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**Chromatin Fiber Profiling and Transcription Factor Footprinting by Genome-Scale Single Molecule Sequencing from Opioid Use Disorder and Control Brain**

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We established in our laboratory a Fiber-seq protocol for the human brain, which allows for the first time to profile single chromatin fibers, directly from brain nuclei, uninterrupted for 10-20kb on a genome-wide scale without the need for PCR or other amplification. This type of single molecule long-read sequencing provides, at base pair resolution, information on m5CpG methylation patterns, nucleosomal positioning and the activity-status of nucleosome depleted regions by transcription factor footprinting. Furthermore, we bypass additional limitations posed by conventional nucleolytic assays (incl. ATAC-seq) which typically built chromatin landscapes from short-read sequencing (0.1-0.15kb) of PCR amplified DNA, which leaves an estimated 50% of human nuclear genome underexplored because of poor annotation of low-complexity repetitive loci, duplicated regions, tandem arrays, and complex structures.

Here, we apply, for the first time, our fiber-seq protocol to the frontal cortex of a cohort of opioid use disorder and control brains. We will present initial results on single fiber-level epigenomic dysregulation in diseased brains, with multiomic integration of endogenous CpG methylation, and nucleosomal positioning at promoter and enhancer regions including potential changes in transcription factor footprints.

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