Epigenetic insights: Signios Bio's comprehensive approach to single-cell ATAC-seq, CUT&RUN and multiome analysis

Epigenetic mechanisms play an important role in regulating gene expression. Advances in high-throughput sequencing technologies has enabled genome-wide studies to map epigenetic landscape. Epigenetic studies are now routinely carried out using bulk cell/tissue samples as well as single cell samples. At Signios Bio, we have effectively executed and delivered numerous projects on epigenetics studies. We provide an end-to-end solution for single-cell ATAC-seq, single- cell multiome ATAC + gene expression, CUT & RUN, Bulk ATAC sequencing services and bioinformatics analysis pipelines.

Single-cell ATAC-seq and multiome (ATAC + gene expression)

Single-cell ATAC-seq is a technique used to determine genome-wide chromatin accessibility at the single cell level. With single cell ATAC-seq, chromatin profiling of thousands of nuclei can be performed in parallel, resulting in fast, accurate epigenomic profiling. While bulk ATAC-seq has proven to be a powerful method for determining broad characteristics of a cell population, tissues and other biological samples which tend to contain multiple cell types in varying states of cellular processes. To capture this variation, it is necessary to sequence individual cells.

Using Chromium single-cell ATAC solution from 10x Genomics, we can analyze hundreds to thousands of nuclei per run at single-cell resolution. We have extensive experience in nuclei isolation, transposition, single-cell partition, library preparation and sequencing. We also offer 10x single-cell multiome ATAC + gene expression service, enabling simultaneous profiling of gene expression and open chromatin from the same cells. This empowers researchers to correlate regulatory signals with their gene expression profiles at single-cell level for thousands of cells.

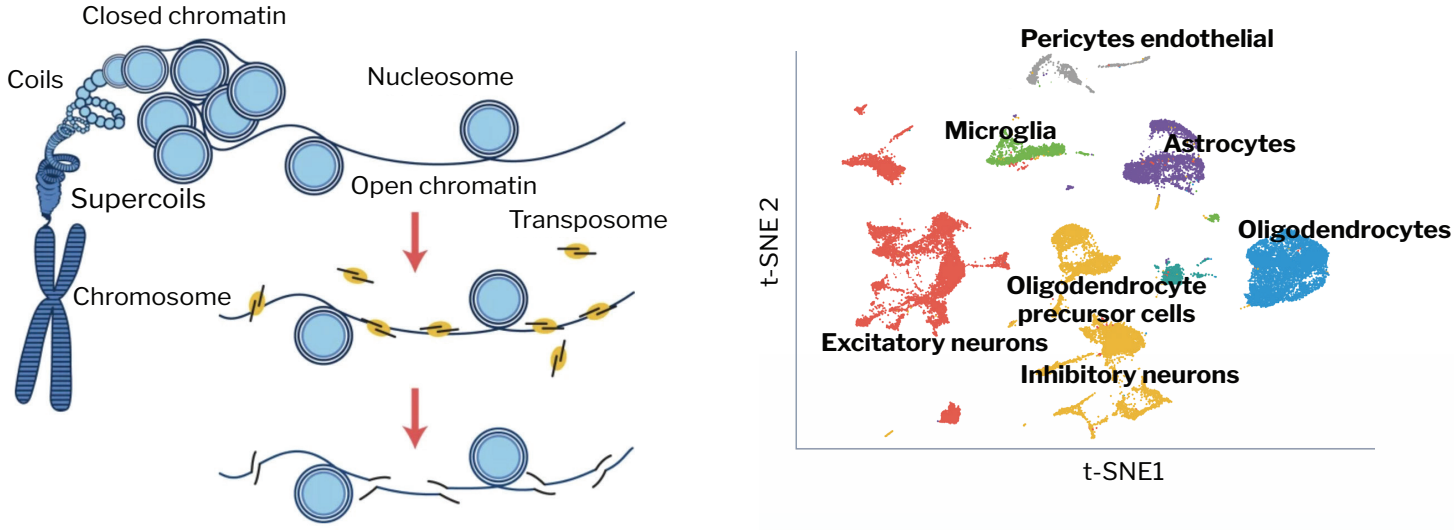
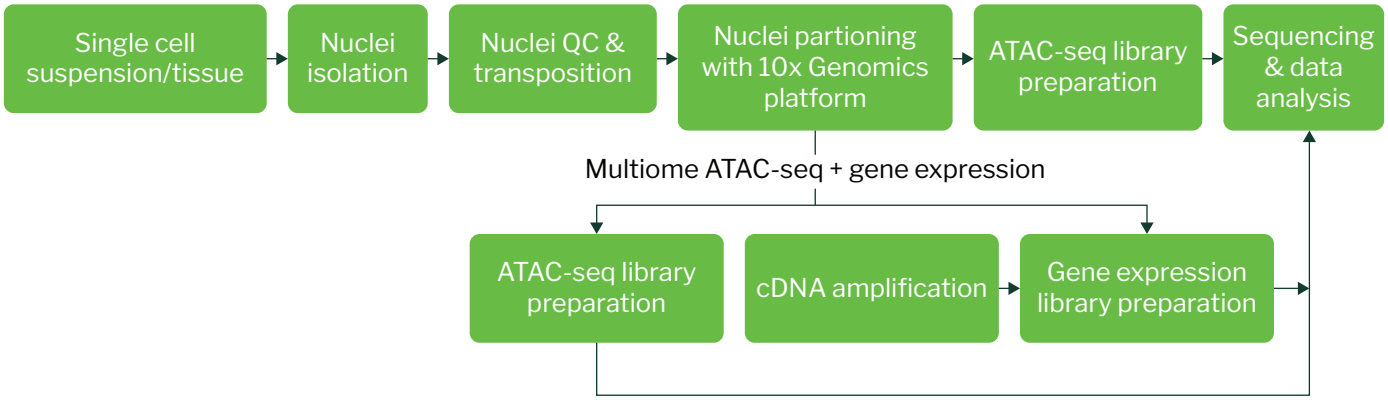


Figure 1. Regions of open chromatin correlate with areas of active gene transcription (Source: 10x Genomics).

Single-cell multiome (ATAC + gene expression) workflow



Sample requirements: We accept flash frozen tissue and cryopreserved cells. Please contact us for further details on project design, consultancy & sample submission.

Frozen tissue submission (for nuclei isolation)

1. Isolate fresh tissue, immediately transfer the tissue to a cryovial placed on dry ice or in liquid nitrogen bat to preserve RNA integrity. Not adhering to this procedure will impact the quality of isolated nuclei.
2. Smaller tissue size is preferable (approx. 50-100 mg) as it can be frozen more quickly than larger tissue pieces.
3. Our recommendation is to use a bath of isopentane and liquid nitrogen to freeze freshly obtained tissue. Tissue should not be placed directly in liquid nitrogen as the temperature difference may damage tissues, leading to air pockets and uneven freezing. This may crack and morphologically damage the tissue. Ship tissue on dry ice.

Frozen cells submission (for nuclei isolation)

1. Our recommendation is to freeze 500 K cells (for rare population of cells) in 1 ml of freezing media per cryovial and prepare 2 cryovials per sample. If cell number is not a limiting factor, freeze one million to 2 million cells in 1 ml freezing media. (A high cell number necessary if enrichment such as dead cell and granulocyte removal is required).
2. Use Cryostor CS10 (Stem cell) or any standard freezing media (Cell culture media + 20% FBS + 10% DMSO or 90% FBS + 10% DMSO) and freeze cells slowly by reducing the temperature at approximately 1ºC/minute using a cryo freezing container such as Mr. Frosty at -80 ºC and then move to liquid nitrogen after 24h for long term storage.
3. Ship cells on dry ice.

Cleavage under targets & release using nucleases (CUT&RUN)

Chromatin immunoprecipitation-sequencing (ChIP-seq) has been a mainstay for investigating protein-chromatin interactions. However, ChIP-seq has several limitations in accurately establishing the transcriptional or chromatin state and usually requires many cells, lengthy protocols, and high-sequencing depth with no guarantee of clear results.

One alternative strategy developed to overcome some of the limitations of ChIP assays is cleavage under targets & Release using nuclease (CUT&RUN). It is a new technology that can be used for chromatin profiling whereby fusion of proteins A and/or G to micrococcal nuclease (pAG-MNase) is recruited to selectively cleave antibody-bound chromatin *in situ*. In CUT&RUN, cells or nuclei are immobilized to a solid support, with pAG-MNase cleaved target DNA fragments isolated from solution. The workflow is compatible with next-generation sequencing (NGS) to provide high-resolution genome-wide profiles of histone post-translational modifications (PTMs) and chromatin-associated proteins (e.g. TFs and chromatin remodelers).

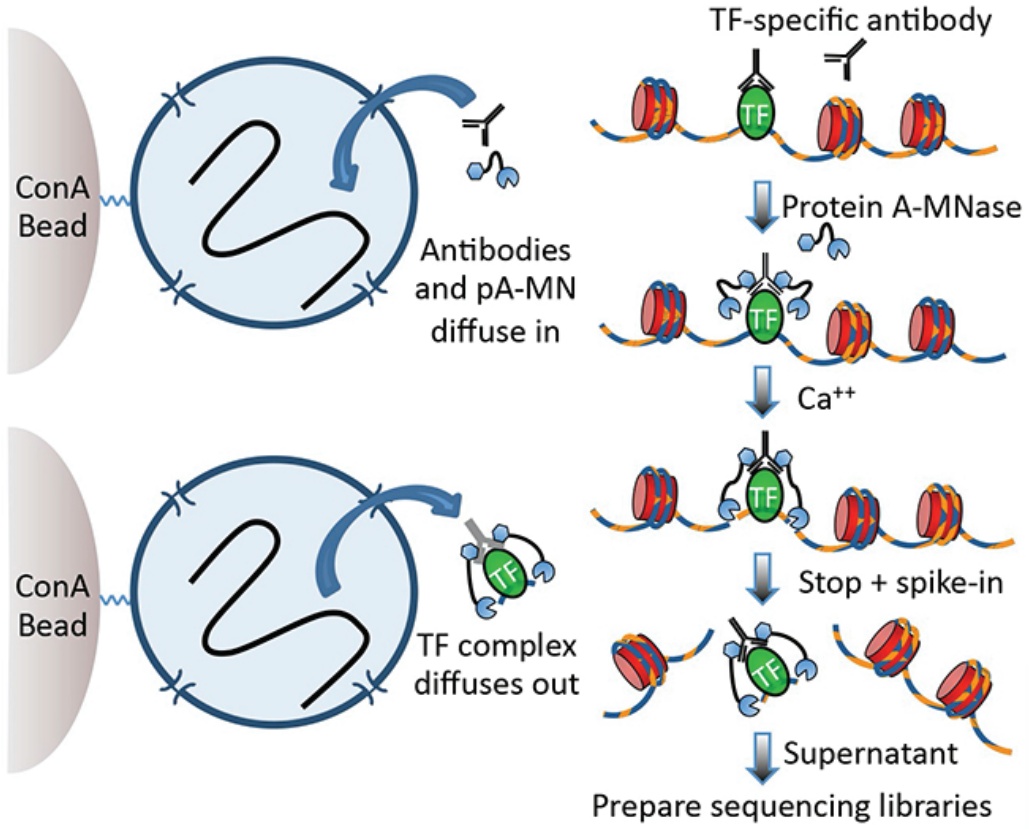


Figure 2. Schematic of CUT&RUN strategy (Adapted from Skene and Henikoff. eLife 2017;6:e21856).

Sample requirements: We accept fresh cells, live cell lines and cryopreserved cells. Please contact us for further details on project design, consultancy and sample submission.

Submission of live cell lines, fresh cells including FACS sorted and rare cell populations

1. Seed cells into one T25 flask. Ship the cells on day 2 or 3 after passage or seed the culture with 600 to 800K cells. Prior to shipping, fill the flasks with warmed media, tighten the cap and seal it with parafilm (do not use vented caps).
2. Ship cell lines by 24-hr delivery service. DO NOT ship cells on ice or cold packs.
3. Our recommendation is to send minimum of 1 to 2 million fresh cells in 1ml culture media with 10% FBS. For rare cell populations, minimum 500 K cells are required. Ship the cells on ice by < 24-hr delivery service or drop-off if local.

Bulk ATAC-seq

ATAC-seq method relies on NGS library construction using the hyperactive transposase Tn5. In the assay, intact nuclei are treated with a hyperactive Tn5 transposase mutant which is able to simultaneously tag the targeted DNA with NGS sequencing adapters and fragment the DNA in a process called tagmentation. The library that is generated can be sequenced by NGS and the regions of the genome with open or accessible chromatin are analyzed using bioinformatics.

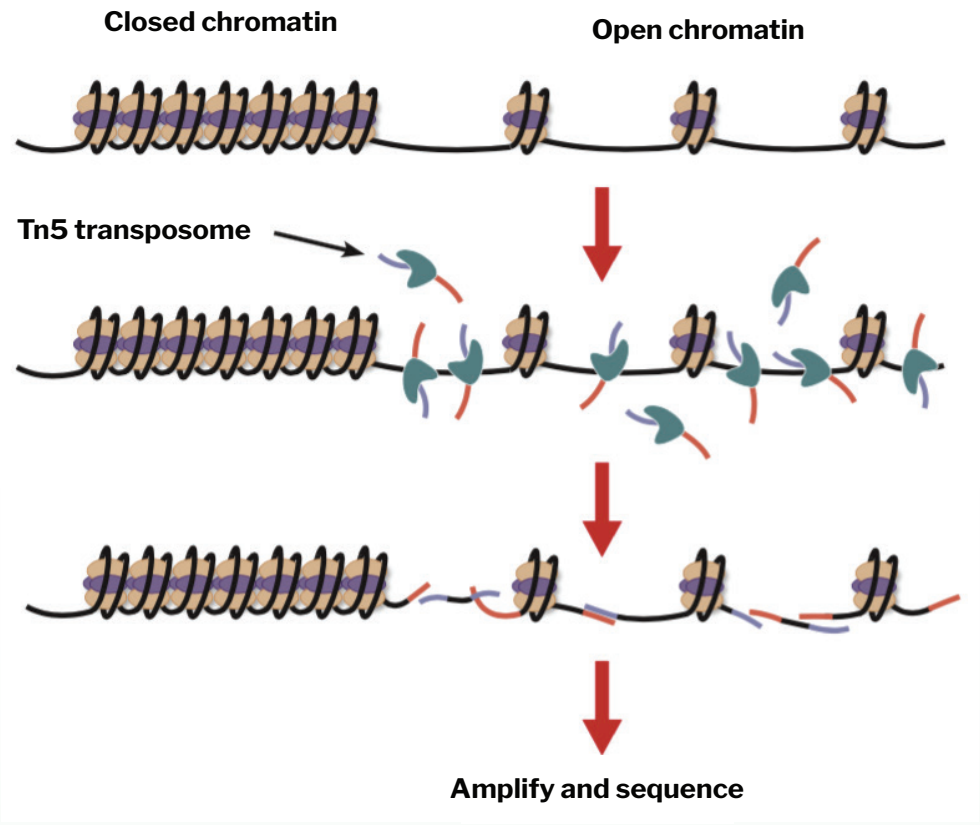


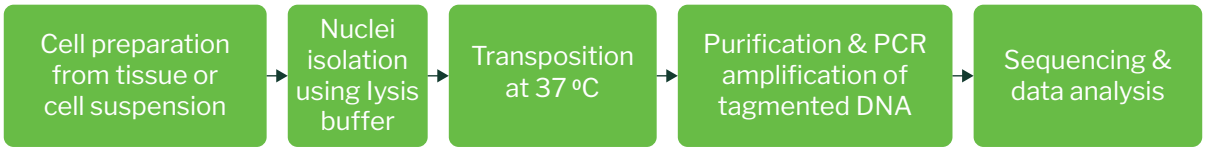
Figure 3. Overview of the ATAC-seq protocol (Source: Active Motif).

The main advantages of ATAC-seq compared to other techniques, such as FAIRE-seq or DNase-seq that investigate similar chromatin features, requires 20-30 mg tissue and a lower number of cells (50,000 to 100,000) per reaction and the relative simplicity of its two-step protocol. The historical methods are also more laborious compared to ATAC-seq, which can be completed in a two-step process within several hours.

Because of the assay's speed, simplicity, sensitivity and applicability to a wide range of sample types, ATAC-seq has become a commonly used epigenetic assay and can serve as a gateway to detailed epigenetic analysis.

ATAC-seq offers an additional benefit, it can also be used to generate a high-resolution map of nucleosome positions as well as transcription factor binding profiles. ATAC-seq could also potentially be used to generate personal epigenetic profiles from clinical samples.

Service workflow



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Fresh tissue preparation and submission

1. We recommend storing freshly isolated tissue (20-50mg) in 15 ml tube with pre-chilled Miltenyi MACS tissue storage solution to prevent apoptosis induction and it has been validated for variety of tissues from both human and mouse. It allows storage up to 48-hr at 4 °C. Therefore, it is important to ship on cold packs with ice within the defined storage time.

Frozen tissue preparation and submission

1. Isolate fresh tissue, immediately transfer the tissue to a cryovial placed on dry ice or in liquid nitrogen bath to preserve RNA integrity. Not adhering to this procedure will impact the quality of isolated nuclei.
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Submission of live cell lines, fresh cells including FACS sorted & rare cell populations

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3. Our recommendation is to send minimum 1 to 2 million cells if there are no limitations in 1ml culture media with 10% FBS. For rare cell populations minimum 500 K cells are required. Ship the cells on ice with ice packs by < 24-hr delivery service or drop-off if local.

Bioinformatic solutions for epigenetic profiling

Signios Bio has developed an extensive array of cutting-edge bioinformatics tools that empower scientists by facilitating the analysis of epigenetic and multi-omics data.
Our bioinformatic pipelines enable end-to-end analysis of epigenetic markers for our bulk and single cell experiments, providing insight into differential peak accessibility, epigenetic footprint, pathway enrichment analysis and more. Results are shared as comprehensive reports which include high-resolution and publication-ready figures.

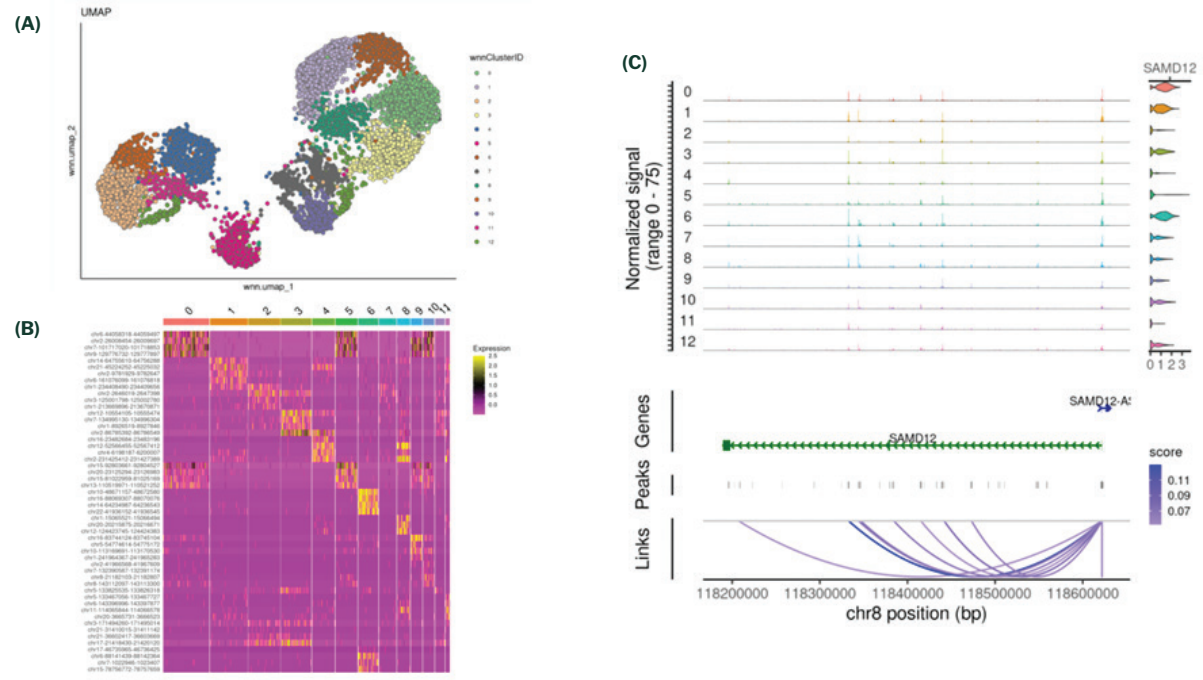

Figure 4. Example figure outputs from our advanced single-cell multiome analysis pipeline.

(A). Cell clustering using WNN unsupervised framework
(B). Heatmap showing differential peak accessibility between clusters
(C). Coverage plot showing expression of *SAMD12* gene (violin plots on the right of the panel) in different clusters and chromatin peak enrichment (central panel).

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