Name: Risa Watanabe Email: risa.watanabe@mssm.edu

Genome-Scale Long-Read Single Molecule Sequencing for Nucleosome Position Mapping and Transcription Factor Footprinting in Opioid Exposed and Control Prefrontal Cortex

Risa Watanabe[1,3], Bicheng Jiang[1], Aman Agarwal[3], Cyril J Peter*[1,2], Xuedi Wang[3], Viviana Evans[1], Travis Dawson[1], Maya Fridrikh[4], Nadejda M. Tsankova[2,5], Robert P Sebra[4], Dan Hasson[3,6], Deborah Mash[7], Schahram Akbarian[1,2,4]

[1]Department of Psychiatry, Icahn School of Medicine at Mount Sinai; [2]Friedman Brain Institute, Icahn School of Medicine at Mount Sinai; [3]Tisch Cancer Institute Bioinformatics for Next Generation Sequencing (BiNGS) core, Icahn School of Medicine at Mount Sinai; [4]Department of Genetics and Genomic Sciences, Center for Advanced Genomics Technology, Icahn School of Medicine at Mount Sinai; [5]Department of Pathology, Molecular, and Cell-Based Medicine, Icahn School of Medicine at Mount Sinai; [6]Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai; [7]University of Miami

We apply a single-molecule chromatin fiber sequencing (Fiber-seq) protocol designed for amplification-free cell type-specific mapping of the regulatory architecture at nucleosome resolution along extended ~10kb chromatin fibers, to neuronal and non-neuronal nuclei sorted from human brain tissue. Specifically, application of this method enables the resolution of cell-selective promoter and enhancer architectures on single fibers, including transcription factor footprinting and position mapping with sequence-specific fixation of nucleosome arrays flanking transcription start sites and regulatory motifs. We uncover haplotype-specific chromatin patterns, multiple regulatory elements cis-aligned on individual fibers, and accessible chromatin at 20,000 unique sites encompassing retrotransposons and other repeat sequences hitherto ‘unmappable’ by short-read epigenomic sequencing. Overall, we show that Fiber-seq is applicable to human brain tissue, offering sharp demarcation of nucleosome-depleted regions at sites of open chromatin in conjunction with multi-kilobase nucleosomal positioning at single fiber resolution on a genome-wide scale. Our initial, disease=relevant studies are focused on the dorsolateral prefrontal cortex of controls and subjects diagnosed with chronic opioid use disorder.