Archival Report

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Practical Guidelines for High-Resolution Epigenomic Profiling of Nucleosomal Histones in Postmortem Human Brain Tissue

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ABSTRACT

BACKGROUND: The nervous system may include more than 100 residue-specific posttranslational modifications of histones forming the nucleosome core that are often regulated in cell-type-specific manner. On a genome-wide scale, some of the histone posttranslational modification landscapes show significant overlap with the genetic risk architecture for several psychiatric disorders, fueling PsychENCODE and other large-scale efforts to comprehensively map neuronal and nonneuronal epigenomes in hundreds of specimens. However, practical guidelines for efficient generation of histone chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) datasets from postmortem brains are needed.

METHODS: Protocols and quality controls are given for the following: 1) extraction, purification, and NeuN neuronal marker immunotagging of nuclei from adult human cerebral cortex; 2) fluorescence-activated nuclei sorting; 3) preparation of chromatin by micrococcal nuclease digest; 4) ChIP for open chromatin-associated histone methylation and acetylation; and 5) generation and sequencing of ChIP-seq libraries.

RESULTS: We present a ChIP-seq pipeline for epigenome mapping in the neuronal and nonneuronal nuclei from the postmortem brain. This includes a stepwise system of quality controls and user-friendly data presentation platforms.

CONCLUSIONS: Our practical guidelines will be useful for projects aimed at histone posttranslational modification mapping in chromatin extracted from hundreds of postmortem brain samples in cell-type-specific manner.

Keywords: Cell type specific, ChIP-seq, Epigenomics, Postmortem human brain, PsychENCODE, Schizophrenia

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The field of neuroepigenomics is shedding light on the mechanisms underlying normal neurodevelopment (1), behavior and learning (2,3), and various neuropsychiatric disorders (4), including schizophrenia (5–8), depression (9), bipolar disorder (7,10), and autism (11). Insight into the mechanisms governing epigenetic regulation in the brain is necessary for a more complete understanding of complex behaviors and psychopathology, hopefully leading to novel therapeutic and diagnostic approaches in the future. Epigenomic regulation is thought to include a large variety of more than 100 amino acid residue-specific histone posttranslational modifications (histone PTMs). This includes monomethylation, dimethylation, and trimethylation; acetylation; crotonylation; polyADP-ribosylation; and small ubiquitin-related modifier modifications of specific lysine residues. Other amino acids are also altered including arginine citrullination, monomethylation and dimethylation, serine phosphorylation, and tyrosine hydroxylation, among others (12). While the large majority of histone PTMs await functional exploration in the nervous system (13), studies conducted in dividing cells and peripheral tissues have shown that many histone PTMs are closely associated with chromatin states, differentiating between active, inactive, or poised promoters and enhancers; gene bodies and exonic or intronic DNA; transcription start sites; and broader regulatory domains including super-enhancers, among others (12,14–16). It has been shown that a significant fraction of the histone PTM landscape shows strong tissue-specific and cell-type-specific regulation in the human brain (17). This observation has important implications for the genetic risk architecture of psychiatric disease. For example, sequences decorated with open chromatin-associated histone methylation and acetylation in fetal brain and in cortical neurons are, on a genome-wide scale, enriched with common polymorphisms conferring heritable risk for schizophrenia, suggesting a potential mechanistic relationship between DNA sequence variation and neural-specific epigenetic modifications (18).

Therefore, comprehensive mapping of histone PTMs in specific regions and cell types of the developing and adult

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human brain could help elucidate genome organization and function in the context of neuropsychiatric disease. However, with the field still in its infancy, establishing practical guidelines early can lay the foundation for large-scale projects such as PsychENCODE, a National Institutes of Mental Health sponsored initiative. PsychENCODE is aimed to create a public resource of genomic data, including histone PTMs, using tissue-specific and cell-type-specific samples from approximately 1000 human postmortem brains (19). Here, we discuss protocols and develop a set of quality control standards for our chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) pipeline designed to effectively produce and disseminate human brain epigenomic maps and datasets. Using two histone PTMs as examples, H3-trimethyl-lysine 4 (H3K4me3), primarily defined by sharp peaks (~1 kb) and found near transcription start sites, proximal promoters and some enhancers, and H3-acetyl-lysine 27 (H3K27ac), more broadly distributed at various *cis*-regulatory sequences, we demonstrate that our ChIP-seq pipeline on human postmortem brain is broadly consistent with the Encyclopedia of DNA Elements (ENCODE)-defined criteria for histone PTMs (20) and provides examples for user-friendly data access platforms. These advances are likely to have significant impact because they open the exciting opportunity to study histone PTM landscapes in postmortem brain with quality controls (QC) similar to those applied to peripheral tissues (which are generally much easier to access) or cell lines and to do so in large cohorts involving many hundreds of brain specimens. We expect that the approaches outlined here, when applied to human brain collections across development and disease states, will ultimately lead to deep advancements in our understanding about the neurological functions, particularly of regulatory and noncoding sequences in the human genome, currently left almost completely unexplored.

METHODS AND MATERIALS

The multistep procedure (Figure 1) begins with isolation, immunotagging, and fluorescence-activated cell sorting of nuclei extracted from tissue. Next, chromatin is prepared by enzymatic digestion with micrococcal nuclease (MNase) (N3755; Sigma-Aldrich, St. Louis, MO) to yield primarily mononucleosomes (the elementary unit of chromatin comprised of a histone octamer with 146 base pair [bp] DNA wrapped around it in two and a half loops). DNA enriched for histone modification of interest is obtained using ChIP with a specific antibody. Then, ChIP-seq libraries are prepared and sequenced by Illumina HiSeq 2500 (San Diego, CA) followed by a data quality control pipeline. Our ChIP-seq pipeline includes QC at each step of the protocol (Figure 1). The entire pipeline, including bioinformatical analyses, has so far been completed for more than 100 ChIP-seq samples. Here, we show a representative example of $n = 8$ ChIP-seq datasets from two prefrontal cortex (PFC) specimens, each processed for $NeuN^{+}$H3K4me3, $NeuN^{-}$H3K4me3, $NeuN^{+}$H3K27ac, and $NeuN^{-}$H3K27ac. A detailed step-by-step protocol, a glossary, and description of postmortem tissue are included in the Supplement.

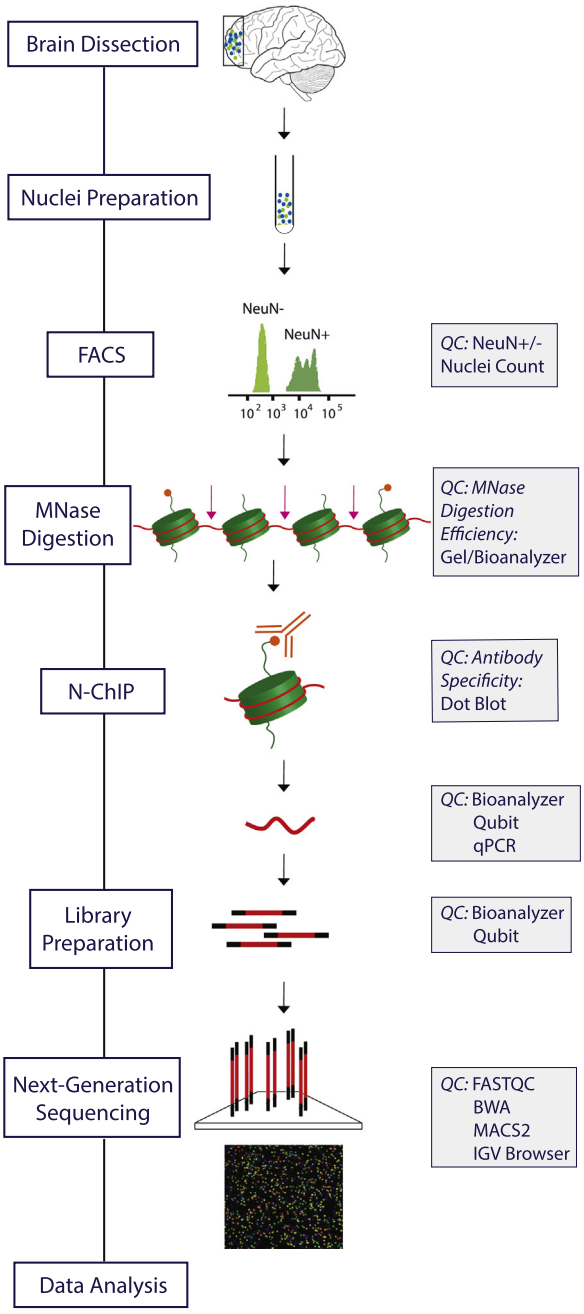

Figure 1. Chromatin immunoprecipitation followed by deep sequencing pipeline. Overview of the pipeline flow chart and corresponding quality controls (QC). FACS, fluorescence-activated cell sorting; IGV, Integrative Genomics Viewer; MNase, micrococcal nuclease; N-ChIP, native chromatin immunoprecipitation; NeuN+, neuronal; NeuN−, nonneuronal; qPCR, quantitative polymerase chain reaction.

Nuclei Isolation, Immunotagging, and Sorting

Approximately 300 mg of cortical gray matter, dissected from fresh frozen brain slabs, is homogenized by douncing followed by nuclei extraction via sucrose gradient ultracentrifugation. The nuclei are recovered from the pellet, resuspended, and