CENTER FOR THE MULTIPLEX ASSESSMENT OF PHENOTYPE

NEWSLETTER

Fall | October 27, 2020

https://www.cmap.gs.washington.edu

WELCOME

The Center for the Multiplexed Assessment of Phenotype, (CMAP) is a Center of Excellence in Genome Sciences, supported by the National Human Genome Research Institute. Our goal is to develop technologies to assess the functional impact of variants in human genes. Linking phenotype to genotype is one of the most pressing problems in biology and our goal is to facilitate variant interpretation to enable genome- guided precision medicine in clinical decision making. We are based at the University of Washington and at the University of Toronto.

CMAP - COVID CHRONICLES

CEGS co-PIs Lea Starita and Jay Shendure were featured in an NBC News illustrated series; Covid Chronicles. See the true story in comic book form here: https://nbcnews.to/33klOhR

SAVE THE DATE

Mutational Scanning Symposium and Workshop

April 5th, 6th and 7th, 2021

Virtual! Free! Registration link below:

https://www.varianteffect.org/aveevents

Towards Variant Effect Mapping of secreted proteins

by Daniel Tabet (Roth Lab)

Over the past decade, large-scale genetic sequencing projects have uncovered millions of variants, many of which are found in genes associated with human disease. However, our ability to interpret the effects of these genetic variants in the clinic has yet to reach the scale required for the widespread application of genome -guided precision medicine. By applying large-scale functional assays, we can create variant effect maps (VEMs) which enable us to proactively study all possible coding sequence variants in a gene of interest. Not all genes are easily amenable to large-scale assays however, one such instance being those encoding secreted proteins.

Our ability to generate VEMs for any gene depends largely on the connection between the genotype of a cell and its selectable phenotype. This connection allows us to perform assays on thousands of variants at once in large pools, assessing the function of a protein and reading the corresponding genotype. In the case of secreted proteins, the connection between genotype and phenotype is lost, preventing us from performing the large-scale pooled assays required to generate VEMs.

To overcome this obstacle, we can envision at least two general techniques, which we describe below in the context of studies on two secreted proteins implicated in coronary heart disease, lipoprotein lipase (LPL) and proprotein convertase subtilisin/kexin type 9 (PCSK9).

The first strategy is to anchor the protein to the cell surface. We are pursuing this for LPL, which acts in the breakdown of triglycerides carried in large lipoprotein particles, allowing them to be cleared from circulation. To carry out this role, LPL is linked to the cell surface by interactions with membrane proteins. However, these interactions are transient, and the protein transfers readily to neighbouring cells, posing an obvious problem for pooled assays. To remedy this, we experimented with GPI anchors, small post-translational modifications that robustly connect the protein to the cell surface. We have since observed that GPI-tagged LPL remains entirely associated to its parental cell and the effects of the modification on the protein appear minimal. While this approach has proven useful for proteins that carry out their function(s) on the cell surface, it is not amenable to all secreted proteins.

The second general strategy involves microfluidic encapsulation of single cells. Here, the cells are, in one sense, still pooled in heterogeneous culture. In another sense, however, each single cell (or single-cell-derived clone) is isolated in its own microfluidic environment. Kevin Wang, a graduate student in the Roth lab, has been working on developing a generalizable platform based on the microfluidic encapsulation of single cells. He is applying this to the study of PCSK9, a secreted protein that poses particular challenges due to its paracrine function. PCSK9 acts in the internalization and degradation of the LDL-receptor, clearing LDL particles from the circulation and preventing high cholesterol levels. To assess the impact of variants on this protein, a GFP-tagged LDL-receptor is used to measure the ability of PCSK9 to bind and target the LDL-receptor for degradation. By encapsulating cells in individual droplets, a micro-environment is created, such that the protein can only act on the cell by which it was secreted, re-establishing the connection between genotype and phenotype.

With the expansion of these techniques, we can unpack the effects of genetic variation on a broad range of secreted proteins, thereby advancing genome-guided precision medicine.

References:

Deep mutational scanning: a new style of protein science. Fowler, D.M. & Fields, S. Nature methods, (2014).

Multiplexed assays of variant effects contributed to a growing genotype-phenotype atlas. Weile, J. & Roth, F.P. Human Genetics, (2018).

AVE ALLIANCE

The Atlas of Variant Effects Alliance is an international collaborative effort with a mission to propel systematic measurements of variant impact on functional elements of human and pathogen genomes, towards diagnosing and treating human disease and understanding genes, gene products and their regulation.

This budding alliance is led by several UW CMAP PIs and dovetails with the scientific mission and goals of our existing center. The vision of the Alliance is to create comprehensive variant effect maps for important regions of human and human pathogen genomes.

To learn more please visit: https://www.varianteffect.org

Atlas of Variant Effects

CEGS 2020 - NHGRI GRANTEE MEETING

Our center participated in NHGRI 's annual CEGS meeting on October 15th and 16th , 2020. After the plenary/ introduction from Doug Fowler we had a series of fabulous 5 minute talks by our researchers Kevin Kuang, Shawn Fayer, Lauren Saunders, and Lea Starita . A set of pre-recorded lightning talks by Kevin Wang, Nick Popp, Yash Ashok, Val Grillo-Alvarado, Kyle Hess , Hyeon-Jin Kim, Ian Smith and Mike Dorrity provided succinct snapshots of research in progress. It was a great opportunity to showcase our work and to engage with other researchers in this community. We look forward to seeing everyone again next year!

THESIS DEFENSE - BRYAN ANDREWS

CMAP researcher Bryan Andrews from the Fields lab defended his thesis "Analysis of protein adaptation from high throughput mutagenesis studies" on August 20th via Zoom. Congratulations Dr. Andrews!