ATAC-Seq Sample Preparation

ATAC-Seq was carried out as described[1]. Briefly, cells were lysed in cell lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.1% IGEPAL CA-630, protease inhibitors) on ice for 5 min and centrifuged at 230 rcf for 5 min at 4 °C. The pellet, containing the nuclei, was re-suspended in 25 ml of 1X Tagment DNA Buffer (Illumina). 50K nuclei were subjected to transposase reaction (Nextera - Illumina) followed by DNA purification. The tagmented DNA was PCR amplified using Nextera indexing primers (Illumina). Nucleosome-free fragments (175-250 bp) were size selected using AMPure XP magnetic beads (Beckman Coulter) and further amplified by PCR to obtain the final libraries. The libraries were sequenced using the Illumina NextSeq platform (paired end, 75nt). All samples passed quality control checks that included morphological evaluation of nuclei, assessment of size distribution via Agilent Bioanalyzer, and real-time qPCR to assess the enrichment of open-chromatin sites.

ATAC-Seq Bioinformatics Analysis

Read trimming, alignment to genome using trimmed reads, calling of peaks, and reporting of single-sample QC metrics were all completed with The Encyclopedia of DNA Elements (ENCODE)[2] Data Coordination Center's ATAC-Seq pipeline (version 1.7.1)[3]. Raw FASTQ reads were trimmed with cutadapt[4] (version 2.5). Trimmed reads were aligned to the GRCh38 genome with Bowtie2[5] (version 2.3.4.3). PCR duplicate reads were marked with Picard[6] MarkDuplicates (version 2.20.7) and removed. Peaks were called with macs2[7] (version 2.2.4). Differential analysis was conducted with DiffBind[8] (version 2.10.0) using the DESEQ2 method[9] and False Discovery Rate (FDR)[10] correction with a threshold of less than 0.1.

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