**Kirkland ATAC Seq**

**Nuclear Isolation and Library Preparation**

Drosophila melanogaster hearts were isolated by \_\_\_\_\_ and frozen using \_\_\_\_ until sequencing. Samples were submitted to the Epigenomics core for Tn5 Transposase ATAC sequencing using a protocol adapted from Buenrostro et a. 2015 (PMID 25559105). Briefly, samples were pulverized using a liquid nitrogen-cooled mortar and pestle, transferred to 1.5 mL tubes and permeabilized in nuclear permeabilization buffer (5% BSA [A7906, Sigma], 0.2% m/V IGEPAL-CA630 [I8896, Sigma], 1mM DTT [D9779, Sigma], 1X protease inhibitor [0505649001, Roche], in PBS [10010-23, Thermo Fisher Scientific]) for 10 minutes at 4 Celsius. Nuclei were filtered using a 30μm Celltrics filter [25004-0042-2316, Sysmex], and centrifuged at 500g for 5 minutes at 4 Celsius. 50 μL of tagmentation buffer (33mM Tris-acetate [ph=H=7.8, BP-152, Thermo Fisher Scientific], 66 mM K-acetate [P5708, Sigma], 11 mM Mg-acetate [M2545, Sigma], 16% DMF [DX1730, EMD Millipore], in molecular grade water [46000-CM, Corning]) and diluted to a concentration of 2,000-5,000 nuclei/mL on ice after counting with a hemocytometer.

0.5 μL of Tagment DNA Enzyme 1 [FC-121-1030, Illumina] was added to each sample and pipetted 5 times to mix. Samples were then incubated 30 minutes at 37 Celsius in a centrifuge at 500g. Next, 100 μL of PB Buffer [Qiagen] and 5 μL of Na-acetate (3M, pH = 5.2) were added to each sample and purified using the MinElute PCR Purification Kit [28004, Qiagen]. Next, samples were eluted using 10uL of EB and amplified by PCR (see supplemental table X for primer sequences). Each reaction included 10 μL of Tagmented DNA, 25 μL NEBNext 2x PCR MasterMix [M0541, NEB], 2 μL of i5 Primer (25 μΜ), 2 μL of i7 Primer, (25 μΜ), and 11 μL of Molecular biology grade water. Thermocycling was performed as follows: 72 Celsius for 5 minutes, 98 Celsius for 30 s, and then eight repetitions of 98 Celsius for 10s, 63 Celsius for 30s, and 72 Celsius for 1 min. Afterwards, temperature was lowered to 12 Celsius indefinitely. 250 μL of Buffer PB [Qiagen] and 10 μL of Na-acetate (3M, pH=5.2) were added to each sample and purified, again using the MinElute PCR Kit and eluted using 20 μL of EB. Size selection was performed using 110 μL of Ampure XP beads [A63880, Beckman Coulter] in 180 μL of BM, mixed ten times by pipetting. Samples were then incubated for 5 minutes at room temperature and separated using a magnetic stand. 300 μL of supernatant for each sample was transferred to a new tube, and 190 μL of beads were added and again incubated for 5 minutes at room temperature. After magnetic separation, beads were washed twice with 200 μL of 70% EtOH. Beads were then resuspended in 20 μL of EB by pipetting 10 times. After another magnetic separation, 17 μL of final library were transferred to new tubes.

DNA amount was quantified using the Qubit dsDNA HS Assay Kit with 1 μL/sample, [Q32851, Thermo Fisher Scientific], and size was quantified using the 4200 TapeStation High Sensitivity D1000 ScreenTape [5067-5584, 5067-5583, Agilent Technologies]. Samples were then sequenced on the 10X Genomics platform.

**Sample Processing from FASTQ**

FASTQ files were submitted through the UCSD Epigenetics ATAC-seq pipeline (https://github.com/epigen-UCSD/atac\_seq\_pipeline), based on the ENCODE pipeline. Briefly, reads were aligned using bowtie2, converted to uncompressed BAM files, sorted and index using: bowtie2 -X2000 --mm --local -1 $fastq1 -2 $fastq2 | samtools view -Su /dev/stdin | samtools sort & index > xxx.PE2SE.bam &.bai 2> align.log. Poorly mapped, (<30 mapping score), duplicate, multimapped, and mitochondrial reads were removed using samtools and picard. Tn5 adapters were removed by truncating + end reads by 4 base pairs and – end reads by 5 base pairs, and then written to final output BAMs.

**Computational Analysis**

BAM files were downloaded from UCSD Center for Epigenomics, sorted and indexed with samtools. Peakcalling was performed using MACS2 using the following commands: callpeak -f BAMPE -g dm - -q 0.01 --nomodel --shift -100 --extsize 200 --keep-dup all. MACS .xls output files and sorted BAMs were used to construct a Diffbind3.0.9 sample sheet for each comparison: 1 week vs 5 week w1118 samples, wildtype vs LamB iR attp40 samples, and wildtype vs LamC iR attp2 samples. Samples were read into R Studio using dba(), count densities per peak were calculated using dba.count(), filtering out peaks with <1 read per sample and a summit width of 100 (as recommended by the Diffbind3 vignette). Differential accessibility was calculated using the EdgeR wrapper of dba.analyze(). BED files were generated for each comparison using dba.report() and annotated using HOMER annotatePeaks.pl. Regions were filtered based on a log2 fold change of 0.32 and FDR of ≤ 0.1. Common features between comparisons were isolated using dplyr’s inner\_join function of the “Nearest.Refseq” column output of HOMER. Plots were generated using ggplot2 and ggrepel packages.

**Supplemental Table X**. Primer Sequences for ATAC amplification

PCR primer [IDT] sequences were the same as in the Nextera Index XT Kit v2 (FC-131-2001, Illumina).

i5-Primer

|  |  |  |
| --- | --- | --- |
| Name | Index Sequence | Full Sequence |
| S502 | CTCTCTAT | AATGATACGGCGACCACCGAGATCTACACCTCTCTATTCGTCGGCAGCGTC |
| S503 | TATCCTCT | AATGATACGGCGACCACCGAGATCTACACTATCCTCTTCGTCGGCAGCGTC |
| S505 | GTAAGGAG | AATGATACGGCGACCACCGAGATCTACACGTAAGGAGTCGTCGGCAGCGTC |
| S506 | ACTGCATA | AATGATACGGCGACCACCGAGATCTACACACTGCATATCGTCGGCAGCGTC |
| S507 | AAGGAGTA | AATGATACGGCGACCACCGAGATCTACACAAGGAGTATCGTCGGCAGCGTC |
| S508 | CTAAGCCT | AATGATACGGCGACCACCGAGATCTACACCTAAGCCTTCGTCGGCAGCGTC |
| S510 | CGTCTAAT | AATGATACGGCGACCACCGAGATCTACACCGTCTAATTCGTCGGCAGCGTC |
| S511 | TCTCTCCG | AATGATACGGCGACCACCGAGATCTACACTCTCTCCGTCGTCGGCAGCGTC |

i7-Primer

|  |  |  |
| --- | --- | --- |
| Name | Index Sequence | Full Sequence |
| N701 | TCGCCTTA | CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGG |
| N702 | CTAGTACG | CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGG |
| N703 | TTCTGCCT | CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGG |
| N704 | GCTCAGGA | CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGG |
| N705 | AGGAGTCC | CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGG |
| N706 | CATGCCTA | CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGG |
| N707 | GTAGAGAG | CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGG |
| N710 | CAGCCTCG | CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGG |
| N711 | TGCCTCTT | CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGG |
| N712 | TCCTCTAC | CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGG |