

Duke Molecular Genomics Core

10x Genomics single cell RNAseq data analysis

BASIC (10x Genomics Cell Ranger)

- 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
- Ambient RNA removal using Cellbender if required
- Deliverable files include the following: Count matrix files, Alignment files, fastq files (if required)

ADVANCED (SoupX, Seurat, SingleR, scType, Doublet finder)

- Cell count correction using SoupX
- Quality control filtering
- Dimensional reduction and cell clustering
- Doublet removal using doublet finder
- Conserved gene marker identification per cell cluster
- Differentially expressed gene identification between samples
- Cluster annotation using automatic prediction algorithm (singleR/scType)
- Plotting initial genes of interest (up to 30)
- Deliverable files include the following: Clustering UMAP 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

- Gene Set Enrichment Analysis (GSEA) between the identified clusters or between the samples
- RNA Velocity analysis using Velocity
- Pseudotime analysis using Monocle
- Cell interaction and signaling network analysis using Cellchat
- Gene ontology enrichment analysis using Enrichr/ClusterProfiler
- Pseudobulk differential gene expression analysis using Muscat
- Gene regulatory network analysis using WGCNA/SCENIC
- Copy number variation analysis using InferCNV
- Figures for papers/grants that aren't part of the initial analysis

IMPORTANT!

- It is your responsibility to store your data long term.
- 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.