SynBio6.0 6th Annual Synthetic Biology Symposium Booklet

May 16-17, 2024, Vancouver, BC, Canada

2024-05-13

Conference Program

Day 1 - May 16th

Morning Session

Time	Event
8:00 - 9:00	**Registration and Coffee and pastries**
9:00 - 9:15	**Welcome Address** **Theme: Microbial Synthetic Biology**
9:15 - 10:15	**Keynote 1:** Dr. Rebecca Shapiro, University of Guelph
10:15 -10:45	**Drug Resistance Evolution in Yeast Harboring Synthetic Gene Circuits**- Daniel Charlebois
10:45 - 11:00	**Coffee Break**
11:00 - 11:15	**Kluyveromyces marxianus – A green cell factory for organic acid production**- Mackenzie Thornbury
11:20 - 11:35	**Screening and characterization of bidirectional promoter in Saccharomyces cerevisiae**- Zimo Jin
11:40 - 11:55	**Methanivore: a comprehensive solution to landfill gas emissions through bacterial metabolic engineering**- Evelyn Chen

Lunch Session

Time	Event
12:00 - 13:30	**Lunch**

Afternoon Session

Time	Event
	Theme: Regenerative Medicine
13:30 - 14:45	**Keynote 2:** Dr. Ron Weiss, MIT
14:45 - 15:15	**Engineering Collective Behavior in Stem Cell Driven Colonie**- Tiam Heydari
15:15 - 15:30	**Coffee Break**
15:30 - 15:45	**A Synthetic Device to Monitor the Interaction between Human Embryonic Stem Cells**- Omar Bashth
15:50 - 16:05	**Theme: Microbiome Engineering**
16:10 - 16:25	**Engineered Saccharomyces boulardii: A Probiotic Approach for Combating Pathogen-Derived Gastrointestinal Diseases**- Laura Enekegho

Time	Event
16:30 - 18:00	**Poster Session 1 (Odd Numbered)**

Day 2 - May 17th

Morning Session

Time	Event
8:00 - 9:15	**Coffee and pastries**
	Theme: Genome Engineering
9:15 - 10:15	**Keynote 3:** Dr. Leopold Parts, Wellcome Sanger Institute
10:15 - 10:30	**PHYCUT: A multiguide CRISPR editing system for glycoengineering of Phaeodactylum tricornutum**- Emily struckless
10:30 - 10:45	**Surveying the Diversity of GIY-YIG Nuclease Domains for Gene Editing**- Kurt Loedige
10:45 - 11:00	**Coffee Break**
11:00 - 11:30	**Enzyme reaction cascades to screen and engineer oxidoreductases**- David Kwan
11:30 - 12:00	**Engineering elephant TP53 mutations**- Emil Karpinski

Lunch Session

Time	Event
12:00 - 13:30	**Lunch**

Afternoon Session

Time	Event
	Theme: Systems Biology
13:30 - 14:45	**Keynote 4:** Dr. Pulin Li, Whitehead Institute, MIT
14:45 - 15:15	**Exploring duplicated gene retention using single-cell imaging of protein dynamics**- Elena Kuzmin
15:15 - 15:30	**Coffee Break**
15:30 - 16:00	**Environmental protein interaction dynamics at a proteome scale**- Dayag Sheykhkarimli
16:00 - 16:15	**Invisible heroes: Role of a non-catalytic auxiliary protein as a(n) (iso)flavonoid metabolic flux conductor**- Lee-Marie Raytek
16:15 - 16:30	**Linked RNAs – new tools for the in vitro selection of a model Origin of Life**- Hendrik Boog
16:30 - 16:45	**Concluding Remarks**
16:45 - 18:15	**Poster Session 2 (Even Numbered)**

Evening Session

Time	Event
18:30 - 20:30	**Gala Dinner**

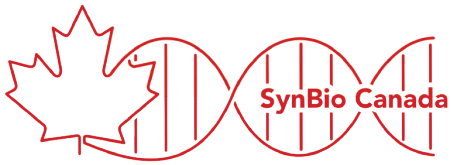
Gold	
Silver	  
Bronze	     

Figure 1: Sponsors

Figure 2: Map to AMS Student Nest

How to Reach the Venue

The conference will be held at the **AMS Student Nest**, located at **6133 University Blvd, UBC, Vancouver, BC V6T 1Z1**. Here are the recommended transportation options to reach the venue:

By Public Transit

- **Bus:** The UBC campus is well-served by multiple bus routes. The most convenient buses are the #99 B-Line, #25, #33, #41, and #49, all of which stop at UBC Exchange, a short walk to the AMS Student Nest.
- **SkyTrain:** While there is no direct SkyTrain service to UBC, you can take the Canada Line to Broadway-City Hall Station and transfer to the #99 B-Line bus which goes directly to UBC.

By Car

- **From Downtown Vancouver:** Take W 4th, W 10th or SW Marine Drive west to UBC.
- **Parking:** Available at the Health Sciences Parkade, which is a 5-minute walk from the venue, or at the North Parkade, which is a 10-minute walk.

By Bicycle

- **Bike Racks:** Available around the AMS Student Nest. UBC is bike-friendly with multiple bike lanes leading directly to the campus.

By Air

- **Nearest Airport:** Vancouver International Airport (YVR).

- **Transport to Venue:** From YVR, you can take the Canada Line from the airport to Broadway-City Hall Station and then transfer to the #99 B-Line bus to UBC. Alternatively, a taxi or rideshare from the airport will take about 30 minutes.

Additional Tips

- **Campus Shuttles:** UBC also operates several shuttle buses that can help you navigate around the campus.
- **Walking:** If you're staying on campus or nearby, UBC is pedestrian-friendly, and the AMS Student Nest is centrally located, making it accessible by foot from most parts of the campus.

For real-time directions and traffic updates, using Google Maps or a similar navigation app is recommended to help ensure a smooth arrival at the venue.

Talks

1: Novel CRISPR-based functional genomic tools for fungal pathogens

Rebecca S. Shapiro
University of Guelph

Opportunistic Candida pathogens are a leading cause of fungal infections, and new functional genomic tools enable our ability to better study the biology of these important pathogens. Here, we describe the development and optimization of CRISPR-based tools for functional genomic analysis in diverse Candida pathogens, and their applications to the characterization of antifungal drug resistance and fungal virulence. We have developed two powerful technologies: CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa), for applications in Candida species. We demonstrate the ability of these systems to robustly repress or induce gene expression in C. albicans, including in drug-resistant clinical isolates. We are able to efficiently and rapidly generate large numbers of fungal mutant strains that over- or under-express any gene of interest, providing a powerful new tool for functional genomic analyses in fungal pathogens. We are applying these CRISPR-based functional genomic libraries for comparative functional genomic analysis across diverse strain backgrounds to study antifungal drug resistance on a large scale, and understand mechanisms of fungal-host colonization and virulence.

2: Drug Resistance Evolution in Yeast Harboring Synthetic Gene Circuits

Harold Flohr, Sanjina Aurin, **Daniel A. Charlebois**
University of Alberta

Antimicrobial (drug) resistance is an emerging global health threat. Mathematical models and synthetic gene networks or "circuits" are tools that can be used to study the evolution of drug resistance [1]. Previous research on microorganisms harboring synthetic drug resistance gene circuits controlling drug resistance genes has focused on constant drug conditions. In yeast, certain gene circuit motifs have been shown to enhance drug resistance [2,3] and that a tradeoff between gene expression cost and drug resistance benefit leads to optima on the fitness landscape [4]. In mammalian cells, it has been shown that the motif of the synthetic gene circuit can affect adaptation and evolution to a drug [5]. However, how gene circuit motifs affect resistance evolution in fluctuating drug conditions and if this microevolution can be quantitatively predicted remain to be elucidated.

We adapt a computation model [4] to generate the distribution of "knockout", "tweaking", and "extra circuit" mutations in fluctuating drug conditions. Simulations of the computational model predict increased genetic heterogeneity during fluctuating drug exposure compared to constant drug exposure. To test these predictions, we perform experimental evolution experiments in fluctuating drug conditions on yeast harboring a positive feedback drug resistance gene circuit at range of induction levels. Our preliminary experimental findings indicate that 1) gene expression variability in the experimental groups (constant and fluctuating drug conditions) is smaller than in the control groups (no drug condition), 2) that shifts in gene expression

distributions under drug selection correspond to increases in fitness (growth rate), and 3) that fluctuating drug exposure results in greater fitness drops than constant drug exposure.

Overall, this research combines quantitative models, synthetic gene circuits, and experimental microbial evolution to advance our fundamental understanding of antimicrobial resistance and to uncover new treatments to combat drug-resistant infections.

References

1. K. Farquhar, H. Flohr, D.A. Charlebois, Front. Bioeng. Biotech., 2020.
2. D.A. Charlebois, G. Balazsi, M. Kaern, Phys. Rev. E., 2014.
3. B. Camellato, I.J. Roney, A. Azizi, D.A. Charlebois, M. Kaern, Eng. Biol., 2019.
4. C. Gonzalez, J.C. Ray, M. Manhart, R.M. Adams, D. Nevozhay, A.V. Morozov, G. Balazsi, Mol. Syst. Biol., 2015.
5. K. Farquhar, D.A Charlebois, M. Szenk, J. Cohen, D. Nevozhay, G. Balazsi, Nat. Commun., 2019.

3: Kluyveromyces marxianus – A Green Cell Factory for Organic Acid Production

Mackenzie Thornbury, Adrien Knoops, Iain Summerby-Murray, Sydney Johnson, James Dhaliwal, Jaya Joshi, Lauren Narcross, Vincent Martin
Concordia University

Organic acids, such as fumaric acid, feature heavily in manufacturing due to their ease of polymerization into diverse industrially relevant compounds. Currently, many of these organic acids are derived from chemical conversion of fossil fuels in energy intensive and greenhouse gas-producing processes. To mitigate these environmental concerns, our group is engineering the yeast Kluyveromyces marxianus to act as a platform for organic acid biosynthesis from renewable feedstocks. K. marxianus is an attractive host because it is the fastest growing eukaryote, able to utilize a broad range of substrates, thermotolerant to 50 ℃, and acid-tolerant to pH 2.5. Leveraging these characteristics, we aim to valorize the lactose present in milk permeate, a milk industry waste-product, by converting it into the dicarboxylic organic acid fumarate. Genetic tools available for manipulating K. marxianus have historically been limited. However, we have expanded this toolkit using by creating CRISPR activation, inhibition, and deletion (CRISPR-AID) tools and demonstrating their use independently and in combination. Our aim is to leverage these tools to induce fumaric acid production through a combination of rational design and high-throughput screening. To facilitate our efforts, we have constructed a comprehensive CRISPR-AID genome-wide guide library. This library serves as a valuable resource for screening desired phenotypes, such as higher fumaric acid production and improved organic acid tolerance for this project. Currently, we are in the process of building and testing this library, initially assessing acid tolerance through a growth phenotype. Subsequently, we plan to employ a biosensor approach for screening fumaric acid production. By using the CRISPR deletion tools, we have applied a rational design strategy to enhance fumaric acid production. Specifically, we've increased fumaric acid production from 0 to 260 mg/L by deleting the endogenous fumarase, preventing fumarate from re-entering the TCA cycle. Further work is underway to express a highly active fumaric acid synthesis pathway in this strain. Collectively, these techniques can result in a new platform for organic acid production from dairy by-products while also providing new genetic tools to unlock the metabolic engineering potential of K. marxianus.

4: Screening and Characterization of Bidirectional Promoter in Saccharomyces cerevisiae

Zimo Jin, Morten Raadam, Md Mohsin Patwary, Yueming Dong, Codruta Ignea
McGill University

Divergent expression is a common phenomenon for all eukaryotes, in which one bidirectional promoter could induce the expression of two distinct transcripts in the opposite direction. Extensive studies have been focused on bidirectional promoters in various hosts, however, the exact mechanism of such bidirectional expression is still unclear. Recently, several metabolic engineering toolboxes have been developed using the bidirectional expression system, demonstrating rapid multi-gene co-expression could be achieved in a

modular way. However, few well-characterized bidirectional promoters existed for metabolic and genetic engineering. In this study, we identified 167 native bidirectional promoter candidates (less than 1000 bp in length) in the genome of Saccharomyces cerevisiae, based on the single shared nucleosome-depleted region between two opposite gene orientations. The promoter strength was profiled using RNA-Seq based on the abundance of transcripts, and forty bidirectional promoter candidates' strengths were further experimental examined by both fluorescence reporter assay and diterpene production assay. We found that the majority of the bidirectional promoter candidates have a biased promoter strength at different sides, which could have promising applications for fine-tuning metabolic flux. For proof of concept, we developed a carotenoid production-based system for the fine-tuning of metabolic flux using a bidirectional promoter in S. cerevisiae. Our results demonstrate that high levels of beta-carotene could be achieved by manipulation of promoter strength. Future promoter engineering by manipulating transcription factors binding motifs within the selected bidirectional promoter led to an additional 30% to 50% increase in the final titer of compounds of interest compared to the parental counterpart. Our results suggested that repressor binding plays an essential role in determining the strength and stability of the bidirectional transcripts. We believed that this promoter engineering strategy could be used to enhance the promoter activities of other bidirectional promoters in S. cerevisiae or other organisms.

5: Methanivore: A Comprehensive Solution to Landfill Gas Emissions through Bacterial Metabolic Engineering

Ally Cheung, Amogh Manivannan, Ruochen Liu, Xinyi (Marry) Xuan, Min Yi (Mindy) Yu, Santiago Plata Salazar, Jeff Chen, Cynthia Wu, **Evelyn Chen**, Nithya Gopalakrishnan, Jaden Bhogal, Dhanya Jagannathan, Rodrigo Reyes Feregrino, Sarvnaz Ale Mohammad, Alston Lo, Chau (Chloe) Nguyen, Eric Raju, Sean Yam, Liliane Kreuder, Jessica Wang, Lauren Altomare, Derya Ozsokmen, Esha Mohan, Elma Chowdhury, Kenneth Sulimro, Ishaan Gupta, John Hiscock, Akriti Sharma, Ananya Sharma, Edward Lombo, Joe Chen, Grace Miao, Henrik S. Zimmermann
University of Toronto

Landfill gases (LFG) constitute 23% of Canada's methane emissions, a potent greenhouse gas that significantly contributes to global warming. Traditional methods like methane combustion release ecologically harmful carbon dioxide. We present an innovative approach utilizing a genetically engineered Escherichia coli strain (T-B18) designed to metabolize methanol through the optimized ribulose monophosphate pathway (RuMP). Our process begins with the separation of methane from LFG using cellulose acetate membranes, followed by its conversion to methanol in a catalytic reactor, which then serves as a feedstock for T-B18.

To enhance the methanol consumption efficiency of T-B18, we employed Genome-Scale Metabolic Models and computational techniques, such as Flux Balance Analysis, to identify critical genetic modifications, including knockouts and over-expression using CRISPR-Cas9, plasmid cloning, and adaptive laboratory evolution. We targeted genes such as TpiA and frdABCD for modification. The methanol assimilation capacity of T-B18 was quantified using High-Performance Liquid Chromatography (HPLC) to measure methanol concentrations in cell cultures before and after genetic modifications.

The results show that the engineered T-B18 strain exhibits a significant increase in methanol consumption efficiency compared to the native strain, positioning it as a promising candidate for sustainable on-site applications at landfill sites.

6: Synthetic Biology Foundations and Application to Programmable Organoids

Ron Weiss
Massachusetts Institute of Technology

Mammalian synthetic biology has recently emerged as a field that is revolutionizing how we design and engineer biological systems for diagnostic and medical applications. In this talk, we will describe our integrated computational / experimental approach to engineering complex behavior in mammalian cells with applications to Programmable Organoids derived from hiPS cells. In our research, we apply design principles from electrical engineering and other established fields. These principles include abstraction, standardization,

modularity, and computer aided design. But, we also spend considerable effort towards understanding what makes synthetic biology different from all other existing engineering disciplines by discovering new design and construction rules that are effective for this unique discipline. We will present Programmable Organoids, a new platform for drug discovery that enables rapid and effective drug screening. Based on programmed guided multistep differentiation into synthetic mammalian tissues having multiple cell type architectures that are similar to human organs, Programmable Organoids mimic the response of a target organ to both positive and negative effects of drug candidates. Factors that can be non-destructively measured include cell state, viability, and function. Because they are synthetic, Programmable Organoids can host a large array of live-cell biosensors, built-in to one or more cell types, providing a rapid and real-time spatial readout of pathway-specific biomarkers including miRNAs, mRNAs, proteins, and other metabolites. Organoids programmed with both general and disease specific sensors then provide detailed information that can be used to identify candidates for further analysis. We envision a programmable common platform that can be used for testing multiple drug candidates, as well as for regenerative medicine.

7: Engineering Collective Behavior in Stem Cell Driven Colonies

Tiam Heydari, Joelle Fernades, Daniel Aguilar-Hidalgo, Peter Zandstra
The University of British Columbia (UBC)

In this study, we investigated coordinated behavior within colonies of pluripotent stem cells by examining the relationship between ordered system states and their entropy levels. We discovered a collective order pattern dependent on cell density, leading to a coordinated developmental state of stem cells in response to BMP4 ligand supplementation. We created a simplified mathematical model to understand how cell colonies behave collectively. This model combines aspects of cell mechanics, how genes are expressed, and the way cells communicate with each other, all based on the study of changes in gene expression over time. Inspired by the concept of how herd dogs guide sheep, we applied our model to identify and implement mechanisms for directing group behavior. Our finding demonstrate that influencing just a few guide cells can determine the determine the behavior of the entire colony. Our framework offers a new approach to studying and controlling collective dynamics in biological systems via synthetic biology, with implications for applications in stem cell research and regenerative medicine.

8: A Synthetic Device to Monitor the Interaction between Human Embryonic Stem Cells

Omar Bashth, Janella Schwab, Nika Shakiba
The University of British Columbia (UBC)

Human pluripotent stem cells (hPSCs) have the remarkable capacity to self-renew and differentiate into any cell type of the body, rendering them powerful substrates to model human development in vitro. However, these cells live in a social network where they physically interact with one another, exchanging signaling molecules to coordinate fate and function. To elucidate their contact-mediated interactions, we genetically engineer hPSCs to express a synthetic ligand or receptor ("synthetic Notch"), enabling the activation of a fluorescent reporter protein upon cell-cell contact. Our engineered hPSCs show a strong and stable localization of the ligand ("sender cells") and receptor ("receiver cells") on their outer membrane. Co-culture of senders and receivers in pluripotent culture leads to high efficiency of reported expression (~50% of receiver cells), validating the use of the synthetic Notch in hPSCs. To verify the required physical interaction for activating the fluorescent reporter, we conducted separate cultures of sender and receiver cells using culture insert, enabling us to monitor their expansion and migration over time. Subsequently, upon removal of the culture insert, we observed activation exclusively within receiver cells situated at the border of interaction with sender cells. This confined activation underscores the requirement of direct cell-cell contact to trigger the reporter expression. We next took advantage of micropatterning technologies to allow our co-cultured sender and receiver hPSCs to self-organize into spatially defined lineages resembling the three germ layers of ectoderm, mesoderm and endoderm. Our results show robust activation of the fluorescent reporter with distinct localized enrichments of receiver cells within the pattern. By isolating the fluorescently activated and non-activated receivers, we are conducting transcriptome analysis to explore the impact of cell-cell interactions on hPSC

fate during lineage commitment. These investigations shed light on the interplay between cell-cell interactions and hPSC fate decisions during lineage commitment, offering valuable insights into the mechanisms governing developmental processes. Further, this will enable improved engineering pipelines, where cell-cell interactions can be engineered and controlled to improve the efficiency and reproducibility of cell fate programming for regenerative medicine.

9: Engineered Saccharomyces boulardii: A Probiotic Approach for Combating Pathogen-Derived Gastrointestinal Diseases

Laura Enekegho, Dr. David Stuart
University of Alberta

The battle against infectious gut diseases remains a significant problem in healthcare and agriculture. The pathogen, Clostridium perfringens, causes highly inflammatory diseases in humans and farm animals, with high associated mortality rates. In poultry birds, it is responsible for the necrotic enteritis (NE) disease, which is characterized by inflammation of the small intestines, followed by acute death. Necrotic enteritis significantly hampers the productivity of the poultry industry, as billions of dollars are spent annually on treatments for the disease. As such, C. perfringens contributes to a worldwide issue of poultry food shortage, presenting an essential need for better therapeutics to combat the diseases it causes. Antibiotics are commonly used to help fight infections caused by C. perfringens. However, long-term antibiotic usage has been shown to contribute to gut dysregulation and promote environments where pathogens flourish. This presents a significant issue as modern medicine heavily relies on antibiotics to treat pathogen illnesses. In response to this, our group is engineering the probiotic yeast, Saccharomyces boulardii, as an alternative candidate for fighting the necrotic enteritis disease. S. boulardii is an attractive probiotic because it thrives well in the gut environment, is a fast-dividing microbe, and can be used to produce a broad range of peptides. Leveraging these characteristics, we aim to engineer this probiotic yeast to produce antimicrobial proteins that can bind to C. perfringens in the gut and promote its clearance. To do this, we use genetic modification tools such as CRISPR inhibition and homologous recombination to create engineered S. boulardii strains. These strains can produce heterologous proteins at a high rate and secrete anti-microbial peptides in the gut. Thus far, by targeting the yeast secretory system, we have successfully designed multiple S. boulardii strains with increased capacity of protein production. We have also used integrative genomic tools to introduce endolysin, an antimicrobial gene, into the yeast genome. When secreted, this endolysin peptide promotes killing of C. perfringens by targeting and breaking down its cell wall. We have observed that our S. boulardii probiotic producing the endolysin peptide kills C. perfringens in vitro. Further work is underway to show that incorporating this engineered probiotic in poultry feed can help treat birds infected with the necrotic enteritis disease. Altogether, we hope to show that these approaches can result in a novel therapeutic that is more affordable and effective at treating pathogenic infections compared to current treatment options.

10: Genetic Tools for the Spatial Characterization of Gene Expression in the Commensal Gut Bacterium Bacteroides thetaiotaomicron

Giselle McCallum, Juan Burckhardt, Jerry He, Charlotte Clayton, Alice Hong, Laurent Potvin-Trottier, Carolina Tropini
The University of British Columbia (UBC)

The human gut hosts a diverse and dynamic collection of bacteria that are critical to our health. Changes in community composition are associated with many diseases, yet the role of individual species' spatiotemporal dynamics remains relatively unexplored. The gut is comprised of diverse and heterogenous ecological niches ranging in oxygen levels, pH, antimicrobial peptides, and other factors that affect bacterial growth and function. Characterizing spatial differences in bacterial abundance and function is critical to understand the link between health and microbiota composition and will be critical for the implementation of emerging technologies such as engineered probiotics or targeted therapeutics. As tools to measure bacterial function in situ with spatial resolution are lacking, we aim to develop a biosensor that reports host cell gene expression levels via fluorophore expression to characterize spatial differences in gene expression in the human commensal bacterium Bacteroides thetaiotaomicron (B. theta) using confocal microscopy. To this end, we have engineered

several repressible promoters that can drive fluorophore expression when activated, which form the basis of a modular B. theta-compatible transcriptional reporter to quantify expression from native B. theta promoters. Preliminary biosensors reporting expression from an oxidative stress-induced promoter (PahpC) have shown detectable changes in GFP (~5-fold change) in response to peroxide-induced oxidative stress in vitro. We are now validating this reporter in an in vivo mouse model, in which we will characterize differences in oxidative stress response throughout regions of the gut, as a function of distance from the epithelium, and in models of inflammation. In the future, this modular reporter will allow the interrogation of B. theta gene expression levels throughout the gut and under various perturbations to the environment, ultimately helping to characterize the bacterial microbiome's dynamic response to the diverse and varying biogeography of the gut.

11: Randomizing the human genome

Leopold Parts
University of Cambridge

While protein-coding genes are characterized increasingly well, 99% of the human genome is non-coding and poorly understood. This gap is due to a lack of tools for engineering variants that affect sequence to the necessary extent. To bridge this gap, we have developed a toolbox to create deletions, inversions, translocations, and extrachromosomal circular DNA at scale by highly multiplexed insertion of recombinase recognition sites into repetitive sequences with CRISPR prime editing. Using this strategy, we derived stable human cell lines with several thousand clonal insertions, the highest number of novel sequences inserted into single human genomes. Subsequent recombinase induction generated an average of more than one hundred megabase-sized rearrangements per cell, and thousands across the whole population. The ability to detect rearrangements as they are generated and to track their abundance over time allowed us to measure the selection pressures acting on different types of structural changes. We observed a consolidation towards shorter variants that preferentially delete growth-inhibiting genes and a depletion of translocations. We isolated and characterized 21 clones with multiple recombinase-induced rearrangements. These included viable haploid clones with deletions that span hundreds of kilobases as well as triploid HEK293T clones with aneuploidies and fold back chromosomes. We mapped the impact of these genetic changes on gene expression to decipher how structural variants affect gene regulation. The genome scrambling strategy developed here makes it possible to delete megabases of sequence, move sequences between and within chromosomes, and implant regulatory elements into new contexts which will shed light on the genome organization principles of humans and other species.

12: PHYCUT: A Multiguide CRISPR Editing System for Glycoengineering of Phaeodactylum tricornutum

Emily Stuckless, Dr. Samuel Slattery, Dr. David Edgell, Kira Dempsey, Warren Lament
Schulich School of Medicine and Dentistry

Since the publication of its genome sequence in 2008, the diatom Phaeodactylum tricornutum has garnered much interest as a model microalgal species with applications in biofuels and recombinant protein production. A lack of system for creating simultaneous gene knock outs has hindered efforts to re-engineer the genome on a large scale. We developed the CRISPR-based multiguide editing system PHYCUT (PHaeodactylum tricornutum csY4 Cas9 mUltiplex Tool), which co-expresses SpCas9, an sgRNA array, and the Pseudomonas aeruginosa cas protein Csy4. This system allows sgRNAs to be expressed on a single transcript, creating an easily cloneable modular system that is delivered on an independently replicating plasmid via bacterial conjugation. We used PHYCUT to knock out genes to humanize the N-linked glycosylation pathway of P. tricornutum. P. tricornutum is an excellent candidate for the heterologous expression of therapeutically relevant glycoproteins such as monoclonal antibodies. However, the addition of a(1,3)-linked fucose residues onto its N-glycans precludes their use in humans. The P. tricornutum genome contains 3 copies of core a(1,3)-fucosyltransferases (FucTs). Using PHYCUT I simultaneously expressed guides targeting each of the 3 FucT copies and found that 27% of exconjugants screened showed editing at all 3 loci. Notably, 37% showed defined-length deletions of ~3.4 kb between FucT copy A and B which are adjacent to each other

on chromosome 9. Exconjugants were further subcloned and sequenced and a selection of various genetic mutants were followed for phenotypic characterization, however no biallelic knock out of all 3 FucT copies was identified. Glycoproteins secreted by mutant strains showed a median decrease of 43% in a(1,3)-linked fucose compared to wild type, with the 4 best strains showing an 83% mean decrease. No growth defect was seen for these strains. Mass spectrometry will be used to validate changes to glycosylation both globally on total secreted protein and specifically to the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. The RBD is a glycoprotein that we have previously overexpressed in P. tricornutum for diagnostic purposes and was used as a purifiable glycoprotein to monitor glycosylation changes being made. Our results show that PHYCUT can efficiently target multiple genes at once using a simple and cheap conjugative delivery system. We modified the N-linked glycosylation pathway of P. tricornutum, achieving the expression of more human-like glycoproteins which could be used to create accessible and environmentally friendly biotherapeutics.

13: Surveying the Diversity of GIY-YIG Nuclease Domains for Gene Editing

Kurt W. Loedige
Western University

Gene editing involves making specific modifications to an organism's genetic material by introducing targeted double-stranded breaks in DNA using endonucleases. The CRISPR/Cas9 system is commonly used for this purpose. GIY-YIG homing endonucleases can be combined with Cas9 to create dual-cutting targeted nucleases. TevCas9 is a dual endonuclease comprising the nuclease and linker domains of the I-TevI GIY-YIG homing endonuclease, along with Cas9 from Streptococcus pyogenes (SpCas9) or Staphylococcus aureus (SaCas9). This allows the RNA-guided CRISPR/Cas9 system to cut target DNA twice. In our most recent publication, we discovered that the 2-nt, 3' DNA overhang generated by the I-TevI nuclease domain of TevSaCas9 can hybridize with a cis-localized modified repair SaCas9 gRNA, facilitating directional insertion of up to 900 bp of DNA in human cells (rep-editing). While the preference of the I-TevI nuclease domain for cleavage sites resembling its cognate motif (5'-CN↑NN↓G-3') appropriately spaced relative to a Cas9 PAM is a key component of TevCas9's targeting and activity, it can limit the accessible target sequences for rep-editing. To overcome this, we explore the diversity of cleavage motif preferences within the GIY-YIG nuclease domain superfamily to find domains that can be combined with Cas9 (GIY-Cas9s) and have distinct preferences from the I-TevI nuclease domain. Screening of uncharacterized nuclease domains has revealed 17 domains with strong cleavage activity on a randomized DNA substrate library in vitro. Further investigation of the most active domains, I-BamI and I-SphI, using a novel Oxford nanopore sequencing method confirms their cleavage site preferences as 5'-TTTTT-3' and 5'-NANCG-3' respectively. Employing them in vitro enables editing of target sequences inaccessible to TevSaCas9. This research aims to expand the versatility of GIY-Cas9s and rep-editing, thereby broadening the gene editing toolbox and facilitating efficient editing of therapeutically relevant gene sequences currently inaccessible to TevCas9.

14: Enzyme Reaction Cascades to Screen and Engineer Oxidoreductases

David H. Kwan, Trisha Ghosh, Jacob Sicheri
Concordia University

Enzymes play a pivotal role in "green chemistry" as tools for biocatalysis. Oxidoreductase enzymes are especially useful for carrying out key electron transfer (redox) steps towards a wide range of chemical transformations (e.g., asymmetric hydrogenation, oxygenation, hydroxylation, epoxidation, or Baeyer-Villiger oxidation) that might not otherwise be available to chemists through conventional (nonbiological) synthetic approaches. The ability to screen oxidoreductase activity is important in identifying useful biocatalysts from nature, and also towards engineering novel ones through directed evolution. Many valuable redox enzymes are dependent upon NAD(P)H as an electron donating co-substrate (or conversely, upon NAD(P)+ as an electron acceptor), and the common method to detect their activity is to monitor the change in absorbance at 340 nm as NAD(P)H is converted to NAD(P)+ (or vice versa). The limited sensitivity of this method presents a challenge in detecting very low levels of oxidoreductase activity, and this can prove very difficult to begin engineering enzymes as improved biocatalysts when the rates of natural enzymes may be slow for a desired

redox reaction. We have recently developed a fluorescence-based, enzyme cascade-coupled system to detect oxidoreductase activity with orders of magnitude more sensitivity than conventional absorbance-based assays. While recycling NAD(P)H from NAD(P)+, the coupled enzyme cascade triggers cleavage of a fluorogenically labeled probe, releasing a strong fluorescent signal. This allows detection of very low levels of a specific oxidoreductase activity that we may wish to magnify by directed evolution using our assay in high-throughput screening.

15: Engineering Elephant TP53 Mutations

Emil Karpinski, Nikil B. Badey, Asaf Ashkenazy-Titelman, Esther Mintzer, George M. Church
Harvard Medical School

Tumor protein p53 (TP53) has been called the "guardian of the genome" and plays an important role in controlling cell growth and repair in response to external or internal DNA damage or stress. In comparison to most mammals which possess one copy of the TP53 gene, elephants also possess many retrogenes, with Asian elephants having a canonical TP53 ortholog and 29 additional retrogenes across three other chromosomes. Previous work in African elephants has implicated the presence of these retrogenes in elephants' unique resistance to cancer. Here we begin to characterize the role of TP53 and its retrogenes in Asian elephant fibroblasts through the use of paired CRISPR-Cas9 deletions to evaluate their contributions to cell growth and DNA damage response. Using various combinations of five sgRNAs we've successfully managed to disrupt the canonical TP53 gene, all 29 retrogenes, or all loci simultaneously. We subsequently note an increased through nonsignificant growth rate in TP53 knockout cells, however this pattern is not observed when only the retrogenes are deleted, despite these retrogenes being implicated in TP53 expression. Ongoing work involves more thoroughly characterizing the role of TP53 and its retrogenes more in-depth using RNAseq, and the potential use of these mutations for immortalization.

16: Learning rules of cell-cell communication from synthetic systems

Pulin Li
Massachusetts Institute of Technology

Cells within a tissue frequently exchange information with one another through secreted signaling molecules that travel over tens to hundreds mm. Being able to decipher the communicative relationship amongst cells is crucial for understanding how tissues are formed in embryos, how they perform physiological functions in adults and how things go wrong in pathological states. However, even with the rapidly expanding tools for collecting information about individual cells within tissues, our capability of deciphering the communicative relationship amongst cells is still quite limited. I will discuss how we use synthetic systems to learn the rules of cell-cell communication, which could help us "listening in to" the conversations in real tissues.

17: Exploring Duplicated Gene Retention Using Single-Cell Imaging of Protein Dynamics

Elena Kuzmin
Concordia University / McGill University

Gene duplication is common across the tree of life, includingyeast and humans, and contributes to genomic robustness. In this study, we examined changes in the subcellular localization and abundance of proteins in response to the deletion of their paralogs originating from the wholegenome duplication event, which is a largely unexplored mechanism of functional divergence. We performed a systematic singlecell imaging analysis of protein dynamics and screened subcellular redistribution of proteins, capturing their localization and abundance changes, providing insight into forces determining paralog retention. Paralogs showed dependency, whereby proteins required their paralog to maintain their native abundance or localization, more often than compensation. Network feature analysis suggested the importance of functional redundancy and rewiring of protein and genetic interactions underlying redistribution response of paralogs. Translation of non-canonical protein isoform emerged as a novel compensatory mechanism. This study provides new insights into paralog retention and evolutionary forces that shape genomes.

18: Environmental Protein Interaction Dynamics at a Proteome Scale

Dayag Sheykhkarimli, Jennifer J. Knapp, Nishka Kishore, Ashyad Rayhan, Da Kuang, Roujia Li, Betty Liu, Maria Nguyen, Oxana Pogoutse, Guillaume Dugied, Miha Škalič, Claudia Colabella, Atina G. Cote, Marinella Gebbia, Florent Laval, Kerstin Spirohn, Tong Hao, Mario Leutert, Siyang Li, Marta Verby, Nikko Torres, Brandon Ho, Grant W. Brown, Uroš Petrovič, Judit Villén, Michael A. Calderwood, David E. Hill, Marc Vidal, Evangelia Petsalaki, Nozomu Yachie, Dae-Kyum Kim, Frederick P. Roth
The University of British Columbia (UBC)

Although protein interactions are crucial for maintaining cellular homeostasis during perturbations, the dependence of protein interaction on environmental change has not been explored at proteome scale. Here we apply fluorescence-Barcode Fusion Genetics-Yeast Two-Hybrid (fBFG-Y2H) to generate an atlas of direct yeast protein-protein contacts, including a 'pilot' atlas of over a million protein pairs assayed in 31 environments, and four proteome-scale (~16 million pairs assayed) maps in baseline, DNA damage, carbon starvation, and oxidative stress environments. Orthogonal assays showed our interactions to have quality on par with interactions observed at least twice in the literature. Our validated atlas (the Environmental Yeast Reference Interactome or EYRI) contains a union of ~1500 high-quality interactions, ~30% of which were dynamic across environments. Proteins involved in environmentally-dynamic interactions were both less extensively studied and depleted for members of multi-protein complexes. Transcriptomic and phosphoproteomic measurements, obtained under matching environments, enabled the systematic discovery of interaction-regulatory models as exemplified by our analysis of regulated oligomerization of the major CTP synthetase Ura7. EYRI represents a unique resource for exploring the environment-dependent regulation of eukaryotic protein interactions.

19: Invisible Heroes: Role of a Non-Catalytic Auxiliary Protein as a(n) (Iso)flavonoid Metabolic Flux Conductor

Lee-Marie Raytek, Brandon Saltzman, Meha Sharma, Mehran Dastmalchi
McGill University

One of the major pillars of synthetic biology is the engineering-inspired, modular assembly of various biologically functional "parts" in a heterologous host cell to produce high-value targets. However, such rationally designed biosynthetic "assembly lines" suffer from pitfalls associated with stripping away the context of the native cellular environment. The plant kingdom is abundantly rich with beneficial and medicinal compounds, produced at the species-, tissue-, developmental-, and/or condition-specific levels, to provide us with a biochemical playground for engineering microbes. For instance, the phenylpropanoid-derived isoflavonoids are legume-characteristic metabolites functioning as symbiosis-driving signals, antimicrobial phytoalexins, as well as phytoestrogens in the human diet. An integral biosynthetic enzyme positioned just upstream of the first committed step of isoflavonoid synthesis is chalcone synthase (CHS), which is ubiquitous in land plants and is notorious for exhibiting promiscuous activity. Ex planta, CHS can catalyze the formation of undesirable lactone by-products – leading to "carbon loss" in the form of reduced chalcone formation. To rectify this, plants possess a non-catalytic auxiliary protein, chalcone isomerase-like (CHIL), which serves as a conductor of metabolic flux through CHS. We have demonstrated the species-specificity of CHILs from four plant phyla: bryophytes (Marchantia polymorpha), lycophytes (Selaginella moellendorffi), angiosperm monocot (Oryza sative; rice), and legumes (Glycine max; soybean), in complementing the catalytic activity of soybean CHS alone, and in coupled-assays with downstream enzymes in vitro. Inclusion of GmCHIL resulted in up to 50% reduction of by-product formation in standard and coupled enzyme assays. Functional analysis of CHILs in flavanone and isoflavone-producing Saccharomyces cerevisiae further supported this species-specificity, and boosted titers by supressing by-product formation. Generally, metabolic bioengineering largely focuses on elucidating and assembling biosynthetic components of a given pathway, although critical rectifying or flux-enhancing proteins or chaperones may additionally be involved in the native system. Because these functions are much more challenging to identify and diagnose, they are rarely implemented into synthetic biological systems; however, they represent significant bottlenecks that should be targeted for titer maximization.

20: Linked RNAs – New Tools for the In Vitro Selection of a Model Origin of Life

Hendrik Boog, Jana Caine, Peter Unrau
Simon Fraser University, Department of Molecular Biology and Biochemistry

How life emerged is one of the most fundamental, unsolved questions in modern biology, which is addressable by the methods of synthetic biology. While fossil evidence of the Origin of Life might be long gone, the synthesis of an artificial Origin of Life is likely to give key insights into the processes that made life on Earth possible. The best current model of the Origin of Life centers around RNA which can act as both catalyst and genome. An RNA polymerase ribozyme (RPR) able to extend an RNA primer into an RNA duplex, just like a DNA polymerase extends a replication fork, might have marked the transition between chemical evolution and early biological evolution capable of RNA-dependent RNA replication. The clamping polymerase (CP) ribozyme (Cojocaru, Science 2021) searches for an RNA promoter and changes its conformation from an 'open' RNA primer binding form to a 'closed' topologically entrained form upon finding the promoter. Since the 5' and 3' ends of the CP are close together in the closed form, I ligated the CP into a circle that intertwines with a circular template (cT1) to form a topologically linked catenane CP:cT1. Such structures were not characterized before. I have modified CP.m to ligate into CP:cT1 catenanes with linking numbers of either 1 or 2 to compare with the unmodified CP:cT1. These engineered constructs suggest that the ribozyme forms a catenane with linking number of 1. I also demonstrated that CP:cT1 catenane is catalytically active. Even though the CP catenanes are slightly less active than the original clamped construct, this will allow me to perform a high-performance in vitro selection where a linkage between genotype (as encoded in a pool of RNA polymerases ribozymes) and phenotype (as observed by the templated copying of the topologically entrained template) can be reliably implemented. As the ribozyme and template are permanently topologically linked, the evolution of a double-strand invading RPR that mimics the rolling circle replication of certain RNA viruses may be possible. This form of RNA replication uses an RNA replication fork highly analogous that used by DNA replication. This type of replication may have played a fundamental role early in the evolution of life.

Posters

1: Synergistic Integration of Deep Learning, Artificial Intelligence, and Synthetic Biology: Advancing Precision in Gene Therapy and Regenerative Medicine

Ilia Rezazadeh Dehaghani, Parmida Rostami
Tehran University of Medical Science

This paper delves into the intricate interplay between deep learning, artificial intelligence (AI), and synthetic biology, specifically exploring their combined applications in gene therapy and regenerative medicine. Synthetic biology, with its capacity to engineer biological systems through the design and construction of novel genetic circuits, holds immense potential for addressing complex biomedical challenges. By integrating deep learning and AI methodologies, researchers aim to optimize the intricate design parameters of synthetic genetic circuits, precisely modulate gene expression dynamics, and engineer bespoke cellular systems tailored for therapeutic applications. Leveraging vast repositories of genomic data, gene expression profiles, and cellular interactions, deep learning models enable predictive modeling of optimal genetic architectures conducive to desired therapeutic outcomes. Furthermore, AI algorithms empower the identification of novel gene targets, regulatory pathways, and biomaterial characteristics, thereby enhancing the efficiency, safety, and specificity of therapeutic interventions. This synergistic amalgamation of computational and biological methodologies heralds a transformative era in personalized medicine, facilitating the development of tailored treatments for genetic disorders, tissue injuries, and degenerative diseases, with profound implications for patient care and well-being.

2: Evidence of Leakage in Every Genomic DNA Trained Sequence-to-Expression Deep Learning Models Trained to Date and Ways to Overcome It

Abdul Muntakim Rafi, Brett Kiyota, Carl de Boer
The University of British Columbia (UBC)

Neural networks (NN) have proven to be an immensely powerful tool in predicting the function of the genome, with many recent successes in understanding gene regulatory logic in particular. However, how the genomics sequences should be split to train and test these models remains unclear. Removal of homologous sequences has been proposed when training using multiple species. Within the same species, current practice is to split the data chromosomally. We show how chromosomal splitting can lead to overestimation of model performance. We also provide a scalable solution hashSplit to split genomics sequence based datasets into train test sets without data leakage.

3: Unlocking the Catalytic Potential of BTS1 for the High-Level Production of Diterpenoids

Bureau J-A, Dong E, Bretagne D, Ignea C
McGill University

Microbial production platforms offer a sustainable production method for highly valuable and difficult to source compounds. The compounds yield is often restricted due to inherent limitations in the specific microbes used. Sesquiterpenoids are produced at much higher yield than diterpenoids in yeast due to relative higher availability of their precursor, farnesyl pyrophosphate (FPP) and geranyl geranyl pyrophosphate (GGPP) respectively. The low production titer of diterpenoids is a matter of high importance due to the high therapeutic potential of the compound class. To address this limitation a directed evolution strategy was employed to improve the GGPP production of yeast's native GGPP synthase – Bts1p. Exploiting GGPP natural role in yeast, Bts1p was evolved under introduced temperature sensitivity, rendering excess GGPP required for cell viability. An additional competing carotenoid production pathway was introduced to further increase the evolutionary pressure as well ass offering a colorimetric selection system for identification of high performing variants. The evolved Bts1p variants displayed mutations at 6 distinct residues, of which 2 were identified as surface residues. The highest performing mutants lead to an over 5-fold improvement in diterpenoid production attributed to increased substrate affinity, as confirmed by in vitro kinetic analysis. Notably, the most efficient variants harbored combined mutations at residues in close proximity to the active site and at the enzyme surface, indicating long-range modulation of the enzyme binding pocket.

4: Genetic Network Rewiring Between Distantly Related Eukaryotic Species

Vanessa Pereira, Rohan Dandage, Sakhil Amikishiev, Elena Kuzmin
Department of Biology, Centre for Applied Synthetic Biology, Centre for Structural and Functional Genomics, Concordia University, Montreal, Canada

Synthetic lethality represents an extreme example of a genetic interaction that occurs when a combination of mutations in different genes results in lethality, which would not be expected from the combined effects of individual viable single mutants. The extent of genetic interaction network conservation differs from genome sequence conservation between species. Two distantly yeast species, S. cerevisiae and S. pombe, diverged ~500 Mya and despite 75% genome conservation, they display 29% genetic interaction network conservation. Other distantly related eukaryotes such as C. elegans and H. sapiens diverged ~600 Mya. Here, we investigate genetic network rewiring by studying the genetic interactions that underlie conditional essentiality of single mutants between S. cerevisiae, S. pombe, C. elegans, and H. sapiens, whereby a gene is essential (ES) in one species but nonessential (NES) in another. We have extensively studied the 2853 S. cerevisiae – S. pombe orthologs, where ~15% are conditional ES. From 269 conditional NES S. cerevisiae genes (S. pombe ES, S. cerevisiae NES), we identified 124 cases which are rewired by synthetic lethal digenic interactions that modify conditional NES single mutants to synthetic lethal double mutants. Single mutant fitness, phenotype rate and genetic interaction degree differentiate conditional NES genes that were rewired by synthetic lethal interactions suggesting that they are functionally important in S. cerevisiae. To understand the functional

relationship between conditional NES genes and their rewiring synthetic lethal interactions, we overlapped them with common functional standards and found that they were co-expressed, co-localized, co-annotated, shared protein-protein interactions and showed similar phenotypic profiles suggesting that genetic rewiring of ES genes is local. When extending these findings to C. elegans and H. sapiens, 14-23% of orthologs are conditional ES between species. Preliminary results reveal that 14-17% of conditional NES genes in C. elegans are rewired by synthetic lethal interactions where the rewiring interactions and conditional NES genes are co-annotated, co-localized and share protein-protein interactions, indicating a functional connection similar to yeast. Understanding the rewiring of gene essentiality and how it is modulated by genetic interactions in distantly related eukaryotes may reveal principles of genetic network conservation and shed light on synthetic lethal therapeutic strategies for human disease.

5: Precise Assessment of Cancer Cell Growth and Survival by Artificial Intelligence

Nader Al Nakouzi, Jason Wong, Nasrin Khazamipour, Joya Saade, Soroush Alibagi, Daria Golanarian, Negin Farivar, Mads Daugaard
SnapCyte Solutions Inc.

Introduction: Cancer research requires accurate methods for measuring analytical parameters like cell culture confluence, cell count, colony numbers, viability, and motility. These methods must be unbiased and user-independent for reproducible data. Cell analytics involves manual processes (e.g., manual cell counting) or reagent-based approaches (e.g., viability kits). In recent years, semi-automated systems have been introduced that can either count cells, measure cell growth by density tracking, and/or determine cell viability. However, these methods are often time-consuming, require reagents and labeling, and may involve costly instrumentation. Artificial Intelligence (AI) has made strides in clinical and laboratory research, holding promise for swift integration into cancer research. Here, we present the development and validation of SnapCyte™, an AI performing accurate, unbiased, label- and reagent-free cell analytics from basic cell culture images, independent of specialized instrumentation.

Materials and Methods: Cell images were generated using diverse cell lines (MCF7, PC3, Hela, Raji...) cultured in various vessels with and without treatments (Taxane, Cisplatin, Heatshock...). For the cell count dataset, adherent cells were detached using trypsin, stained with trypan blue and loaded into a standard hemocytometer. Images were acquired using the SnapCyte™ adaptor and diverse microscopes (Leica, Hund, Zeiss, Nikon) and cell phones. Multiple datasets of 500 annotated images each were created, with images masked for confluency or for singular live and dead cells by experienced scientists. Object segmentation utilized the UNet architecture for localizing cells in cell culture images and iterative training was applied to achieve the required accuracy.

Results: After multiple training iterations, SnapCyte™ AI detection models achieved 99% precision for confluency and >95% precision and recall for cell count. SnapCyte™ surpassed standard methods (Crystal Violet, WST1, MTT, Presto blue, CyQuant, Incucyte®, and Bio-Rad TC20 cell counter), displaying high accuracy and smaller standard error variation than reagent-based assays. Compared to IncuCyte® Bio-Rad TC20, SnapCyte™ demonstrated similar accuracy and greater user-independent results. Furthermore, SnapCyte™ acquired data in under 10 minutes, with non-invasive measurements, allowing direct use of cells in downstream assays.

Conclusion: We have developed and validated an AI model for advanced cell analytics. Our data show that the SnapCyte ™ AI is at par or better than existing reagent and instrument-based solutions in assessing cell confluency, number, and viability. This technology offers a fast, accurate, and unbiased cell analytics platform that is resistant to user variations, and independent of reagents and costly equipment.

6: Continuous Directed Evolution of Opioid Receptors in Yeast

Christien Dykstra, Manpreet Vilkhu, Bjorn Bean, Vincent Martin
Concordia University

Opiates and their analogs remain preeminent in pain management worldwide and are considered essential medicines. Improving our mechanistic understanding action of the u-opioid G-protein coupled receptor is salient in opioid pharmacology and for designing better, safer pain therapies. To accomplish this, this research project will focus on generating and characterizing gene-variant libraries of the u-opioid receptor (uOR) in response to various ligands at different potencies. Leveraging our group's cell-based biosensing system in Saccharomyces cerevisiae, uOR activation is coupled to quantitative growth or fluorescence-based outputs. Our yeast sensor also comes equipped with a mutagenesis platform termed OrthoRep (Orthologous Error-prone Replication) which continuously introduces mutations on the uOR open reading frame during replication and division. Current advances in this work are the successful coupling of mutagenesis and uOR-induced growth responses and fluorescent and mutant library generation over multiple passages. We have also optimized a robust pipeline for the management of escapers, high-throughput sequencing automation, and variant characterization in fresh background strains. This work demonstrates the possibility of mutating heterologous GPCRs in our model organism to further gain insight into the underlying mechanisms of drug affinity and receptor function. Our next steps are to characterize mutants with different opioid agonists and to deploy improved biosensing abilities to metabolic engineering of enhanced opiate production in yeast.

7: Studying in vitro and in vivo Interactions of Cas9-gRNA Using a Novel FRET Based RNP Labeling Strategy

Iqra Yaseen and Peter Unrau
Department of Molecular Biology and Biochemistry, Simon Fraser University

RNA orchestrates many cellular processes and often forms RNA protein (RNP) complexes to enable function (e.g., CRISPR-Cas9:gRNA, the spliceosome etc.). In the CRISPR-Cas9 gene-editing system, the RNP complex screens double-stranded DNA (dsDNA) and upon successful assembly of the Cas9:gRNA complex to the target site, the Cas9 protein cleaves the dsDNA. This specific and programable targeting of the Cas9-gRNA complex makes it a diverse tool for gene editing, gene regulation and imaging. FRET has been used to study protein-protein, RNA-RNA and even RNA-Protein interactions. Here we develop a FRET-based fluorogenic aptamer-fluorescent protein reporter system to study RNP complexes. The structure of the unimolecular gRNA, developed by the Doudna lab, consists of 3 stem-loops: helix loop 1, 2 and 3. Using in vitro dsDNA cleavage assays, we determined that the RNA Mango II tag could be inserted into all three stem-loops of the gRNA without impeding function. FRET between the C-terminal EGFP-Cas9 donor and a Mango-gRNA acceptor is only observed when they form a complex. To optimize the dipole-dipole interaction of the fluorescent protein donor and the fluorogenic aptamer acceptor, the stem length of each RNA Mango II aptamer insertion was systematically increased, by testing a total of 110 gRNA constructs. The best construct was in helix loop 1 with a FRET efficiency of 20%. Further optimization of the FRET signal was possible by switching the commercially available EGFP-Cas9 with a brighter mNeonGreen fluorescent protein which increased the overall FRET efficiency to 41%. Our studies so far, show that RNA-Protein FRET can be used to study Cas9-gRNA assembly. However, this FRET-imaging methodology can also be used to track Cas9-gRNA complex in cells via microscopy and further be used in FACS to streamline the generation of cell lines. To this end, we are currently developing a FRET imaging strategy with Cas9-mNeonGreen and mango-gRNA. By being able to image both the Cas9 and gRNA via FRET, we can understand the cellular concentration and localization of the complex that can potentially help in understanding off-target activity, which is a significant limitation associated with CRISPR technology.

8: Investigating the Role of Amino Acid Residues in u-opioid Receptor Function Using Site Saturation Mutagenesis in a Yeast Biosensor

Manpreet Vilkhu, Christien B. Dykstra, Bjorn D. M. Bean, Malcolm Whiteway, Vincent J.J. Martin
Concordia University

Opioids are potent pain relievers that bind to specific G protein-coupled receptors (GPCRs) in the brain and other areas of the body, inducing an intracellular response that results in analgesia. Although the World Health Organization recognizes opioids as essential pain medication, they can also be highly addictive and cause severe side effects. Consequently, there's an urgent need for analgesics that mitigate the addictive

risks of current opioid formulations. This requires a deeper understanding of opioid receptor function and the intricate mechanisms contributing to addiction. While the structure of the human u-opioid receptor (MOR) has been elucidated, the specific roles of individual amino acid residues remain unclear, posing a critical knowledge gap. Addressing this gap will not only enhance our understanding of opioid addiction but also illuminate GPCR-ligand interactions, essential for developing safer analgesic alternatives. Scanning site saturation mutagenesis (SSSM) can aid in understanding the functional components of u-opioid receptor residues. SSSM involves systematically mutating each amino acid residue in the receptor and screening the variants for receptor function. While SSSM requires high-throughput screening, which can be challenging in mammalian cells, it is possible in yeast. Our laboratory has recently developed a humanized yeast-based opioid biosensor by functionally connecting MOR to an endogenous signalling system resulting in increased expression of green fluorescence protein (GFP) in the presence of opioid agonists. However, this biosensor has low sensitivity and selectivity. To address these limitations, we designed an SSSM library targeting the core domain of the receptor for testing in our yeast biosensor. The library variants underwent screening using the opioid analog DAMGO and were sorted based on fluorescent intensity. Subsequently, next-generation sequencing was employed to decipher the unique roles of each amino acid in binding and activation. Our preliminary results suggest that substitutions within the extracellular loop 2 of MOR enhance the GFP response of the biosensor compared to the wild-type receptor. Currently, we are screening and characterizing additional variants to pinpoint specific mutations influencing MOR functionality. Our future work aims to unravel which amino acids within MOR play pivotal roles in its interaction with various opioid analogs. This study not only advances our comprehension of the structure-function relationship of GPCRs but also contributes to the development of a robust cell-based pharmacological screening platform with enhanced sensitivity and selectivity. Such advancements are crucial for quantifying opioid production and discovering novel ligands from pharmacological libraries.

9: Culture Wars: Empirically Determining the Best Approach for Plasmid Library Amplification

Nicholas Mateyko, Carl de Boer
University of British Columbia

DNA libraries play a crucial role in various biological experiments, such as cDNA library screening, CRISPR screens, and massively parallel reporter assays. These libraries are generally stored within plasmids that are amplified in E. coli to obtain sufficient material for an experiment. Maintaining uniformity within these libraries is essential to ensure consistent testing across all library elements, and the method used to culture these libraries during amplification is thought to impact their uniformity. In this study, we evaluated five common culturing methods: liquid culture, semisolid agar, cell spreader-spread plates with high or low colony density, and bead-spread plates. We found that the simplest method, liquid culture, was equivalent to or better than other methods for amplifying a high complexity library of uniform length with low transformation coverage. However, the choice of culturing method had minimal effect on the uniformity of amplification for a lower complexity library with a wide size range and high transformation coverage. These results suggest that in most cases, researchers can save time and money by using liquid culture to amplify plasmid libraries without sacrificing library uniformity.

10: Enhancing Research Using UBC-Life Sciences Institute's Scientific Core Facilities

Tom Pfeifer, Stephane Flibotte, Guang Goa, Jason Rogalski, Armando Alcazar, Andrew Johnson
LSI-UBC

The Life Sciences Institute (LSI) at UBC provides state-of-the-art research Core Facilities to make technology and expertise available to all internal UBC researchers as well as external users. Our Core facilities have state of the art equipment and are staffed with skilled researchers and technicians, who facilitate efficient and effective delivery of key technologies, generate technological knowledge, and train people across various disciplines. They are also a wonderful way of temporarily extending your workforce without having to train or hire additional personnel. These Core's consist of: Biofactorial, a high throughput biology group providing

access to libraries and automation equipment for most needs; Bioinformatics, providing consultation, training and analysis services; LSI Imaging, providing access to state-of-the-art equipment for super resolution and confocal microscopy, high content imaging, live cell imaging, and analysis software; ubcFLOW, providing access to high-end flow analyzers, and flow sorting services; Proteomics Facility offering a broad range of services, from mass confirmations to large-scale quantitative proteomic screens, along with all the associated bioinformatics; and Metabolomics providing access to state of the art MS for detailed profiling of metabolites. This poster highlights the Cores capabilities that can help enable the next stages of research programs.

11: A Combined Synthetic Biology and Mathematical Modeling Approach for the Large-Scale Supply of Human Pluripotent Stem Cells

Ali Shahdoost, Nava Leibovich, Ren Takimoto, Rina Sakata, Soh Ishiguro, Ivana Barbaric, Nozomu Yachie, Sidhartha Goyal, Nika Shakiba
School of Biomedical Engineering, UBC

The unlimited potential of human pluripotent stem cells (hPSCs) for self-renewal and differentiation into all body cell types positions them as a crucial resource for regenerative medicine cell therapies. To meet the escalating demand for these therapies, bioprocessing pipelines must scale up to produce trillions of hPSCs. However, prolonged cultivation of hPSCs often leads to the emergence of variants with genetic and epigenetic abnormalities. These variants not only display a cancer-like growth pattern that dominates cultures but also reduce differentiation efficiency and are unsafe for clinical applications. Current methods for identifying these variants are inadequate; standard assays like karyotyping only detect a narrow range of known genetic changes, leaving many harmful mutations undetected. Additionally, once detected, there is no effective method to eliminate these variants from cultures, often necessitating the disposal of affected hPSC batches.

In response to these challenges, this project introduces a novel bioengineering strategy that utilizes a CRISPR-based DNA barcoding technology combined with computational modeling. This innovative approach identifies variant hPSCs by analyzing growth rates, which act as a phenotypic fingerprint, thus bypassing traditional genotypic assays. The central goal of this initiative is to develop and implement a technology capable not only of tracking but also eliminating the problematic variants from cell cultures. This technology promises to be the first standardized method for detecting and removing problematic hPSC variants, potentially revolutionizing hPSC bioprocesses across the biotechnology industry. This advancement is especially significant as cell therapies emerge as a new class of treatments in Canada and globally. By enabling a large-scale, reliable supply of hPSCs, this approach could replace all current methods, which have significant limitations, and it is poised to be implemented in bioreactors and suspension cell cultures, enhancing safety and efficiency in cell therapy manufacturing.

12: A Novel Mechanism of N-terminal Protein Degradation

Lina Alhourani, Richard Fahlman
University of Alberta

Protein degradation is essential for maintaining the intact proteome under both healthy and stressed conditions. Disruption of proper protein degradation could result in the accumulation of hazardous intracellular aggregated species and eventually diseases. Proteins starting with Met are considered stable degrons, but some will still be degraded by the different branches of the N-end rule depending on the amino acids following the initiator Met. I found a new type of N-terminal-dependent degradation using mammalian cells, and genetic tools to mutate the N-terminal part of expressed proteins, I noticed that proteins expressing MK- and MR-N-terminal motifs showed the least stability of translated reporters. To test if this type of degradation is a proteasomal-dependent type I treated the cell model with a proteasome inhibitor that was able to block the degradation. I tested two exogenous reporters expressing the same N-termini in comparison to a control. I was able to show that this type of degradation is not substrate-based dependent. There is limited knowledge regarding such type of N-terminal-dependent degradation. Therefore, my project aims to investigate this type of N-terminal-dependent instability in mammalian cells not only Hela cells. Together this study aims to reveal a novel mechanism that regulates the stability of proteins.

13: Toward A Large-Scale Gene Regulatory Network Inference for Human Cells through Divide-And-Conquer Approach

Herbert Yao, Yusuke Kijima, Brett Kiyota, Nozomu Yachie
UBC

The gene expression landscape, shaped by transcription regulation, is crucial for controlling various aspects of a cell's life, including its growth, differentiation, aging, and death. This intricate control system is often conceptualized as gene regulatory networks (GRNs), which are mathematical models representing how genes regulate themselves and each other. Constructing a comprehensive and simulatable GRN model is a primary objective in modern biology, as it would significantly enhance our understanding of cellular processes and their roles in development and disease within the human body. However, the field of GRN faces challenges due to gaps in knowledge, data availability, and computational complexity. In this project, we propose a novel divide-and-conquer methodology to construct large-scale GRNs using data from single-cell RNA sequencing (scRNA-seq). Our approach involves initially sampling gene expression profiles from extensive scRNA-seq datasets to create multiple low-resolution or local GRNs. These smaller GRNs are then assembled into a comprehensive sample-level GRN model. This unified model undergoes a process of simulation and data assimilation to ensure it accurately reflects the scRNA-seq data. Additionally, we aim to extend this framework to incorporate GRNs from various scRNA-seq datasets, constructing a global GRN model. Such method to achieve a simulatable cell model that comprehensively interprets cellular behaviors at the scale of the entire body has not been established previously. We believe our approach marks a significant advancement in biological research, paving the way for groundbreaking developments in whole-cell and tissue-level modeling of mammalian systems.

14: A Multi-Kingdom Genetic Barcoding System for Precise Target Clone Isolation

Soh Ishiguro, Kana Ishida, Rina C. Sakata, Hideto Mori, Mamoru Takana, Samuel King, Omar Bashth, Minori Ichiraku, Nanami Masuyama, Ren Takimoto, Yusuke Kijima, Arman Adel, Hiromi Toyoshima, Motoaki Seki, Ju Hee Oh, Anne-Sophie Archambault, Keiji Nishida, Akihiko Kondo, Satoru Kuhara, Hiroyuki Aburatani, Ramon I. Klein Geltink, Yasuhiro Takashima, Nika Shakiba, and Nozomu Yachie
UBC

Clonal heterogeneity underlies diverse biological processes, including cancer progression, cell differentiation, and microbial evolution. Cell tagging strategies with DNA barcodes have recently enabled analysis of clone size dynamics and clone-restricted transcriptomic landscapes of heterogeneous populations. However, isolating a target clone that displays a specific phenotype from a complex population remains challenging. Here, we present a new multi-kingdom genetic barcoding system, CloneSelect, in which a target cell clone can be triggered to express a reporter gene for isolation through barcode-specific CRISPR base editing. In CloneSelect, cells are first barcoded and propagated so their subpopulation can be subjected to a given experiment. A clone that shows a phenotype or genotype of interest at a given time can then be isolated from the initial or subsequent cell pools stored throughout the experimental timecourse. This novel CRISPR-barcode genetics platform provides many new ways of analyzing and manipulating mammalian, yeast, and bacterial systems.

15: Multiplexable Production of Monoclonal Pseudoviruses for High-Throughput Viral Variant Screening

Samuel King, Soh Ishiguro, **David Mackay**, Brian Guo, Morris Huang, Myles Osenton, Arman Adel, and Nozomu Yachie
UBC

Synthetic virology has enabled the production of pseudotyped viruses and their use in medical therapies and evolutionary studies of viral variants. Pseudotyping a regulated lab viral strain with surface glycoproteins of interest is a safe alternative to working with live virus strains. However, it remains difficult to create and analyze synthetic variants at a large scale, given that each variant must be individually packaged with monoclonal surface proteins, preventing a library-scale production of viral variants with the current pooled

packaging methods. Here, we describe a multiplexable viral packaging technology to produce and screen monoclonal synthetic viral variant libraries in single pooled packaging and infection steps. We employed large serine integrases to simultaneously reassemble barcoded viral variant genomes and activate pseudotype variant expression upon integration into a single landing pad locus in host cells. Following the single integration events per cell, the landing pad cells each effectively become monoclonal viral variant factories, whereby the pseudotyped viruses can be harvested and used in a large-scale assay of choice, such as serum neutralization or drug screening. We envision the technology will create new possibilities in synthetic virology, such as enhanced pandemic preparation procedures, drug testing, and therapeutic development.

16: Modeling Functional Evolutionary Trajectories of Protein-Coding Sequences by High-Dimensional Genotype-Fitness Landscapes

Sofia Romero and Nozomu Yachie
UBC

Sequence homology-based analysis enables the extrapolation of functional annotations between homologs, prediction of protein structures, as well as the reconstruction of the protein evolutionary trajectories. While the sequence-based phylogeny estimation captures evolutionary paths, the neutral theory of molecular evolution suggests that sequence evolution does not always impact the functional evolution since most DNA changes are not adaptive or subjects to natural selection. Besides functionally beneficial mutations that incrementally evolve protein function, mutations that are initially neutral can either become beneficial through subsequent mutations or develop into molecular constraints. In contrast, certain mutations that initially yield beneficial effects in evolution may later be masked by subsequent mutations, displaying them seemingly neutral for biological observation. Our current view of gene evolution might underestimate such "evolutionary buffering mutations" and "evolutionary masked mutations." Therefore, the understanding of evolution and evolvability of proteins remains premature in this regard. Here, we plan to measure high-dimensional fitness landscapes of yeast and human histone subunits in yeast cells and associate them with their sequence evolutionary trajectories. We will establish a system to provide single, double, and higher-order synthetic mutations to a pair of yeast, human and ancestral histone subunits and measure their fitness. Aside from the measurement of the evolutionary landscape, we also envision that such a high-throughput genotype-fitness landscape would provide a wealth of resources for functional and structural prediction as well as protein engineering.

17: High-Content Cell Lineage Tracing of Mouse Embryogenesis

Nanami Masuyama, Yusuke Kijima, Hideto Mori, Hirofumi Nishizono, Mikiko Negishi, Madina Kagieva, Shoma Matsumoto, Eiichi Okamura, Arman Adel, Rina Sakata, Hiromi Toyoshima, Kentaro Kakoi, Soh Ishiguro, Yoko Tanimoto, Osamu Masui, Motoaki Seki, Keiji Nishida, Haruhiko Koseki, Hiroyuki Aburatani, Masatsugu Ema, Seiya Mizuno, Nozomu Yachie
University of British Columbia

Mammalian development starts from a single fertilized egg that proliferates to form complex structures in the body. Several approaches have been reported to trace cell lineages of metazoan species, whereby chromosome-embedded DNA barcodes are continuously mutated by CRISPR-Cas9 genome editing and inherited from mother to daughter cells. In such a system, the mutation patterns of the descendant barcodes allow the reconstruction of the developmental lineage, akin to evolutionary phylogeny estimation. However, the cell lineage resolutions of previous methods remain limited, mainly because DNA barcodes are short, and Cas9 induces a limited pattern of mutations mainly by deletions and is toxic to cells. Here, we will present a high-content lineage tracing method Barclock that we have developed in the last several years. The Barclock system employs CRISPR base editing for the massively parallel recording of cell lineage information into multiple hundreds of gRNA targeting sequences and scRNA-seq for reading the recorded memory information. We envision obtaining the high-content map of mammalian developmental cell lineage for the first time.

18: Development of a High-Throughput Platform for Transcription Factor Characterization

Will Cheney, Carl de Boer
University of British Columbia

Transcription factors are integral modulators of gene expression. They function by binding to specific DNA sequences and regulating transcription. A comprehensive understanding of gene regulation and potential effects of mutation is reliant on our ability to identify sequences a transcription factor will bind. Notably, transcription factor binding motifs, encapsulating the optimal binding sites, are unknown for approximately 60% of all eukaryotic transcription factors. Current techniques for identifying motifs are labour-intensive and have limited scalability with increasing transcription factor throughput. Here, we augment our previously described Gigantic Parallel Reporter Assay (GPRA), enabling the characterization of binding motifs for a diverse library of transcription factors. A GPRA leverages randomized synthetic promoters to control expression of a fluorescent reporter. Sorting by reporter expression followed by sequencing allows us to identify important regulatory sequences, including of transcription factor motifs. By adding an exogenous

19: High-throughput Investigation of Underlying Mechanisms of Priority Effects to Facilitate Microbiome Engineering

Leah Fulton, Sarzana Hossain, Melissa Chen, Ziniu Chen, Steven Hallam, Kayla King, Cara Haney University of British Columbia

Microbes exist in complex communities known as microbiomes that can be free-living or in close association with a host. From promoting the growth of plants to nutrient cycling in the ocean, microbial communities perform important services to their host or environment. As a result, microbiome engineering through the manipulation or introduction of microbes has the potential to improve the health of humans, agriculture, and the environment. However, priority effects where native microbes exclude introduced strains, can limit success of engineering strategies; thus understanding what allows microbes to invade a community is essential to improving microbiome function. We hypothesise that closely related strains will have more niche overlap and thus have strong inhibitory priority effects while more dissimilar strains will co-exist regardless of arrival order. To test this hypothesis, we developed a high throughput model system to investigate priority effects among Pseudomonas bacteria of varying genetic diversity on the roots of the model plant Arabidopsis. Our preliminary results showed that our system could accurately track priority effect outcomes and that the successful establishment of a closely related Pseudomonas pair depends highly on their timing and arrival order. To understand the underlying mechanisms that allow for microbial community invasion, in a second approach we will build a tool that allows for transfer DNA from one Pseudomonas strain to another to screen for genes that facilitate microbes to overcome priority effects. Understanding the ecological and genetic factors that can predict community outcomes is key to implementation of microbiome engineering strategies.

20: CRISPR-based Diagnostics for the Rapid Detection of Candida Species and Antimicrobial Resistance Mutations

Amy Heathcote, Xena Li, Robert Kozak, Nicole Weckman
University of Toronto

Antimicrobial resistance (AMR) is an increasing global threat, projected to cause 10 million deaths per year by 2050. Of particular concern is the emergence of AMR in Candida species. These organisms are known for their highly transmissible and invasive nature, particularly in healthcare settings, where infections can often be associated with poor outcomes. This has drawn the attention of the World Health Organization who has included Candida auris on their pathogen priority list due to the pathogen's multi-drug resistance, including possible resistance to all classes of antifungals used for treatment. It is therefore imperative that we dedicate our efforts to advancing new technologies that will enhance AMR surveillance, management of outbreaks, and antimicrobial stewardship. Current techniques in fungal culture and susceptibility testing require multiple rounds of culture and traditional phenotypic-broth based assays in centralized reference laboratories, causing delays in diagnosis and patient treatment. To overcome these challenges, we propose a highly sensitive

and specific CRISPR-based diagnostic for the detection of genetic biomarkers associated with antifungal resistance in Candida species. CRISPR-based diagnostics offer numerous advantages including cell-free, rapid, isothermal, and cost-effective results that can be tested directly at the point of care. Conventional CRISPR-based diagnostics detect short nucleic acid sequences to diagnose an infectious disease, but further research is required to advance these protocols to robustly detect single nucleotide polymorphisms associated with antifungal resistance. Here, we present several CRISPR assay designs and investigate their effectiveness in identifying specific targets within 5 commonly encountered clinical Candida species, as well as possible resistance mutations associated with azole and echinocandin antifungal agents. Assay designs will be streamlined into a user-friendly protocol and validated using DNA targets isolated from Candida cultures and samples with clinically confirmed resistance. This research will demonstrate a novel approach for rapidly analyzing clinical samples and establish a foundation for future endeavours aimed toward improving AMR and infectious disease diagnostics.

21: Salmonella-based Delivery of Immunogenic Proteins to Mammary Cancer Cells as a Novel Approach for Immunotherapy

Meilin An, Ania Bogoslowski, Josef Penninger
Department of Medical Genetics, Life Sciences Institute, University of British Columbia, Vancouver, BC, Canada

Conventional anti-cancer therapeutics face a multitude of challenges such as high levels of adverse effects in normal tissue and cells, inability to penetrate deep into tumor tissue and the development of drug resistance in tumor cells due to prolonged usage of chemotherapeutics. Therefore, there is a necessity to develop novel and effective anti-tumor therapeutics that could supplement or serve as substitutes to conventional therapies. Natural tumor-targeting bacteria are promising antitumor agents because they possess inherent abilities to selectively colonize, penetrate, and regress tumors. Another attractive aspect of utilizing bacteria for cancer therapies is the possibility to attenuate and/or genetically engineer the bacteria to acquire improved antitumor activities. Our lab has developed an efficient system for the direct cytosolic delivery of protein into cells through the engineering of the syringe-like type III secretion system of a non-pathogenic strain of Salmonella typhimurium (S. typhimurium) called ESB2519. We are currently using ESB2519 to transfer OVA protein directly to tumour cells in vitro and investigating OT-I CD8 T cell responses. Our findings will generate a novel immunotherapeutic approach for the treatment of breast cancer by using Salmonella to deliver immunogenic antigens as an alternative to neoantigen-mediated cancer immunotherapy.

22: With a Little Help from Our [Tiny] Friends: Modifying Gut Bacteria to Detect Malabsorption

Juan Camilo Burckhardt, Giselle McCallum, Jerry He, Charlotte Clayton, Alice Hong, Carolina Tropini Department of Microbiology and Immunology, School of Biomedical Engineering, University of British Columbia

Gut osmolality, reflecting particle concentration in intestinal fluid, is vital in gut physiology, governing functions such as water absorption and cell structure. Gut osmolality imbalances frequently occur in conditions such as inflammatory bowel disease, food intolerances, and laxative use. These imbalances arise as impairments in nutrient absorption that lead to an accumulation of particles in the gut lumen. Despite its importance, human intestinal osmolality remains understudied. Existing stool-based methods cannot capture the osmolality of different gut regions, limiting their use as a diagnostic tool. Furthermore, gut disease diagnosis usually necessitates invasive endoscopic procedures. Previous research from our lab revealed that certain bacterial families persist during gut osmotic upshifts. These osmotically-tolerant bacteria sense the gut osmotic environment and respond to its changes by regulating the transcription of specific sets of genes. Thus, we proposed to detect malabsorption by leveraging the genetic mechanisms that these commensals employ when responding to changes in the gut's physical environment. We repurposed regulatory sequences of osmolality-responsive genes from the genetically tractable, osmotically-tolerant, and prevalent commensal Bacteroides thetaiotaomicron to create a biosensor for localized and fine-scale osmolality measurements. Through transcriptomics analyses, we identified B. theta genes selectively expressed across increased osmolality both in vitro and in vivo. Subsequently, we cloned the promoters from the identified genes into a synthetic

reporter fluorophore circuit, creating osmolality biosensors in B. theta. We selected biosensors with a graded response to osmolality, and through further optimization, we achieved a 15- to 25-fold fluorescence difference in high osmolality conditions compared to baseline. Currently, we are validating our optimized biosensor in vivo by measuring its signal in the intestines of mice experiencing disrupted gut osmolality. Through this work, we are creating a platform for commensal-based microbial diagnostics that respond to disease-induced gut changes, improving our understanding of gut physical conditions.

23: Exploring the Role of Commensal Biofilms in Gut Microbial Community Dynamics

Jerry He, Carolina Tropini
University of British Columbia

Biofilms are a sessile bacterial lifestyle prevalent in a wide range of ecological niches. Traditionally defined as multicellular aggregates immersed in a self-produced matrix of extracellular polymeric substances, these aggregates experience direct cell-to-cell contact and molecular concentration gradients, resulting in distinct phenotypes compared to planktonic cultures. In human health, gut biofilms are of particular interest due to their high microbial density and role in human health and development. While current knowledge regarding commensal gut biofilms is limited, studies have alluded to possible roles of commensal biofilms in colonization resistance, highlighting the importance of understanding how biofilms affect gut community dynamics. We hypothesize that expression of biofilm genes in the gut promotes bacterial colonization and increases community resilience to changes in the gut environment. Here, we employ a synthetic biology approach to dissect and manipulate biofilm-specific gene networks in Enterocloster bolteae, a prevalent commensal biofilm-forming bacterium. To identify potential biofilm-associated genes in E. bolteae, transcriptomics was performed on the bacterium, grown as a biofilm or planktonically. Preliminary analysis highlighted genes that were associated with processes including quorum sensing, phosphotransferase systems, sugar utilization, and carbohydrate-associated ABC transport, all of which possess known ties to biofilm formation. To functionally characterize these genes, we are constructing knockout/knockdown mutants using CRISPR-Cas9 gene editing tools. These mutants will serve to validate the roles of these genes in E. bolteae biofilm formation, community resilience, and their effects on gut community dynamics. Altogether, this work will provide novel insights into possible roles of biofilm formation in the gut microbiome.

24: Engineering Escherichia coli for Increased Production of Outer Membrane Vesicles Carrying Therapeutic siRNAs

Kana Oshima
University of Alberta

complementary messenger RNA and can interfere with the expression of the target gene. In cancer therapy, siRNA has emerged as a powerful tool to inhibit tumor progression by effectively suppressing the oncogene expressions. However, there are some challenges in the clinical use of siRNA, such as poor membrane permeability and stability. Developing siRNA delivery systems for clinical applications is crucial to overcoming these challenges. Outer membrane vesicles (OMVs) are nanosized extracellular vesicles naturally produced by gram-negative bacteria. OMVs have gained interest in drug delivery as they can penetrate various cellular barriers, including human cells. Their ability to transport various biomolecules, including DNA and RNA, and prevent them from degradation makes OMVs potentially viable therapeutic vehicles for siRNAs. This study aims to develop a novel bacterial therapy by engineering non-pathogenic Escherichia coli to secrete OMVs carrying therapeutic siRNA. To achieve efficient delivery of siRNAs, we introduced mutations in the nlpI and yrbE genes which increase OMV production. Our experimental design utilized the E. coli KEIO deletion collection that contains these mutations marked with an excisable kanamycin resistance gene. Phage transduction was used to combine these mutations in an E. coli strain that carries an inducible T7 RNA polymerase and a deletion of rnc14 encoding RNase III that allows for increased RNA accumulation. These genomic mutations were confirmed by PCR. OMV production was quantified by protein content and nanoparticle tracking analysis. To test the functionality of the E. coli system, we designed synthetic GFP-specific DNA fragments that yield siRNAs and cloned them into a T7 promoter vector that lacks a

translation initiation site. The siRNA-encoding plasmids were introduced into the OMV hyper-producing E. coli strain and their expression was confirmed by RT-PCR. RT-PCR was also performed on RNAs extracted from the supernatant of the E. coli culture and its results confirmed the E. coli system secretes the siRNA out of the cells. We are currently investigating the packing efficiency of siRNA into OMVs by isolating the OMVs and measuring the amount of GFP-specific siRNA within them. The E. coli system will be tested on GFP-expressing cell cultures to determine if they reduce the expression of GFP. The abundance of GFP will be assayed by fluorescence microscopy and western blot. These results will elucidate the extent of its therapeutic potential and application. Future work aims to optimize the E. coli system to increase siRNA packaging into OMVs and uptake of OMV into human cells. Our study will construct a novel bacterial-based therapeutic that has the potential to secrete OMVs carrying siRNAs for specific inhibition of target genes.

25: Rapid Assembly of Guide RNA Arrays for Multiplexed CRISPR-Cas9 Applications

Asfar Lathif Salaudeen, Carl de Boer
University of British Columbia

CRISPR-Cas9 technology has revolutionized genome engineering, enabling applications from genome-wide perturbations to transcriptional regulation. Despite these advancements, one significant challenge persists, the efficient and simultaneous targeting of multiple genomic loci. In this study, we developed a streamlined method using polymerase chain assembly (PCA) and Golden Gate Assembly to rapidly assemble gRNA arrays with up to 10 gRNA units in a single day. Using a GFP reporter system, we demonstrated the advantages of using gRNA arrays as opposed to individual gRNA expression vectors and the functional activity of each gRNA positions in the assembled array. Our approach significantly reduces the time and complexity of building multiple gRNA arrays that can be used in CRIPSR-Cas9 mediated genome engineering applications.

26: Scaling Up Massive Parallel Reporter Assays with Bulk Quantitative Magnetic Cell Sorting

JJ Hum, Carl de Boer
UBC

Variations in regulatory DNA sequence affect gene expression and phenotype. Massive Parallel Reporter Assays (MPRAs) are a key tool for understanding regulatory DNA, enabling simultaneous testing of how thousands of cis-regulatory elements (CREs) affect expression of a reporter gene. The resulting sets of regulatory sequences and associated expression levels are ideal data for training gene regulation models. However, model accuracy is limited because the vast combinatorial interaction space of DNA binding proteins cannot be captured at current experimental scale. More data is required to improve predictive models of sequence to gene expression, necessitating an improvement in large-scale reporter assays.

I aim to improve MPRAs by developing a bulk quantitative magnetic activated cell sorter (MACS). Bulk sorting time with MACS is independent of cell quantity, significantly increasing throughput compared to sort-seq MPRAs where sorting time increases with cell quantity. MACS MPRA also captures cell-to-cell variation, unlike bulk RNA-seq MPRA. In MACS MPRA, cells express a cell-surface reporter protein that can be magnetically labeled with superparmagnetic particles (SPMNPs). Cells with high reporter expression will capture more SPMNPs and be more strongly attracted towards a magnet. I have designed a prototype MACS where cells with high reporter expression are attracted horizontally towards bins on an opposite magnetic face, while cells with low reporter expression will fall into bins below the cell intake. This creates an axis where expression level is related to the horizontal and vertical bin location. After sorting, cells are harvested from bins and sequenced to link CREs to expression level.

I am currently validating the MACS design using HEK293T cells tranisently transfected with a reporter plasmid containing eYFP and a cell surface marker that can be labelled with SPMNP-linked antibodies. Cells' variable plasmid copy number will be used as a proxy for gene expression to correlate magnetic and fluorescent signals and optimize MACS design. Once the sorter design is optimized, MACS MPRA will be evaluated by comparison to bulk RNA-seq MPRA using a reporter plasmid contains a minimal promoter, cell

surface marker, barcode sequence, and an enhancer library chosen for a wide range of expected expression. Validating the MACS MPRA will enable high throughput testing of CREs limited only by the number of cells we can grow, resulting in more accurate models for predicting regulatory DNA function.

27: Variant Effect Prediction Using Deep Neural Network in Autoimmune Diseases

Sambina Islam Aninta, Abdul Muntakim Rafi, Carl de Boer UBC School of Biomedical Engineering

Autoimmune diseases represent a diverse group of conditions characterized by the immune system's aberrant response against normal host tissues. In general, autoimmune diseases result from a complex interaction between multiple gene pathways and environmental factors. In order to identify new genes and pathways that are linked with the disease, many genome-wide association studies (GWAS) have been done. Several of the variants identified reside in the non-coding regions of the genome, modifying the sequences of enhancers and promoters and thus disrupting transcription factor binding sites without changing the protein coding region. Even though genome-wide association studies (GWAS) have identified hundreds-of-thousands of variants associated with autoimmune diseases, the causal variants for >99% of these trait associations have yet to be identified and functionally validated. Therefore, we lack an understanding of how many of these expression modulating variants (emVars) identified from GWAS can result in autoimmune disease. To establish a mechanism connecting thousands of these disease-causing variants in cis-regulatory elements (CRE) to their effects on gene regulatory pathways, massively parallel reporter assays (MPRAs) have been performed on primary human T cells. Utilizing this MPRA data, we were then interested to develop a deep neural network model that can predict the effect of these causal variants. Complex gene regulation makes it a challenging task to predict how single nucleotide polymorphism (SNP) in the upstream regulators can change expression. Leveraging state-of-the-art deep neural network architecture optimized for sequence-to-expression prediction from the DREAM challenge, we pretrained the model on T-cell MPRA data and further fine-tuned a siamese-style network to predict the difference in expression between alternate and reference allele. The model's ability to accurately predict causal variation will then be evaluated on the held-out test data and analyze if these predicted emVars enrich for variants with a high posterior inclusion probability (PIP). The model will then be further tested on its ability to predict variant effects from T cell-related traits that were not included in the MPRA data it was trained on. The model's ability to enrich for variants with high PIP will suggest it can identify causal variants even in untested variants in similar cell types. Furthermore, through comprehensive model interpretation frameworks such as DeepLift, we will analyze how changes in DNA sequence can influence gene expression patterns. Overall, we demonstrate how deep neural network approach can be used to establish a causal mechanism on the genetic basis of autoimmune diseases.

28: MitoCode: Using Mitochondrial DNA to Track Population Dynamics of Human Induced Pluripotent Stem Cell Villages

Najmeh Nikpour, Thristan Paulo B. Taberna, Yeganeh Dorri Nokoorani, Peter W. Zandstra, Nika Shakiba, Carl G De Boer UBC de Boer Lab

Induced pluripotent stem cells (iPSCs) are generated by direct or indirect overexpression of transcription factors to reprogram specialized cells into a pluripotent state. Theoretically, iPSCs have the capacity to become any cell type in the body. Since their discovery by Yamanaka in 2006, iPSCs have been used in drug screening, cell therapies, disease modeling, and studying development. In parallel, it has been well documented that iPSC lines differ in their properties, including growth rates and differentiation efficiency. These distinct properties and heterogeneity among iPSC lines have been hypothesized to be driven primarily by genetic variations. The increased number of iPSC lines from different donors offers an opportunity to simulate and capture the differential biological response that stems from human diversity. However, there is a lack of elegant tools that would enable these studies. The cell village model, where iPSCs from unrelated donors are cultured in one dish can be used to study context-specific and inter-individual genetic variation effects in vitro, aiming to lower costs and reduce experimental noise. To study iPSC donor composition dynamics during expansion and differentiation, one method involves using a library of gene-perturbing guide RNAs with DNA barcodes. DNA sequencing at multiple time points estimates the relative frequency of

each barcode, revealing the population dynamics and donor proportion within the cell village. However, genetic perturbations can alter cell biology or lead to mutations thus causing undesirable molecular phenotype shifts and permanent epigenetic changes. To investigate population composition in iPSC villages, we are using mitochondrial DNA (mtDNA) analysis during expansion and differentiation, circumventing current limitations. Previous studies sequencing mtDNA from multiple iPSC lines of varying donor characteristics found no evidence of de novo mutagenesis. Heteroplasmy levels of stable neutral mitochondrial variants remained unchanged throughout culture. We hypothesize that mtDNA variants could be correlated with iPSC donor identity and measure each iPSC line contribution to the cell village. To this end, we are tracking the relative abundance of individual iPSC donors in a cell village using both the DNA barcoding approach and our proposed methodology to compare the feasibility, accuracy, and robustness of these methods. We will repeat this experiment for differentiating iPSCs to hematopoietic progenitor cells and cardiomyocytes to check the robustness of the proposed method during differentiation. If successful, this approach makes studying the dynamics of cell populations in cell village studies faster, more accessible, and more reliable as it exploits inherent features of the cells, thereby eliminating the risk of DNA alterations.

29: Evolution of a Cannibalistic Enzyme from a Non-Catalytic Ancestor

Xingyu Cara Fan, Charlotte M. Miton, Janine N. Copp, Nobuhiko Tokuriki
University of British Columbia

Nature has evolved proteins to acquire a springboard of over 8000 catalytic functions 1. Elucidating the molecular mechanism underlying such functional divergence is a perennial question. While evolutionary biochemistry has provided considerable insights into the evolution of novel enzymes from promiscuous ancestors, the emergence of de novo catalysis has seldom been investigated 2,3. BluB, an enzyme involved in vitamin B12 (B12) biosynthesis, constitutes a compelling case to study this phenomenon 4. It belongs to the flavin mononucleotide (FMN)-dependent nitroreductase (NTR) superfamily, a group of enzymes that generally utilize FMN as a cofactor to perform oxidoreduction 5. In contrast, BluB catalyzes the aerobic fragmentation of reduced FMN (FMNH2) into 5,6-dimethylbenzimidazole (DMB), the lower ligand of B12 (EC 1.13.11.79). A comprehensive study of the NTR superfamily revealed that BluB likely diverged from a common ancestor of NTRs with FMN-binding activity. The evolutionary transition from FMN-binding to FMN-fragmentation represents a functional switch between two mutually exclusive molecular functions. Thus, BluB has been referred to as a "cannibalistic enzyme." The presented project aims to decipher how and when this functional switch occurred. Using Ancestral Sequence Reconstruction (ASR) 6, I will resurrect the ancestral states of BluB to explore the stepwise mutational events giving rise to the function of FMNH2 fragmentation and DMB synthesis. I will additionally compare BluB's phylogeny with the species tree to reveal the evolutionary context of BluB, hence the emergence of aerobic B12 biosynthesis pathway. Our research will offer molecular insights into the evolution of catalysis, aiding de novo protein design. The resurrected nitroreductase ancestral fold will also serve as an evolvable platform for engineering novel biocatalyzers. Moreover, our findings will provide unique opportunities to explore the evolution of complex anabolic pathways such as B12 biosynthesis 7.

References

1. Chang, A. et al. BRENDA, the ELIXIR core data resource in 2021: new developments and updates. Nucleic Acids Res 49, D498–D508 (2020).
2. Kaltenbach, M. et al. Evolution of chalcone isomerase from a noncatalytic ancestor. Nat. Chem. Biol. 14, 548–555 (2018).
3. Clifton, B. E. et al. Evolution of cyclohexadienyl dehydratase from an ancestral solute-binding protein. Nat Chem Biol 14, 542–547 (2018).
4. Hazra, A. B., Ballou, ‡ David P. & Taga, § and Michiko E. Unique Biochemical and Sequence Features Enable BluB To Destroy Flavin and Distinguish BluB from the Flavin Monooxygenase Superfamily. Biochemistry-us 57, 1748–1757 (2018).
5. Akiva, E., Copp, J. N., Tokuriki, N. & Babbitt, P. C. Evolutionary and molecular foundations of multiple contemporary functions of the nitroreductase superfamily. Proc. Natl. Acad. Sci. 114, E9549–E9558 (2017).

6. Thornton, G. K. A. H. and J. W. Reconstructing Ancient Proteins to Understand the Causes of Structure and Function.
7. Martens, J.-H., Barg, H., Warren, M. & Jahn, D. Microbial production of vitamin B12. Appl Microbiol Biotechnol 58, 275–285 (2002).