

ATAC-seq Service Guidelines

ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) allows for assessing genome-wide chromatin accessibility. The technology is based on the use of the transposase Tn5 which cuts exposed open chromatin and simultaneously ligates adapters for subsequent amplification and sequencing.

In order to maximize results we provide strict guidelines for sample preparation (cell collection).

We request 500.000-1.000.000 cells / sample of interest to be processed (200.000 cells as a strict minimum*). Cell viability should be higher than 95% when collected. Two additional test samples (500.000-1.000.000 cells / test sample) are required to perform the nuclei isolation optimization.

*for projects for which this minimum number of cells/sample cannot be obtained, please contact our services team for advices.

ATAC-seq workflow

Cells will be prepared by the customer according to Diagenode's sample preparation guidelines. Diagenode recommends to perform the experiment at least on two biological replicates.

OPTIMIZATION ON TEST SAMPLES (2 test samples requested per cell line/tissue type)

1. Sample QC

- Cell counting
- Cell viability assay

2. Nuclei isolation

- Nuclei isolation : testing 2 lysis conditions
- QC : Nuclei counting and microscopic evaluation

ATAC-SEQ ON SAMPLES OF INTEREST

1. Sample QC

- Cell counting
- Cell viability assay

2. Sample tagmentation

- Nuclei isolation
- QC : Nuclei counting and microscopic evaluation
- Nuclei Lysis
- Tn5 digestion

3. Library preparation

- Library amplification
- Library purification
- QC on the ATAC-seq library : DNA concentration and analysis of library profile
- Libraries pooling

4. Deep sequencing

- Samples are sequenced on an Illumina[®] platform, paired-end 2x50bp
- 40 million raw reads on average.

5. Bioinformatic analysis

- Quality check, alignment to reference genome, identification of enriched regions (peak calling) are included.

Provided files:

- Report with sequencing statistics
- Raw data in FASTQ format
- FastQC reports
- Alignment files in BAM format
- Peak files in BED format

Additional analysis on request:

- **Differential binding analysis:** Identification and annotation (human, mouse, rat, drosophila) of differential chromatin accessibility between samples.

Provided files:

- Report with summary of differential accessibility analysis and plots
 - Files containing differentially accessibility sites or unique peaks and breakdown of those in annotated regions: introns, exons, promoters, 1-to-5 kb upstream-TSS and intergenic regions for human, mouse, rat and drosophila.
- **Annotation in genomic regions:** Annotation of ATAC-Seq peaks with genomic regions: introns, exons, promoters, 1-to-5 kb upstream-TSS and intergenic regions for human, mouse, rat and drosophila.
 - **Gene ontology terms analysis:** Enrichment analysis on gene sets. Gene Ontology terms that are overrepresented in open chromatin regions or differentially accessible regions may indicate the underlying biological processes involved.
 - **Pathway analysis:** Identify biochemical pathways in which genes associated with open chromatin regions or differentially accessible regions may be overrepresented.

- **Visualization of specific genomic regions:** Visualization of results (i.e. sequencing data, peaks) at specific genomic regions (e.g. genes, promoters) in publication-ready images (human, mouse, rat).

Additional information

For sample preparation and sample shipment it is mandatory to follow Diagenode's guidelines. If customer samples do not meet Diagenode's quality requirements, any additional QC of new samples will be charged to the customer. Any delay in sample shipment to Diagenode's facilities might result in delaying customer's project.

Generated files will be available for download during 1 month and stored for an additional period of 3 months on Diagenode's servers. Additional long-term storage of data is available upon request. This offer includes a one hour call to walk you through the results if needed.

Original samples are stored at Diagenode during 4 months after project completion, but will be discarded once this time is exceeded. Return shipment of samples is available upon request.

Any additional service which is beyond the current project scope will be charged to the customer.