Aug 08, 2024

ATAC-seq of primary human fibroblasts cultured on soft and stiff ECM

DOI
dx.doi.org/10.17504/protocols.io.ewov1q1kygr2/v1

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Andrea R Daniel: This protocol was adapted from the work of Brian Cosgrove and Lexi Bounds in the Gersbach lab at Duke University.

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Protocol Citation: Brian D. Cosgrove, Lexi Bounds, Carson Key Taylor, Alan L. Su, Anthony J. Rizzo, Alejandro Barrera, Andrea R Daniel, Gregory E. Crawford, Brenton D. Hoffman, Charles A. Gersbach 2024. ATAC-seq of primary human fibroblasts cultured on soft and stiff ECM. **protocols.io https://dx.doi.org/10.17504/protocols.io.ewov1q1kygr2/v1**

Manuscript citation:
Mechanosensitive genomic enhancers potentiate the cellular response to matrix stiffness
Brian D. Cosgrove, Lexi R. Bounds, Carson Key Taylor, Alan L. Su, Anthony J. Rizzo, Alejandro Barrera, Gregory E. Crawford, Brenton D. Hoffman, Charles A. Gersbach
bioRxiv 2024.01.10.574997; doi: https://doi.org/10.1101/2024.01.10.574997

Protocol status: Working
We use this protocol and it's working

Created: December 20, 2023

Last Modified: August 08, 2024

Protocol Integer ID: 92569

Keywords: ATAC-seq, chromatin structure, enhancer, extracellular matrix, ECM stiffness, chromatin accessibility

Funders Acknowledgement:
NIH
Grant ID: HG012053

Abstract

This protocols describes methods for Omni ATAC-seq to characterize the chromatin structural responses to changes in extracellular matrix stiffness cues.

Materials

Cells: ATCC CRL-2097
Hydrogel dishes: 35 and 150 mm PetriSoft EasyCoat dishes (Matrigen)
ROCK inhibitor: Y-27632 ROCKi (StemCell Tech)

Cell culture and soft hydrogel processing

1 Culture primary human neonatal fibroblasts (HFF cells, ATCC CRL-2097) in DMEM with 10% FBS, 1% AntiAnti, and 1% NEAA (Sigma) on tissue culture plastic (TCP).

2 Use polyacrylamide hydrogel 35 and 150 mm PetriSoft EasyCoat dishes (Matrigen) with an Elastic Modulus of 1 kPa (“soft”) and 50 kPa (“stiff”).

3 Incubate dishes for 5 minutes with sterile PBS, rinse two more times with sterile PBS, followed by the addition of 10 ug/mL fibronectin (Sigma) for 30 minutes at room temperature.

4 Remove fibronectin and rinse dishes twice with sterile PBS, followed by a 20 minutes incubation with complete growth media.

5 Remove media from the dishes and add cell suspensions. Allow cells to attach overnight.

Omni ATAC-seq

6 Seed cells on Matrigen dishes of varying stiffness at slightly variable densities to account for reduced HFF attachment on softer substrates. Culture cells for 20 hours overnight.

	A	B
	Elastic Modulus	Cells plated
	1 kPa	70,000
	12 kPa	45,000
	50 kPa	40,000

7 For Y-27632 ROCKi experiments, the seed cells on stiff hydrogels (as in step 6), but 1 hour prior to harvest add 10uM Y-27632 ROCKi (StemCell Tech) to the growth media of cells.

8 Use the Omni-ATAC-seq protocol to minimize mitochondrial reads from the preps (1).

8.1 Exception to the Omni-ATAC-seq protocol: avoid trypsinization, instead perform on-plate disruption/removal of nuclei using the digitonin present in the lysis buffer. This process better preserves the nuclear mechanical context and connectivity prior to transposition by the Tn5.

9 Following the final PCR, perform a 0.5x/1.8x double-sided SPRI clean step on the libraries. Subject libraries to quality control by determining the number of cycles required to reach 25% of the peak threshold in the diagnostic PCR. In addition, run the amplified libraries out on a High Sensitivity D1000 Tapestation (Agilent) to confirm expected size and perform Qubit dsDNA HS assays to determine a final concentration.

10 Diluted the libraries individually to 6nM and then pool them at equal volumes prior to sequencing on an Illumina HiSeq 4000 using a single lane of 50bp single end reads.

11 Use FastQC (2) to identify read quality, and trim adapter reads using Trimmomatic v0.32 (3) followed by Bowtie (4) alignment (v1.0) of the reads to the reference genome using the settings: -v 2 –best –strata -m 1 with duplicate reads removed using Picard MarkDuplicates (v1.13) and ENCODE hg38 blacklist reads removed using bedtools2 v2.25 (5).

12 Perform peak calling using MACS2 with narrowPeak settings and a threshold of FDR < 0.001 (6), and generate a master peak set as the union set of all called peaks across every sample analyzed (224,906 unique regions total).

13 Make count matrices using featureCounts (7) and use DEseq2 v1.36 for differential accessibility analysis (8). Perform annotation of genomic regions using ChIPSeeker (9), for interactive visualization of processed data use Degust (10) and Rstudio along with ggplot2 and tidyverse plugins to generate data visualizations.

14 Generate sequencing-depth normalized ATAC bigWig files using deeptools bamCoverage v3.0.1 (11). Perform motif analysis using the HOMER suite (12).

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