Feb 18, 2026

Bulk ATAC-seq

DOI
https://dx.doi.org/10.17504/protocols.io.dm6gp12kdgzp/v1

Lexi Bounds[1], Alejandro Barrera[1], Maria ter Weele[1], Siyan Liu[1], Euphy Wu[2], Shengyu Li[1], Revathy Venukuttan[1], Ruhi Rai[1], Wancen Mu[2], Nahid Iglesias[1], Paola Giusti-Rodriguez[3], Timothy Reddy[1], Yun Li[2], Raluca Gordan[2], Andrew Allen[2], Michael Love[2], Patrick Sullivan[2], Greg Crawford[1], Charles Gersbach[1]

[1]Duke University; [2]University of North Carolina; [3]University of Florida

Gersbach Lab

Andrea R Daniel
Duke University

DOI: https://dx.doi.org/10.17504/protocols.io.dm6gp12kdgzp/v1

Protocol Citation: Lexi Bounds, Alejandro Barrera, Maria ter Weele, Siyan Liu, Euphy Wu, Shengyu Li, Revathy Venukuttan, Ruhi Rai, Wancen Mu, Nahid Iglesias, Paola Giusti-Rodriguez, Timothy Reddy, Yun Li, Raluca Gordan, Andrew Allen, Michael Love, Patrick Sullivan, Greg Crawford, Charles Gersbach 2026. Bulk ATAC-seq. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gp12kdgzp/v1

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

Created: February 17, 2026

Last Modified: February 18, 2026

Protocol Integer ID: 243537

Keywords: bulk atac, using neural progenitor cell, neural progenitor cell, npc, seq, seq method, seq this protocol

Funders Acknowledgements:
NIH
Grant ID: HG012053

Disclaimer

This protocol was adapted from the work of Lexi Bounds in the Gersbach lab at Duke University.

Abstract

This protocol describes bulk ATAC-seq methods and analysis using Neural Progenitor Cells (NPCs).

Materials

KAPA Library Quantification Kit (Roche #7960255001)

High Sensitivity DNA ScreenTape Analysis (Agilent #5067)

Bulk ATAC-seq

1 100,000 NPCs are harvested using Accutase and resuspended in 1 mL of cold ATAC-seq resuspension buffer and the Omni-ATAC seq protocol was used to generate ATAC-sequencing libraries (Ref. 1).

2 Following pre-amplification, 3 additional cycles of PCR were performed prior to final amplification and cleanup.

3 Library concentrations and sizes were obtained using the KAPA Library Quantification Kit (Roche #7960255001) and High Sensitivity DNA ScreenTape Analysis (Agilent #5067).

4 Perform ATAC-seq for two biological replicates. The final libraries are pooled to 3 nM and sequenced on a HiSeq 4000 with 1×50bp configuration.

Bulk ATAC-seq analysis

5 The pipeline for processing and analysis of the bulk ATAC-seq data can be found here: https://github.com/Duke-GCB/GGR-cwl/blob/master/v1.0/ATAC-seq_pipeline/pipeline-se-blacklist-removal.cwl.

6 Quality control of FASTQ files is performed using FASTQC (Ref.2).

7 Adapter reads are then trimmed using Trimmomatic.

8 Reads are then aligned to the reference genome using Bowtie (Ref. 3).

9 Duplicate reads are removed using Picard MarkDuplicates and ENCODE hg38 blacklist reads were removed using BEDtools2 (Ref. 4).

10 Peak calling is performed using MACS2 (Ref. 5) with narrowPeak settings.

11 Sequencing-depth normalized ATAC bigWig files were generated using deeptools (Ref. 6) bamCoverage.

Protocol references

1. Corces, M. R. *et al.* An improved ATAC-seq protocol reduces background and enables interrogation of frozen tissues. *Nat. Methods* **14**, 959–962 (2017).

2. Babraham Bioinformatics - FastQC A Quality Control tool for High Throughput Sequence Data. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

3. Langmead, B. Aligning short sequencing reads with Bowtie. *Curr. Protoc. Bioinforma.* **Chapter 11**, Unit 11.7 (2010).

4. Quinlan, A. R. BEDTools: The Swiss-Army Tool for Genome Feature Analysis. *Curr. Protoc. Bioinforma.* **47**, 11.12.1-34 (2014).

5. Zhang, Y. *et al.* Model-based analysis of ChIP-Seq (MACS). *Genome Biol.* **9**, R137 (2008).

6. Ramírez, F., Dündar, F., Diehl, S., Grüning, B. A. & Manke, T. deepTools: a flexible platform for exploring deep-sequencing data. *Nucleic Acids Res.* **42**, W187-191 (2014).