

## Duke Molecular Genomics Core

### 10x Genomics Single Cell Multiome ATAC + Gene Expression Data Analysis

#### **BASIC (10x Genomics Cell Ranger ARC)**

- Conversion of raw sequencing data to Fastq format
- Alignment of sequencing reads to reference transcriptome
- Generation of count matrix and alignment files for each sample for Gene Expression and ATAC Fragments
- Deliverable files include the following: Count/Fragment matrix files, Alignment files, Fastq files (if required)

#### **ADVANCED (Seurat, Signac, SingleR)**

- Quality control filtering
- Normalization, PCA, Clustering (RNA / ATAC weighted nearest neighbors), integration (if multiple samples)
- Differential gene markers per cluster by RNA assay
- Differential ATAC peaks by cluster along with closest genes to found peaks
- Annotation with SingleR using relevant annotated single-cell reference (RNA assay)
- Plotting Feature and Violin plots for genes of interest (up to 30) by cluster for RNA assay
- Plotting Coverage Plots for genes of interest (up to 30) linking gene expression and Tn5 insertion frequency over gene tracks
- Motif Enrichment analysis to link marker genes for each cluster to the accessibility of related motifs enriched in the same cluster

Deliverable files include the following: Clustering UMAP plots, Peak Coverage Plots, Motif Exp plots, QC plots, cell count info in csv and bar graph, heatmap of top 10 genes per cluster, differential gene expression results per cluster in csv and between samples if comparing two samples, avg gene expression per cluster in csv, genes expression in feature and violin plots.

#### **OTHER ANALYSES**

- Ambient RNA removal using Cellbender
- Gene Set Enrichment Analysis (GSEA) between the identified clusters or between the samples
- RNA Velocity analysis using Velocity
- Pseudotime analysis using Monocle

#### **IMPORTANT!**

- It is your responsibility to store your data long term. We will delete data 90 days after transfer.

- You will receive a link with your raw sequencing data. You must download it by the deadline listed in the email.
- Basic analysis results will be shared either via Duke OneDrive, 10X cloud or using sftp download option from temp directory. Advanced analysis and other analysis results would be shared via Duke Box or email. You must download your files within two weeks of receipt.
- Any analysis requested after initial basic and advanced analysis requires submission of a new service request.