Session A Abstracts

Exploring HP1-Mediated Heterochromatin Silencing and the Role of SUMO and PxVxL Motif Interactions
Dillan Lau
Mentors: Alexei Aravin and Qing Tang

Heterochromatin, a highly condensed form of chromatin, plays an important role in gene silencing. Epigenetic markers, modifications which do not alter DNA sequences, can initiate and perpetuate heterochromatin. In particular, histone 3 lysine 9 trimethylation (H3K9me3) is known for constitutive heterochromatin formation and the repression of repetitive DNA elements. Histone methyl transferases catalyze H3K9me3 deposition, while HP1 proteins read this mark, prompting chromatin condensation. This project investigates the model behind HP1-mediated silencing. Unexpectedly, the protein SUMO emerges as vital for H3K9me3 deposition. Additionally, HP1-binding proteins have a consensus PxVxL motif that binds to HP1's chromoshadow domain, but when HP1 is mutated at W174A, the PxVxL interaction is killed and HP1 loses its silencing ability. The theorized model proposes an unknown protein containing the PxVxL motif that can bind to HP1 and interact with SetDB1 in a SUMO-dependent manner. TRIM24, CAF-1, and ATRX were selected as potential candidates and subject to Co-IP assays with and without NEM to test for these qualities. The results have yet to ascertain a definitive model, and further work needs to be done investigating additional complexity and alternative models. For example, SetDB1 can be SUMOylated, which was explored through plasmid transfection and Western Blotting.

Lung-on-a-Chip Device to Access the Effects of Inhaled Nitric Oxide on Hospital Acquired Pneumonia
Maya de Luis
Mentors: Lorenzo Berra, Ellen Rothenberg, and Hatus Wanderley Vianna

A lung-on-a-chip is a novel microfluidic device that offers an in vitro approach to studying and experimenting in the environment of a lung. It simulates the alveolar-capillary interface found in human lungs. This interface is the principal site of respiration and serves as a barrier between air and blood. In the chip, there are two chambers with a thin, porous poly(dimethylsiloxane) (PDMS) layer separating them. The first chamber mimics the environment inside the alveolar with a layer of epithelial cells and air flowing through it. The second chamber mimics the environment inside the capillary with a layer of endothelial cells and blood flowing through it. The chip simulates the mechanical movement of the lung during inhalation by stretching. The primary goal of this project is to build a working lung-on-a-chip device using PDMS and human epithelial and endothelial cells. Inhaled Nitric Oxide (iNO) has been shown to decrease bacterial count in the lungs of patients with hospital acquired pneumonia (HAP) and other lung infections. However, the effects of high dose iNO on HAP has yet to be studied in a clinical environment due to the unknown hazards of the high concentrations of iNO. The lung-on-a-chip device provides a way to experiment with high dose iNO to access its effects and any hazards.

Utilizing STABILON and Exin21 Sequence Motifs to Optimize Anti-GnRH Antibody Production
Grace Wilson
Mentor: Bruce Hay

Feral horses are an exotic invasive species in North America that present a threat to native ecosystems. Adeno-associated virus (AAV) has the potential to be used to develop a low-cost gene therapy contraceptive to induce long-term infertility in this invasive species. AAV is a viral vector that has the ability to deliver and incorporate engineered DNA into target cells. It is possible to induce long-term infertility in mice by using AAV to deliver a gene that encodes for an antibody that binds to GnRH, a peptide hormone required for gamete and sex steroid production in males and females of all vertebrates. In order to develop an effective gene therapy for horses, the anti-GnRH construct of interest must produce a sufficient antibody titer for a mammal as large as a fully developed horse. STABILON and Exin21 are two novel sequence motifs that have been shown to increase protein production and protein stability. We cloned the STABILON and Exin21 sequence motifs into the open reading frame of the anti-GnRH construct, transfected HeLa cells with the constructs, and used an ELISA to evaluate the ability of the sequences to increase anti-GnRH antibody production.

Combination Genomic and Epigenomic Analysis of Plasma Cell-Free DNA Identifies Stemness Features Associated With Worse Prognosis in High-Risk Metastatic Castration-Resistant Prostate Cancer
Savar Sinha
Mentors: Jared Leadbetter and Aadel Chaudhuri

Prostate cancer is the second leading cause of cancer death among American men, causing 34,700 deaths annually. While localized prostate cancers are highly responsive to androgen-directed therapies, some patients develop metastatic castration-resistant prostate cancer (mCRPC), which is resistant to these treatments. Previous tumor whole-genome sequencing studies highlighted aberrations in both the androgen receptor (AR) locus and the recently discovered AR enhancer region as genomic hallmarks of mCRPC. The Chaudhuri Lab sought to replicate these results using cell-free DNA (cfDNA) analysis as a less invasive alternative to tumor sequencing, developing
Perform epigenomic analysis of pretreatment patient cfDNA samples, including genome-wide methylation sequencing, nucleosome profiling, and stemness analysis via EM-Seq, Griffin, and CytoTRACE, respectively, to elucidate the underlying biology of mCRPC. We illustrate that pretreatment plasma cfDNA analysis can be used to risk-stratify patients, mCRPC transcriptional profiles can be predicted from cfDNA epigenomics, and higher-risk mCRPC patients have more stem-like signature profiles that correlate with worse survival outcomes.

**Elucidating the Cranial Neural Crest Gene Regulatory Network in Craniofacial Development**
Taylor L. Simonian
Mentors: Marianne Bronner and Sierra Marable

Neural crest cells are multipotent progenitor cells unique to vertebrates that play a crucial role in embryonic development. A complex gene regulatory network coordinates their specification, migration, and differentiation, imbuing neural crest cells with distinct cellular identity and differentiation potential. In amniotes, the cranial neural crest is the only subpopulation that has the ability to develop into craniofacial cartilage and bone. In this project, we have been investigating the gene regulatory network regulating cranial neural crest differentiation into cartilage of the developing mandible. We are characterizing chondrogenesis in the first branchial arch of chick embryos using hybridization chain reaction and immunofluorescence staining to examine markers of cartilage condensation. In addition, we are investigating the expression pattern and function of the transcription factors like Barx1 and Sox5 in the cranial neural crest using CRISPR-Cas9 technology to perturb these genes. These studies will provide insight into the neural crest gene regulatory network that controls craniofacial development.

**A Comparative Study of Gene Regulatory Network Changes in Healthy and Cancerous Tissues With Single-Cell Atlases**
Yingying Gong
Mentor: Matthew Thomson

Gene regulatory networks control the organization of biological activities and dictate cellular information processing from physiological conditions. The advent of multiplexed single-cell sequencing technology has yielded diverse atlases of transcriptional profiles across different tissue and disease conditions. However, a mechanistic view of how the regulatory networks change in diseased conditions is yet to be established. In this study, we highlight the differences in regulatory networks between healthy and cancerous tissues by dissecting the regulatory networks from an integrated atlas of 2.1 million single cells, including Tabula Sapiens Atlas and datasets across 24 tumor types. We applied D-SPIN, a statistical framework, to identify a set of gene programs that represent core cellular activities, establish a regulatory network model among these programs, and discern how the network is sparsely modified in each cancerous sample. We found the changes in regulatory networks are grouped by the tissue type of cancer samples. Our results suggest that cancers of different tissues have distinct features of regulatory network shifts and thus regulatory networks can serve as powerful tools for both understanding and diagnosis of cancer.

**Early Visual Processing During Hunting in Mice**
Jasmine Wang
Mentors: Markus Meister and Daniel Pollak

The superior colliculus (SC) is a region in the midbrain that affects head and eye movement in response to visual and somatosensory stimuli. While mice are known for their predator evasion, they also have innate hunting behaviors. Like escape, hunting in mice is strongly driven by vision. However, it is not fully understood how mice use natural visual inputs in real time to execute hunting bouts. Here, we use a machine learning tool, DeepLabCut, to perform pose estimation of mouse behavior from videos to investigate behavioral strategies for pursuing a visually salient target, specifically a cockroach. Previous mouse hunting behavior studies relied on accidental interactions between mouse and prey. Our behavioral assay produces such interactions multiple times by moving a magnetically coupled dummy in the hunting arena along a repeatable trajectory. We identified two trajectories from a large stimulus space that elicited high engagement. Mice without whiskers had less steady trajectories when hunting but were nonetheless highly engaged. The hunting assay here engages somatosensation and vision as mice with and without whiskers performed hunting bouts. Future studies will integrate this assay’s insights with neural recordings in the SC to understand how sequences of images impinging on the retina initiate hunting.

**MITF-Mediated Melanoma Phenotype Switching Through Gene Tagging and Knock-In Studies**
Rachel Reyes
Mentors: Eiríkur Steingrímsson and Evangeline B. Raja David Isac

Melanoma, a lethal form of cancer originating from melanocytes, presents a complex challenge due to its cellular heterogeneity and resistance to conventional treatments. Recent studies indicate that the Microphthalmia-associated transcription factor (MITF) plays a pivotal role in the regulation of CDH1 and CDH2 genes, which are associated with phenotype switching and drug resistance. However, the precise mechanisms underlying this switching was incompletely understood. Here we employed Mir-SkMel28-MITF cells for two CRISPR-based
experiments. These cells allow us to modulate MITF expression by inducing miRNA that targets MITF expression. CDH1 was tagged with eGFP, while CDH2 was marked with mCherry, enabling real-time expression tracking and following MITF manipulation. This will be achieved using CRISPR technology, involving the transfection of cells with a guide RNA (sgRNA), Cas9 enzyme, and a vector containing fluorescent protein markers flanking the respective genes. The primary objective of tagging these cells was to facilitate the monitoring of CDH1 and CDH2 expressions and the manipulation of MITF levels through knockdown techniques. This tagged cell line will serve as a valuable resource for future genome-wide knockout studies and help identify genes that regulate CDH1 and CDH2, thereby contributing to a deeper understanding of melanoma biology and the development of innovative therapeutic strategies.

Single Nucleus RNA Sequencing Data Set of Enteric Neurons in the Alpha Synuclein Overexpressing Mouse Model of Parkinson’s Disease: Overcoming Obstacles
Christopher Pukszta
Mentors: Sarkis Mazmanian and John Bostick

In addition to the motor symptoms associated with the death of dopaminergic neurons in the substantia nigra pars compacta during the progression of Parkinson’s disease (PD), there are well documented gastrointestinal (GI) symptoms. Several studies have investigated the enteric nervous system (ENS) of human patients but have not been able to sample from the Myenteric Plexus (MP) which directly controls GI tract contractions. Here we look to assess the transcriptional state of MP neurons via single nucleus RNA sequencing. Using an adeno-associated viral vector (AAV) to deliver a nuclear pore protein fused with GFP under the control of the human synapsin 1 promoter, neuronal nuclei are sorted from other extracted nuclei. This report describes the major hurdles associated with packaging and verifying full expression of a novel AAV genome in the ENS, carefully extracting and preserving nuclei for downstream sequencing, and analyzing the transcriptomic profile of a rare population of cells. Subsequent work with this transcriptomic profile will be to examine protein expression levels and investigate new potential links between the gut microbiome, the immune system, and PD pathology.