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 Jiang¹, Aman Agarwal³, Cyril J Peter^{*1,2}, Xuedi Wang³, Viviana Evans¹, Travis Dawson¹, Maya Fridrikh⁴, Nadejda M. Tsankova^{2,5}, Robert P Sebra⁴, Dan Hasson^{3,6}, Deborah Mash⁷, Schahram Akbarian^{1,2,4}

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