Gladstone Institutes
San Francisco, CA
Katie Pollard, Vijay Ramani, Seth Shipman
$1,300,000

Thousands of people have had their genome sequenced, and yet the genetic mechanisms underlying humanity's most devastating diseases remain unknown, limiting the ability to diagnose and treat patients. One reason for this knowledge gap is the lack of tools for identifying causal genetic variants outside protein coding genes. The noncoding genome constitutes 98% of our DNA, controls protein expression, and harbors immense disease susceptibility. To enable efficient characterization of noncoding genetic variants, the Keck Center for Machine Guided Functional Genomics will develop a closed-loop platform that combines deep learning, experimental mutagenesis using "editrons" (which employ retron and CRISPR technologies), and long-read genomic technologies for assaying genome physiology. This hybrid computational-experimental strategy will enable the investigator, and her colleagues at the Gladstone Institutes, to predict and validate causal noncoding variants in a high-throughput and iterative fashion. The investigator team plans to demonstrate causal variant discovery in loci associated with developmental disorders of the heart and brain, for which their access to whole genome sequencing of thousands of families provides a unique opportunity to interrogate rare and de novo variants. This project will convert the investigators' early-stage technologies into a robust, open-source platform that the Keck Center for Machine Guided Functional Genomics will share freely with others to accelerate discovery science and disease research, towards more effective clinical diagnostics and therapeutics for genetic disorders.

Salk Institute for Biological Studies
La Jolla, CA
Fred H. Gage, Pallav Kosuri
$1,300,000

The goal of this project is to map ribosubstitution events, the erroneous incorporation of ribonucleotides into the chromosomal DNA, over the human lifespan in age-susceptible tissues like the brain and heart, using new sequencing technologies. Ribosubstitutions promote genomic instability through altered DNA chemistry, mechanics, and susceptibility to damage. Despite these potentially serious consequences, ribosubstitutions have not been investigated in the nuclear DNA of post-

originally supplied *in vitro* cell lines. The investigators will expand the utility of their sequencing method through the development of a single-nucleus, multiomic technology to sequence ribosubstitutions along with the transcriptome from any source. Expanding their scope, the team also plans to generate aged human cardiomyocytes from their cohort representing the human lifespan to map genomic ribosubstitutions in another age-susceptible tissue, the heart. Finally, they will identify spatial hotspots for ribosubstitutions, and elucidate their impact on cellular microenvironments, through the development of a new spatial transcriptomics technique to quantify ribosubstitutions in intact sections of human brain and heart tissue. Taken together, this project will produce new technologies for sequence-based and spatial mapping of an emergent cause of genome instability – the incorporation of ribonucleotides in the nuclear genome – which could play an outsized role in the mechanisms of aging.

University of California, San Francisco
San Francisco, CA
Alex Pollen, Trygve Bakken, Matthew Schmitz
$1,300,000

The adaptive radiation of cetaceans involved some of the most remarkable innovations in mammalian evolution. Cetaceans hold titles for longest-lived animal, deepest-diving tetrapod, most cortical neurons, largest brain, and largest animal to have ever existed. Immense size, low-oxygen environments, novel senses, and large brains incur costs that require anatomical, physiological, and cellular specializations. A team of three investigators at the University of California, San Francisco and the Allen Institute for Brain Science hypothesizes that a deep dive into molecular and functional specializations of cetacean cells will reveal cellular adaptations to large brain size, long lifespan, and low oxygen environments. The team will combine unique partnerships with marine mammal conservation organizations, single cell and spatial transcriptomics approaches, reprogramming in non-model organisms, and genome engineering screens. These diverse approaches will be used to 1) determine how cetacean cell types have been reconfigured during independent brain expansion, 2) functionally identify protective mechanisms shielding cetacean cells in challenging conditions, and 3) build a platform to accelerate discovery and transo (i)-5wl (s)7.8 (y)-4(t)11.8 8.4 (2ai)-5((i)-5 (s)7.8)11 (p)1 () b)1(v)-4(at-3.2 (i)5.1 h m)-7.2 (l)-5(y)-4(o)