to *M. anisopliae* culture filtrates. SDS foliar symptoms in *G. max* do not show significant mitigation of disease symptoms when treated with *M. anisopliae* thus far. These results may reveal that *M. anisopliae* has the potential to be applied as a biocontrol agent against a greater diversity of fungal diseases in agricultural crops than was previously known.

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**5) Exploring Biofilm Development and Sanitizer Efficacy in Reducing *Salmonella enterica* Transfer from Stainless Steel to Cucumbers**

Ruth Giblin (Department of Microbiology, Genetics, and Immunology), Ahmed Abdelhamid (Department of Food Science and Human Nutrition)

*Salmonella enterica* is the second-most frequent cause of foodborne illness in the United States and thus a critically important pathogen within the food industry, particularly with fresh produce such as cucumbers. This project aimed to characterize the ability of *S. enterica* serovar Saintpaul (*S.* Saintpaul) and *S. enterica* serovar Newport (*S.* Newport) to form biofilms on food contact surfaces, quantify the transfer of biofilm cells from stainless steel coupons (SSCs) to cucumbers, and evaluate the efficacy of sodium hypochlorite, a known sanitizer for surface decontamination, in reducing biofilm formation and transfer to cucumbers. Biofilms were developed by inoculating SSCs with 1 mL Luria-Bertani broth (106 CFU/mL) for 72 h at room temperature. The SSCs were washed and treated with either sodium hypochlorite (200 ppm) or PBS as untreated control. Cucumber slices (2g) were pressed against the untreated and treated SSCs. Residual bacterial populations on the coupons and transferred cells on cucumber slices were enumerated by spread plating. Results indicated that both serovars formed robust biofilms on SSCs, resulting in a 0.7 log CFU increase per coupon compared to the initial inoculum. Approximately 1.5 log CFU/g transferred from the untreated SSCs to cucumber slices. Sodium hypochlorite treatment reduced *Salmonella* populations by 1.1 log CFU per coupon and decreased pathogen transfer to cucumber by 1.2 log CFU/g.  These findings emphasize the importance of effective sanitation protocols, as biofilm formation at room temperature can significantly contribute to *Salmonella* transfer onto fresh cucumbers.

**6) Demethylation of Zebrafish Embryos Using a Viral Vector**

Victoria Grieve1,2,3, Sean W. Monahan1,4 Caroline Mrsan1,4 William Poulos1,4, Fermin Jimenez1,4 Jose B. Cibelli1,4,5

1Cellular Reprogramming Laboratory,Departments of 2Microbiology, Genetics, & Immunology; 3Integrative Biology, 4Animal Science, 5Large Animal Clinical Science, Michigan State University, East Lansing, MI 48824

# I will be testing if cloning success rates in zebrafish can be increased by demethylating the reprogramming resistant regions of the somatic nucleus being cloned. This will be done by microinjecting zebrafish embryos with a vector that is carrying the Kdm4a gene and a green fluorescent protein, driven by the UAS promoter sequence. The vector will also contain a sequence from the zona pellucida gene promoter region and the Gal4- Estrogen Receptor gene. When tamoxifen is added to the water with a fish that was previously microinjected with this vector, the Kdm4a enzyme will only be expressed in the eggs of the fish. When these eggs are used for Somatic Cell Nuclear Transfer the Kdm4a enzymes in them should remove the methyl groups from H3K9 and erase the reprograming resistant regions in the somatic nucleus and allow reprogramming to take place properly.

**7) Analysis of *Daphnia* Microbiomes in Search of *Spirobacillus cienkowskii***

Anne Lemek, Lindsey Thompson, Nina Wale

Department of Microbiology, Genetics, & Immunology

Pathogens can be difficult to culture, both *in vitro* and *in vivo*, especially when little is known about their life cycle and biology. This may impede research on its infection dynamics, and so finding ways to effectively isolate a pathogen is crucial to studying how it behaves.*Spirobacillus cienkowskii* is one such pathogen. *S. cienkowskii* is a pleomorphic, Gram-negative bacteria that infects *Daphnia*, a genus of freshwater zooplankton found worldwide. Associated infections have high mortality rates and a high bacterial load at the time of death, but little else is known about *S. cienkowskii*. This is in part because it has never been cultured *in vitro*, and maintaining *in vivo* cultures is challenging.To address this lack of isolated cultures, a previous experiment exposed three evolutionarily divergent species of *Daphnia* to lake water thought to contain *S. cienkowskii* at two different temperatures. This experiment produced infected animals, but because infections were visually diagnosed, the causative agent must be identified to determine if this is a valid method of generating *S. cienkowskii* infections. DNA from experimental hosts was extracted and the 16S rRNA gene was amplified, then Illumina sequencing was performed to identify the community composition. Special attention was paid to the dominant bacterial taxa in the samples, as the causative agent should be the most abundant.

**8) Characterization of ventral tegmental area neuromedin S expressing neurons**

\*K. McGrath1, C. M. Rivera Quiles1, O. Dodson1, M. S. Mazei-Robison1

1Neuroscience Program, Michigan State Univ., East Lansing, MI

Despite the presence of treatments for opioid use disorder (OUD), opioids remain the leading cause of overdose deaths in the U.S. Studying neurobiological effects of chronic opioid use could lead to better treatments. The ventral tegmental area (VTA) is critical for motivated behaviors. Specifically, dysfunction of VTA dopaminergic neurons (DA) can contribute to addiction. We previously found that neuromedin S (NMS) gene expression is increased following chronic morphine in VTA DA neurons. Less than 5% of VTA DA neurons express NMS in naive mice, and this percentage increased in NMS-Cre mice that underwent chemogenetic manipulations for cell activation and morphine behaviors. To further study VTA-NMS neuronal expression and function, we’re using a retrograde viral tracer approach to identify their projection targets. Our initial studies suggest that VTA-NMS neurons project to the nucleus accumbens (NAc) and lateral hypothalamus (LHA), but not the prefrontal cortex (PFC). To validate this, we injected retrograde viruses into these regions and validated using immunohistochemistry and cell counts. I found that VTA-NMS neurons project to the NAc, and most of these cells are also dopaminergic. Interestingly, they also project to the LHA, but to a lesser degree, and they don’t project to the PFC. Furthermore, to determine the role of NMS in morphine behaviors, we developed a constitutive NMS KO mouse. Interestingly, NMS KO doesn’t alter morphine behaviors. These findings suggest that VTA-NMS neurons represent a subset of DA neurons, and further study is needed to determine the role of VTA-NMS in morphine behaviors.

**9) Evolution of VGLL3 in *Eublepharis macularius***

Sofya Mishina, Yun Liang, Ph.D.

Liang lab, Department of Physiology, Michigan State University

Lupus, or systemic lupus erythematosus (SLE), is a chronic autoimmune disease that occurs when the body's immune system attacks its own healthy tissues and organs. Symptoms can be treated with steroid drugs but not the condition. An estimated 204,000 people have SLE in the United States, according to the most recent data available. (As stated by CDC) Dr. Yun Liang’s research identified the role of transcription factor of VGLL3 and its role in sex-biased autoimmune diseases, like lupus, by activating inflammation pathways. Moreover, it suggested that female-biased VGLL3 overexpression is due to metabolic stress, a key factor in placental mammals when carrying and giving birth to offspring. (Liang Y., 2016) Dr. Yun Liang’s lab studies the role of VGLL3 in placental mammals (mice and humans). The proposed research focuses on looking at the role of VGLL3 in non-mammalian systems and comparing it with its function in mammals. The chosen model of study is Eublepharis macularius (Leopard gecko), due to there being documented cases of reptilian systems expressing symptoms of SLE (Fredric L., 1978). It is a gray area of research that allows to test the idea of VGLL3 overexpression’s linkage to metabolic stress during pregnancy in a non-placental system by performing qPCR, Western blot, immunofluorescence and immunohistochemistry.

# 10) Can Machine Learning be Used to Effectively Quantify Infectious Pathogens for Individual Hosts?

Jade O’Brien, Ashwini Ramesh, Nina Wale

Department of Microbiology, Genetics, & immunology

# In most scientific fields, infection is a matter of infected or not infected, but our experiment aims to quantify the degree to which a host is infected. With the help of Flow Cytometry and the subsequent imaging analysis software, we compare manual spore counts within individual Daphnia samples to AI masking software trained to identify spore structures. Our research seeks to push the limits of artificial intelligence to minimize manual counting and improve the quality and speed of researchers in immunology and microbiology.

**11) Metallophore Production in Poplar Rhizosphere Bacteria**

Krishen Patel1,2, Alex Demetros2, Imani Pascoe2, Mashal Copperman2, Dr. Sarah Lebeis2,3,4,5

1. Department of Plant Biology 2. Department of Microbiology, Genetics, & Immunology 3. Department of Plant, Soil, and Microbial Sciences 4. Plant Resilience Institute 5. Great Lakes Bioenergy Research Center

Mining practices can cause heavy metal contamination in surrounding soil, affecting plant health. Some bacteria can make compounds called metallophores that trap metal ions, which protect the plants that they live on or near from excess. In these studies, we examine this activity in microbes that associate with metal-stressed poplar trees in the rhizosphere, which is the soil that surrounds the root system. In the poplar rhizosphere microbiome in copper contaminated sites, it is hypothesized that certain bacteria are more adept than others at producing metallophores that sequester copper, also called chalkophores. We chose 29 bacterial isolates, including negative and positive controls for metallophore production, from the phyla Actinobacteria, Proteobacteria, and Bacteroidetes. Isolates were grown on copper chloride plates of varying concentrations, and the growth rates were recorded. Measurements were also taken of bacterial growth on Chrome Azurol S (CAS) assay plates, which test for metallophore presence. Genetic analysis was performed by using Uniprot and BLAST to confirm genes responsible for plant association and metal binding and transport were present in the genome of 8 fully sequenced isolates. Current findings show that about 2/3 of the 29 isolates tested are able to grow at a concentration of 200 ppm on copper chloride plates. The CAS assay results are in progress. Soil bacteria with genes for metallophore biosynthesis can be used for bioremediation in cleaning up heavy metal contamination. Therefore, industrial scale propagation of these bacteria and collection of relevant metabolites could be instrumental in environmental restoration.

# 12) Angiotensin-II Modulates Adiponectin Secretion in the different anatomical locations of Thoracic Perivascular Adipose tissue.

\*A. Shadowens1, C.J. Rendon1, M. Chirivi1, G.A. Contreras1, S.W. Watts2, and A. Lauver2

1Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, 2Department of Pharmacology and Toxicology, Michigan State University

Hypertension (HTN) is the most common cardiovascular disease and a major global cause of death. HTN alters the vessel structure, including perivascular adipose tissue (PVAT), resulting in a loss of its vasoactive properties. However, the mechanisms remain unclear. Aortic PVAT (aPVAT) is distributed in three regions: anterior (aaPVAT) and two laterals (laPVAT) which are primarily composed of adipocytes. The populations of these cells are maintained by adipogenesis of Adipocyte progenitor cells (APCs). APCs in aPVAT regions have distinct developmental origins, but their functional differences are unknown. A healthy population of PVAT adipocytes is required as these cells secrete adiponectin, a vasorelaxant hormone. This study examined the effect of angiotensin-II (Ang-II), a HTN inducer agent, on aPVAT-resident APCs adiponectin secretion. We hypothesized that adiponectin levels would be higher in the lateral sites. APCs from aaPVAT and laPVAT of SD rats (n=9) were isolated using explant outgrowth. During adipogenic induction, APCs were exposed to Ang-II, or Yoda1, a PIEZO1 agonist that mimics mechanosignaling and suppresses adipogenesis. Adipogenesis was assessed with a lipid stain, Bodipy, and nuclei stain. Triglycerides and adiponectin secretion were quantified. Upon exposure to Ang-II, adiponectin secretion increased in laPVAT compared to aaPVAT (p=0.01). Notably, laPVAT exhibited the most positive response to Ang-II treatment (p=0.0004). Lipid accumulation and adipogenic efficiency remained unchanged. These findings suggest that laPVAT may serve a protective role in hypertension by sustaining adiponectin secretion in response to Ang-II, highlighting a potential target for therapeutic intervention. Future experiments will utilize a tracing system to assess changes in the adipogenesis of APCs under ANG-II exposure in vivo.

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# 13) Exploring seasonal variation in genetic population structure of lake trout in Lake Superior

Lucille Wilson1, Emily Bardwell2, Ben Kline2, Nadya Mamoozadeh2, Mariah Meek2

1Department of Microbiology, Genetics, & Immunology; 2Department of Integrative Biology

Lake trout (*Salvelinus namaycush*) are one of only 2 trout species native to Lake Superior. After their population dropped to an extreme low in the mid-1900s, collaborative recovery efforts have restored self-sustaining populations throughout most of the lake. Although lake trout population abundance has increased, more insight is needed into the population structure and genetic diversity of this species. Further, it is not well understood how lake trout genetic population structure changes throughout spawning and non-spawning seasons. Individuals are presumed to return to their native spawning reefs during the fall spawning season due to spawning site fidelity. They may be more dispersed outside of the spawning season, but genetic insight is needed to explore these patterns. This study aims to investigate how lake trout dynamics vary by season by comparing genetic population structure during and outside of spawning season. We will conduct restriction-site associated DNA sequencing on around 2500 wild lake trout individuals caught in the fall and spring. With this data, we will perform population structure analyses to explore if the population structure of lake trout changes based on the season. We may gain insight into how populations disperse among spawning reefs, and how this impacts genetic population structure. The critical genetic information gathered in this study will inform conservation management strategies to maintain healthy and diverse lake trout populations across Lake Superior.

# 14) Molecular Cloning of NanoLuc and eGFP into CD63 Expression Vectors via SLiCE Recombination Technology

# Jayadeep Yedla, Dr. Masako Harada

# Biomedical Engineering

Gene cloning is a fundamental technique in molecular biology, enabling the generation of specific DNA constructs to study gene expression, functions, regulations, and many versatile biological processes. Despite their significance, traditional cloning methods depend on expensive reagents, such as restriction enzymes and DNA ligases which can be time-consuming and inefficient in their function.

Seamless Ligation Cloning Extract (SLiCE) recombination technology has emerged as a powerful alternative that utilizes enzymatic components from *Escherichia coli* cell lysate to facilitate homologous recombination between vectors and insert DNA without additional ligation steps, thus overcoming the limitations of the traditional methods.

This project aims to evaluate the effectiveness of SLiCE recombination technology by cloning two target plasmids - pcS-NanoLuc-CD63 and pcs-eGFP-CD63 - which are valuable in extracellular vesicle (EV) labeling. The experimental overflow of the project will include creating plasmids using PCR amplification of vector and insert, quantification of the amplicons, and SLiCE-mediated homologous recombination. The recombinant plasmids will be transformed into competent *E. coli* cells, after which the integrity of the plasmids will be tested using colony PCR. MiniPrep will be performed to isolate the desired plasmids, and they will be sequenced using Sanger sequencing to assess the sequence fidelity. SLiCE efficiency will be verified by colony screening and supported by the results derived from Sanger sequencing.

Our results demonstrated that SLiCE recombination successfully facilitated plasmid construction, establishing its potential as a cost-effective, time-saving, and reliable alternative to the traditional cloning method. Ultimately, this project contributes to understanding the benefits of homologous recombination-mediated cloning for future research.

Thank you to all the presenters and mentors that participated in this showcase,

and to Dr. Vic DiRita and the MGI department for their continuing support!

Best wishes to the graduating seniors for a bright future!

