Study of the regulatory programs of spermatogenesis through the integration of single-cell RNA and ATAC

Projects in Bioinformatics - Fall 2025

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Contents

1 Introduction

Spermatogenesis is a complex process that permits the differentiation of stem cells into mature spermatozoa, and is of high relevance in studying infertility conditions and cross-species differences in the biological processes.

1.1 Goals for the project

Initial:

- learn basics of git
- learn sc workflow with scanpy, muon and scvi-tools
- work with real messy data
- Answer:
  - Cell states & trajectories: Can we recover a clean spermatogenic trajectory (spermatogonia → spermatocytes → spermatids) and supporting somatic lineages?
  - Peak→gene linkage: Which distal elements likely regulate stage-specific genes?
  - TF programs: Which TFs show coordinated motif accessibility + target expression? (e.g., STRA8, A-MYB, TAF7L)

Actually done:

- learn basics of git
- learn how to do Conda environment reproducibility
- small shell scripts to run on SLURM.
- learn single cell workflow with scanpy, muon and scvi-tools
- work with real messy data
- Answer: Cell states & trajectories: Can we recover a clean spermatogenic trajectory (spermatogonia → spermatocytes → spermatids) and supporting somatic lineages?
- Succefull celltype annotation of both scRNA-seq and scATAC-seq.
- Cell topics and DARs for scATAC-seq for further analysis

1.1.1 Data availability

All environment files, notebooks and scripts as well as the repport files for this project are available on a public github repository: SamueleSoraggi/PIB-johan-olesen.

1.2 Workflow Overview

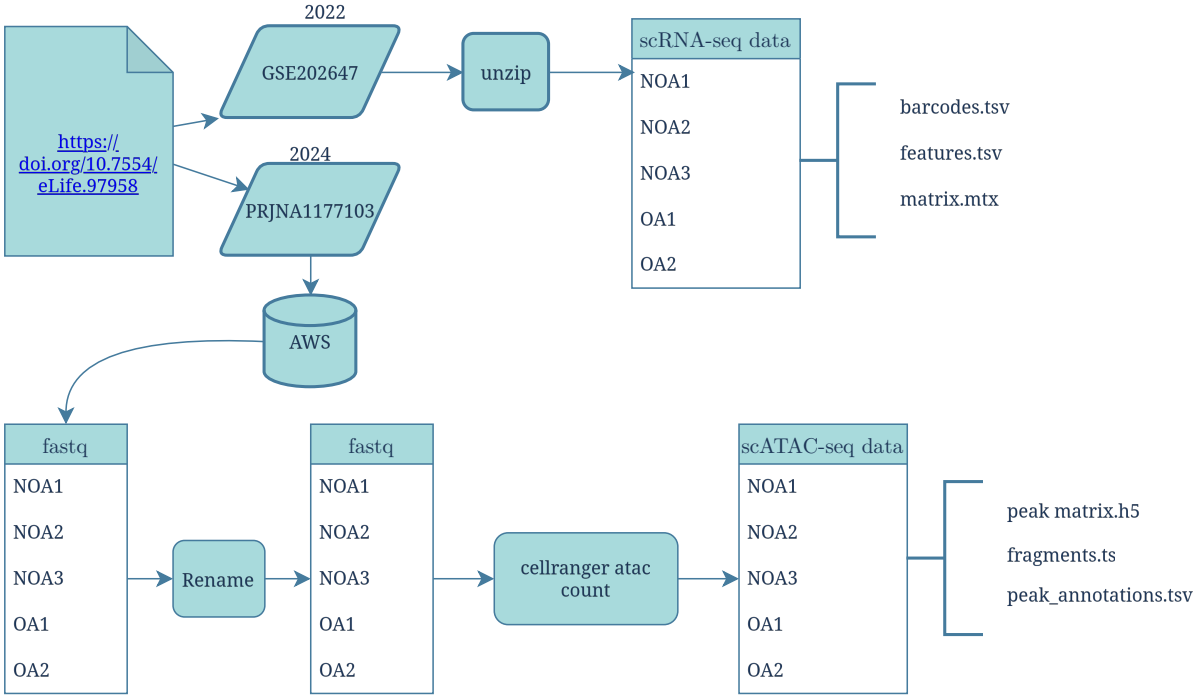

Figure 1: Stage 1 schematic of data aquisition and preparation.

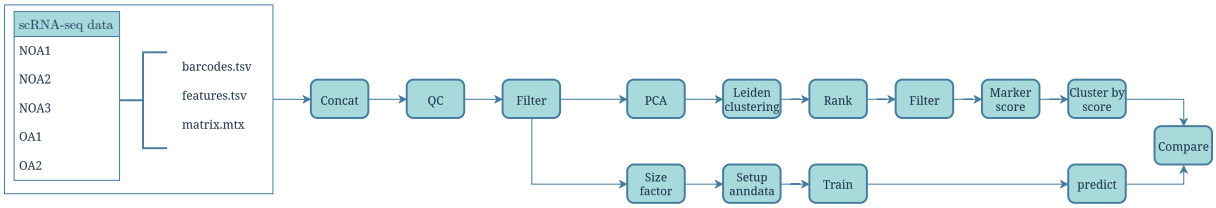

Figure 2: Stage 2 schematic of scRNA-seq celltype annotation with a *semi-manual* way and CellAssign model.

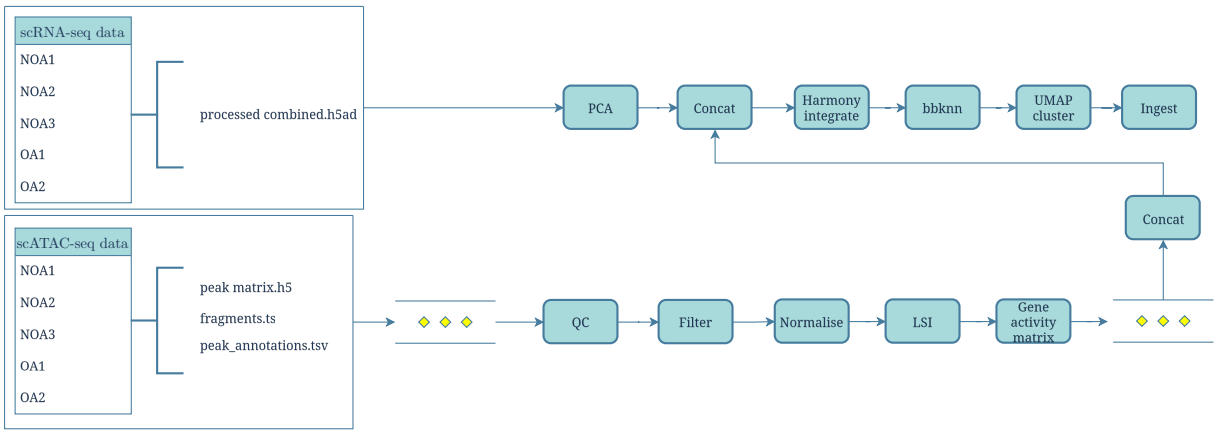

Figure 3: Stage 3 schematic of attempt at label transfer by integrating scRNA-seq and scATAC-seq.

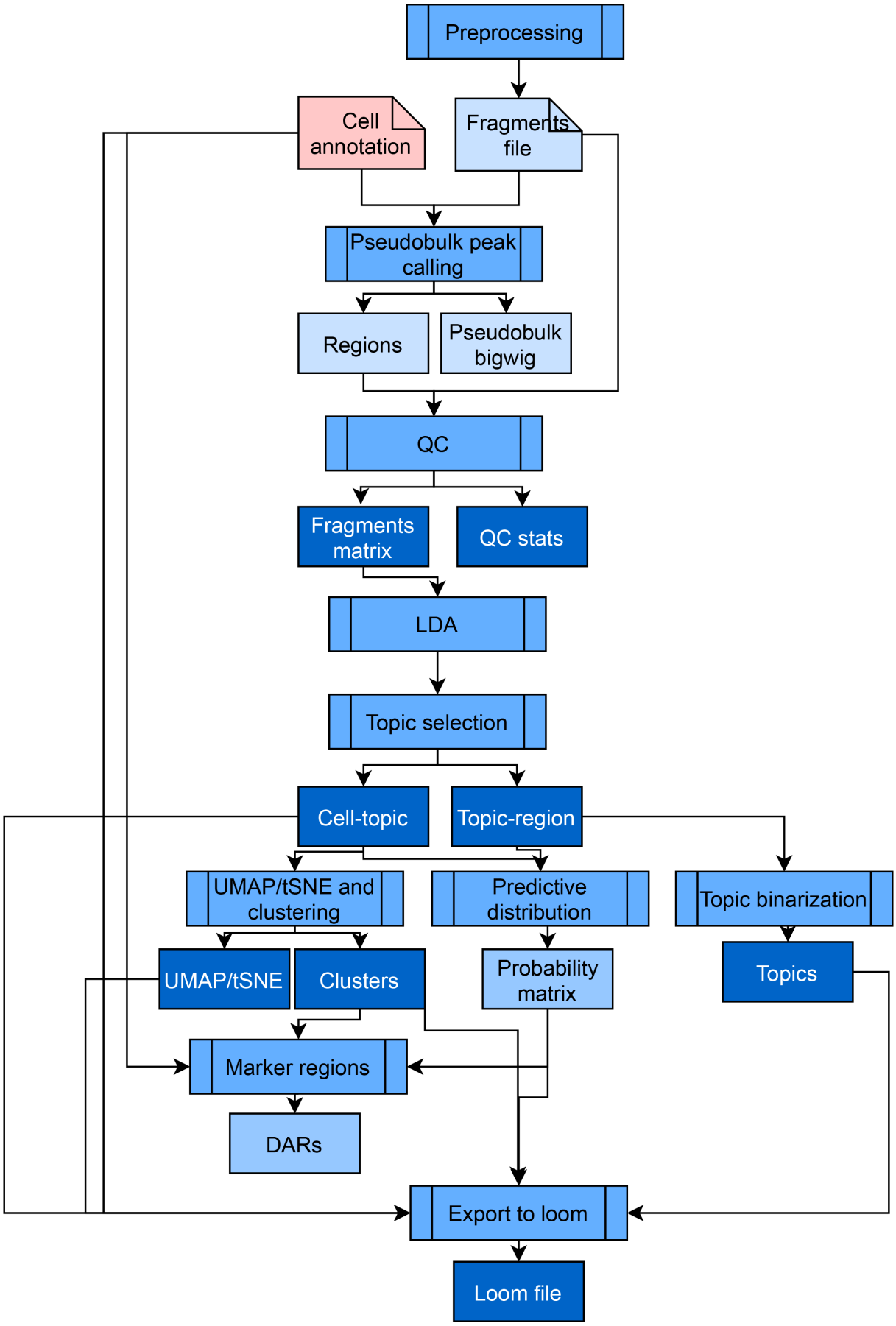

Figure 4: Stage 3 schematic of pycistopic workflow from PyCistopic documentation. [1]

Flowcharts drawn with `draw.io`.

1.2.1 Environment setup with Conda

First step was to get a working environment setup for the analyses. For this Conda was used to create a environment with the required packages, relying on `pip` for the most up-to-date packages.

For the tutorial run and scRNA-seq labelling the environmnet `torch_env.yml` [2] was used. This environment includes the scverse's anndata [3], mudata [4], scanpy [5], muon [6] and scvi-tools [7] packages, as well as full PyTorch [8] CUDA capabilities for scvi-tools.

For the second scATAC-seq workflow another environment was used because of versioning requirements; `cistopic_env.yml`, consisting of the SCENIC+ [9] suite.

1.2.2 Tutorial run

To start of before real testis data had been found. A quick run through of the tutorial run of multiome 10X PBMC [10] by Bredikin to get a quick overview of how to work with single cell data and anndata objects, and to check Conda environment worked.

Was succesful in creating the same analysis as the tutorial.

2 Stage 1: Data aquisition and preparation

For the first stage of the project, we will be focusing on acquiring and preparing data for analysis. This includes downloading and organizing the necessary datasets, as well as preprocessing the data to ensure it is ready for downstream analysis. The workflow is illustrated on **Figure 1**.

For the real dataset set out in the goals, Wang et al [11] have made their scRNA-seq and scATAC-seq data available.

The scRNA-seq data was available under NCBI Gene Expression Omnibus ID **GSE202647** from 2022, and was already ready for analysis.

The scATAC-seq data was available under NCBI BioProject ID **PRJNA1177103** from 2024. This was only the raw read data, so the Cellranger ATAC [12] pipeline was run for each of the five samples. For Cellranger to be able to run the data had to be structured and named in a specific way. Using the guidelines available from the offical 10X Cellranger documentation and matching the read length of each of the four files per sample was renamed accordingly.

The `count` function was run with Cellranger ATAC version 2.2.0 on each sample with reference data *refdata-cellranger-arc-GRCh38-2024-A* using SLURM. This resulted in the `peak_matrix.h5`, `fragments.tsv` and `peak_annotations.tsv` for each sample ready for analysis.

Ideally, we wanted the data from scRNA-seq and scATAC-seq to be cell matched from their barcodes, but finding public datasets for testis with that criteria was difficult. Instead we will be matching cells by celltype instead. The data in Wang et al's study comes from the same five donors, but sequenced at seperate time points.

3 Stage 2: Celltype annotation of scRNA-seq data

For stage 2 the main goal is to get the scRNA-seq data processed and annotated with cell types.

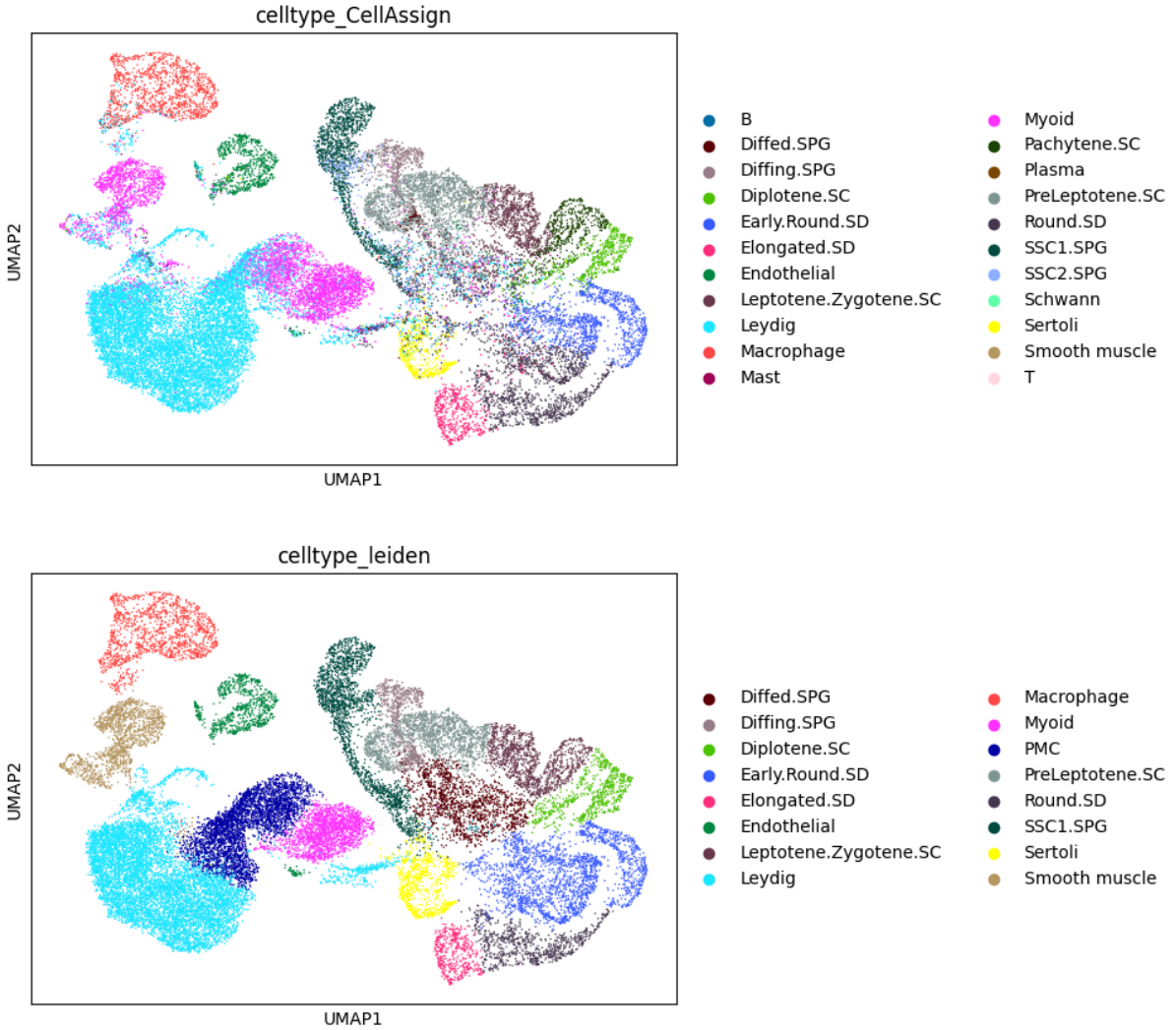

Figure 5: Celltype annotation result using either CellAssign model or leiden clustering using the same set of marker genes.

Cell Type	Marker Genes
Sertoli	SOX9, GATA1, ACSL4, WT1, GAS6, VIM, CD99, APOA1
Leydig	CFD, IGF1, IGFBP5, INSL3
Myoid	MYH11, ACTA2
Macrophage	CD68, CD163, MSR1, CD14
Endothelial	VWF, SOX17
B	CD52
Mast	TPSB2
T	CCL5
Plasma	IGLC2
PMC	DPEP1
Schwann	S100B
Smooth muscle	TAGLN
SSC1.SPG	ID4, UTF1, TWIST1, KRT18, AES, ENHO, C19orf84, SIX1, PIWIL4
SSC2.SPG	ASB9, L1TD1, NANOS3, FAM25G, CITED2

Cell Type	Marker Genes
Diffing.SPG	MKI67, ACTL8, KLHL15, DMRT1, PABPC4
Diffed.SPG	SSX3, TEX19, PNMA6E, PEG10, TKTL1, BEND2
PreLeptotene.SC	DPH7, PRDM9, PRAP1, KIF1A, MAGEA9B, FAM9C, ATP6AP1, OTUD6A, CCNB3
Leptotene.Zygotene.SC	LINC00668, AP000350.6, LINC01120, AL138889.1, LINC00865, C18orf63
Pachytene.SC	PCDHB6, POM121L12, POM121L2, C9orf57, AL133279.3, AC093326.2, AL353581.1, PCDHB5, PCDHB2, AC135012.2, MNS1, CCDC42
Diplotene.SC	ART5, KLB, B3GALT4, ELMO3, RTN4RL2, AC073263.2, LINC00588, WNT6, LINC01309, AL121936.1, KRT72, LDHC
Early.Round.SD	FAM24A, SPACA1, LINC01351, ABRA, LINC00703, LINC02502, PPP1R27, H1F0, TPRG1, LRRC39, C1orf87, TMIGD3
Round.SD	TNP2, LINC02314, LINC01921, PRSS37, FBXO39, LINC02400, DHRS3, FAM205C, CXorf65, SCP2D1, LINC01548, CCDC179, AC010255.3, SPEM3
Elongated.SD	TSSK6, CABS1, SPATA3, CCDC196, TSPAN16, PHOSPHO1, SPEM2, TEX44, LRRD1, SPEM1, GLUL

4 Stage 3a: Celltype annotation of scATAC-seq data with label transfer

5 Stage 3b: Celltype annotation of scATAC-seq data with pycistopic

6 Conclusion

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Appendix A Cellranger

Naming scheme for Cellranger ATAC count [13]: `[Sample Name]S1_L00[Lane Number][Read Type]_001.fastq.gz`, where `Read type`:

- `I1`: Dual index i7 read (optional)
- `R1`: Read 1
- `I2`: Dual index i5 read
- `R3`: Read 2

`Lane Number` does not matter. `Sample Name` can be anything.

Example of NOA1 sample:

- SRR31097965_S1_L001_I1_001.fastq
- SRR31097965_S1_L001_I2_001.fastq
- SRR31097965_S1_L001_R1_001.fastq
- SRR31097965_S1_L001_R2_001.fastq

Appendix B scRNA-seq

Cell Type	Marker Genes
Sertoli	SOX9, GATA1, ACSL4, WT1, GAS6, VIM, CD99, APOA1
Leydig	CFD, IGF1, IGFBP5, INSL3
Myoid	MYH11, ACTA2
Macrophage	CD68, CD163, MSR1, CD14
Endothelial	VWF, SOX17
B	CD52
Mast	TPSB2
T	CCL5
Plasma	IGLC2
PMC	DPEP1
Schwann	S100B
Smooth muscle	TAGLN
SSC1.SPG	ID4, UTF1, TWIST1, KRT18, AES, ENHO, C19orf84, SIX1, PIWIL4
SSC2.SPG	ASB9, L1TD1, NANOS3, FAM25G, CITED2
Diffing.SPG	MKI67, ACTL8, KLHL15, DMRT1, PABPC4
Diffed.SPG	SSX3, TEX19, PNMA6E, PEG10, TKTL1, BEND2
PreLeptotene.SC	DPH7, PRDM9, PRAP1, KIF1A, MAGEA9B, FAM9C, ATP6AP1, OTUD6A, CCNB3
Leptotene.Zygotene.SC	LINC00668, AP000350.6, LINC01120, AL138889.1, LINC00865, C18orf63
Pachytene.SC	PCDHB6, POM121L12, POM121L2, C9orf57, AL133279.3, AC093326.2, AL353581.1, PCDHB5, PCDHB2, AC135012.2, MNS1, CCDC42
Diplotene.SC	ART5, KLB, B3GALT4, ELMO3, RTN4RL2, AC073263.2, LINC00588, WNT6, LINC01309, AL121936.1, KRT72, LDHC
Early.Round.SD	FAM24A, SPACA1, LINC01351, ABRA, LINC00703, LINC02502, PPP1R27, H1F0, TPRG1, LRRC39, C1orf87, TMIGD3
Round.SD	TNP2, LINC02314, LINC01921, PRSS37, FBXO39, LINC02400, DHRS3, FAM205C, CXorf65, SCP2D1, LINC01548, CCDC179, AC010255.3, SPEM3
Elongated.SD	TSSK6, CABS1, SPATA3, CCDC196, TSPAN16, PHOSPHO1, SPEM2, TEX44, LRRD1, SPEM1, GLUL

Appendix C scATAC-seq

Appendix D pycistopic workflow