

Project 2

In this project you will analyze chromatin accessibility data from human bone marrow and peripheral blood cells. It comprises single-cell ATAC-seq data from 3 samples which are matched to the scRNA-seq data you analyzed in project 1.

The paper that analysed the dataset that also included this data was published by [Granja et al. \(2019\)](#) and the original data can be found [here](#).

Deadline: 23.12.2022 23:59

You are allowed to work in groups of two people. The groups do not have to be the same as for project 1.

To be able to attend the final exam, you must achieve at least 50% of all points in the assignments.

If you have any Problems, please contact Irem (irembgunduz@gmail.com) or visit the tutorials.

Submission:

- You will have to submit **one** tar.gz file that includes
 - The code as an R script
 - PDF file including all images and responses to the questions
- The code must be well commented and must run without an error to obtain any points
- If you use any other sources for your answers, don't forget to give the reference

Introduction in ArchR

We will use the programming language R and the ArchR software package. You can install ArchR by following the guidelines on this website: <https://www.archrproject.com/>

ArchR will also work on Windows systems except for some functionalities that you will not have to use in this project.

Install ArchR **before** the first tutorial, you already need it in week 1.

The lectures on scATAC analysis and the documentation of the ArchR package provide useful hints for conducting the analysis.

Download the data

You can download the dataset for this project under the following links (see also page 8):

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM4138888>

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM4138890>

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM4138891>

Week 1:

Task 1: Preprocessing and quality control (5P)

Task 1.1: Set up the environment

Download the data from the given website. Set the reference genome used in this project to: 'hg19'.

Optionally: You can also use parallel processing (requires package parallel). On windows, parallelization is not possible.

Task 1.2: Read the data into an appropriate data structure and apply filtering

Read the downloaded fragment files into arrow files.

Use lenient filtering on number of fragments and TSS enrichment (e.g., filterFragments > 500, filterTSS > 4). Make sure to compute a tiling matrix with appropriate window size (1000 bp or 500 pb).

You will come back to this step in Task 1.6 to evaluate if stricter thresholds are necessary.

Hint: For the filtering parameters, have a closer look at all the parameters that can be given to the function used for creating the error files.

Task 1.3: Identify doublets

Perform Doublet identification. If you observe doublets, remove them from the data and report your findings.

Task 1.4: Collect all samples into a joint data structure

Create one project that includes all samples. Use table 1 below to add the sample metadata to the project.

Inspect the cell metadata:

- How many cells does your project include?
- What is the median TSS-value and the median of the number of fragments?
- What are the dimensions of your dataset?

Task 1.5: Quality control

Report/plot the following measurements:

- How many cells do you have for each sample?
- Plot the fragment length distribution of all samples in a single plot
- Plot the distribution of TSS enrichment scores in each sample
- For each sample, plot number of fragments vs TSS enrichments

Inspect the plots and report your findings. Do you observe outlier samples/samples with low quality?

Note: If done correctly, some of these plots should have automatically been generated in the creation of arrow files.

Task 1.6: Filter the dataset

Based on your observations in the previous task, apply stricter filtering based on the number of fragments, TSS enrichment score, and remove doublets.

Have a look again at the questions from task 1.4 and answer them again for the new parameters.

Week 2:

Task 2: Dimensionality Reduction (5P)

Task 2.1: Iterative LSI

Apply dimensionality reduction using ArchR's iterative latent semantic indexing approach.

Why do we use LSI and not PCA for the dimensionality reduction of scATAC-data?

Task 2.2: UMAP with sample annotation and QC metrics

Compute data-visualization with UMAP coordinates. Create plots of the cells in UMAP space. Color each UMAP-plot by one of the following variables: Sample, TSS enrichment and number of fragments.

Task 2.3: Dealing with batch effects

Do you observe batch effects between cells in the different samples? If so, correct them. Create UMAP representations and inspect. Have the batch effects been resolved?

Task 3: Clustering (3P)

Apply Louvain clustering to all cells in the dataset. Create a UMAP plot coloring by clusters.

How many cells does each cluster contain? What are the sample proportions in each cluster?

Task 4: Peaks (8P)

Task 4.1: Peak calling

Prepare the data for peak-calling. Explain what you did and why this is necessary.

Compute a joint peak set, grouping by clusters and add the peak Matrix to your project.

Task 4.2: Cluster marker peaks

Task 4.2.1: Identify cluster-specific peaks using reasonable cutoffs. Which parameters are used for which variable and what do they describe?

Task 4.2.2: Create a Heatmap showing accessibility in the marker peaks

Task 4.2.3: Create browser plots around the following genes: CD19, CD34, CD3D, CD69

Week 3:

Task 5: Gene activity (3P)

Task 5.1: Compute gene activity scores using chromatin accessibility

Note: depending on the parameters that you used this might have already been done during data import

Task 5.2: Identify Marker genes

Identify cluster marker genes. Explain the parameters and cut-off values you used.

Task 5.3: Using MAGIC

Plot UMAP embeddings for the first 5 marker genes found in the previous task. Plot the visualization with and without smoothing using Magic.

What is the main idea behind MAGIC (1-2 sentences).

Task 6: Transcription Factor motif activity (8P)

Task 6.1: Compute TF motif activity

Pick a suitable TF motif annotation (database).

Questions:

1. Which annotation did you choose?
2. How was the annotation obtained?

Task 6.2: Plot UMAP embeddings for marker TFs

Which are the TFs that are most variable? Plot the top 2 marker motifs in a separate UMAP plot.

Task 6.3: Motif activity

Plot distribution of motif activity of marker TFs in the different clusters

Task 8: Integration with gene expression (7P)

Task 8.1: Data integration

Integrate the annotated scRNA-seq data from Project 1. Link gene expression to accessibility. Plot the scATAC-seq data in the UMAP space and overlay it with gene expression levels for the highlighted marker genes in table 2.

Task 8.2: Correlation Coefficients

Compute the correlation coefficients between the gene expression from the scRNA-seq data and the gene activity from the scATAC-seq data.

Which genes exhibit the highest / lowest agreement?

Task 8.3: Cluster labels from gene expression

Assign cell type labels using the integrated gene expression data and rename the clusters accordingly. Compute a confusion matrix of ATAC clusters with RNA-inferred cell type labels. Report what you observe in terms of agreement.

Task 8.4: Peak-gene linkage

Task 8.4.1: Compute peak-gene linkage using gene expression and chromatin accessibility in nearby enhancers.

Task 8.4.2: Plot side-by side heatmaps of candidate regulatory element accessibility and gene expression

Task 8.4.3: Identify potential enhancers for the highlighted marker genes from Table 2. Plot the links for the marker-genes using genome browser views.

How many potential enhancers do you observe for each gene?

Week 4:

Task 9: Differential accessibility (5P)

Task 9.1: Differential peak accessibility

Compute differential peak accessibility between T cells and monocytes. Create MA and volcano plots of differential accessibility.

Task 9.2: TF motif enrichment

Compute TF motif enrichment in differentially accessible peaks.

Which TFs are enriched in peaks specific to T cells and monocytes respectively?

Plot TF motif activities for the identified TFs as a heatmap or in UMAP space.

Task 10: TF footprinting (6P)

Task 10.1: Obtaining footprints on feature set

Obtain footprints for the top motifs from your enrichment analysis.

Task 10.2: Normalization for Tn5 Bias

Task 10.2.1: Perform a normalization for the Tn5 Bias.

Questions:

1. Explain the need to correct for Tn5 bias.
2. Which normalization method did you use?

Task 10.2.2: Plot the aggregate footprints for T cells and monocytes. Describe and explain what you see.

Task 11: (Bonus) Co-accessibility (+5P)

Task 11.1: Co-accessibility of peaks

Compute the co-accessibility of the peaks.

Task 11.2: Identify potential enhancers for marker genes

Use the highlighted marker genes from Table 2 and plot the linkage of peaks to TSS of these genes.

How many potential enhancers do you observe for each gene?

Table 1:

| Sample | Type | Donor | Sex |
|-----------|------|-------|-----|
| BMMC_D5T1 | BMMC | D5 | F |
| CD34_D7T1 | CD34 | D6 | M |
| CD34_D8T1 | CD34 | D7 | M |

Table 2:

| | |
|------------------|--------------------------------------|
| Early Progenitor | CD34 |
| Erythroid | GATA1 |
| B cells | PAX5, MS4A1, MME, CD19 |
| T cells | CD3D , CD8A, CD4, TBX21, ILR7 |
| Monocytes | CD14 , MPO, CEBPB |
| NK | TBX21 |
| LMPP | CD38 , CD52, CSF3R |
| GMP | ANXA1, AP351 , APLP2 |
| PRO-B | CD19, CD34 |

COVID-19 is an emerging, rapidly evolving situation.
 Get the latest public health information from CDC: <https://www.coronavirus.gov>.
 Get the latest research from NIH: <https://www.nih.gov/coronavirus>.
 Find NCBI SARS-CoV-2 literature, sequence, and clinical content: <https://www.ncbi.nlm.nih.gov/sars-cov-2/>.

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Sample GSM4138888

Query DataSets for GSM4138888

Status Public on Oct 25, 2019
 Title scATAC_BMMC_D5T1
 Sample type SRA

Source name Healthy Human Bone Marrow
 Organism [Homo sapiens](#)
 Characteristics disease state: Healthy
 tissue: Bone Marrow
 Sex: F
 age: 18-55
 Extracted molecule genomic DNA
 Extraction protocol Nuclei were extracted from cells that were cryopreserved (fresh frozen in either Bamberker freezing media or 10% DMSO with 90% serum). Thawed cells were not filtered for viability prior to loading into droplets.
 10X Genomics Single Cell ATAC Kit version 2

Library strategy ATAC-seq
 Library source genomic
 Library selection other
 Instrument model Illumina NovaSeq 6000

Data processing The Cell Ranger RNA Software Suite (version 3.0.0) was used for processing sequencing information and single cell barcodes
 Matrix was filtered for mitochondrial and ribosomal RNA.
 Cells were filtered unique molecular identifies greater than 1000 and number of genes greater than 400.
 genome build: hg19/Grh37
 processed data files format and content: scRNA-rds files are a binary R file that contain a scRNA-seq counts sparse matrix; scADT-rds files are a binary R file that contain a scADT-seq counts matrix associated with the scRNA-seq sparse matrix

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Platform ID [GPL24676](#)
 Series (1) [GSE139369](#) Single-cell, multi-omic analysis identifies regulatory programs in mixed phenotype acute leukemia

Relations
 BioSample [SAMN13110931](#)
 SRA [SRX7053023](#)

| Supplementary file | Size | Download | File type/resource |
|--|--------|--|--------------------|
| GSM4138888_scATAC_BMMC_D5T1.fragments.tsv.gz | 1.6 Gb | (ftp) (http) | TSV |

[SRA Run Selector](#) ?

Raw data are available in SRA

Processed data provided as supplementary file