

## **Assignment #6**

### **Investigating the transcriptional heterogeneity of cell types in AML using scRNA-seq**

#### **Introduction**

Blood cells are classified by two major cell types: 1) lymphocytes, which include T-cells, B-cells, and natural killer cells, and 2) myelocytes, which include neutrophils, monocytes, and macrophages. Acute myeloid leukemia (AML) is a cancer of the blood caused by mutations of immature cells of the myeloid lineage. There are many different healthy and malignant cell types within the blood of AML patients, so it is important to characterize these distinct cell types to better understand AML and devise novel treatments. Single cell RNA sequencing (scRNA-seq) is a technique which resolves the transcriptome of each individual cell in a sample.

For assignment #6, you will analyze scRNA-seq data from healthy and AML patients taken from van Galen et al. (2019)<sup>1</sup>, performing QC, clustering, visualization, and DGE analysis between single cells.

#### **Steps required**

1. **Perform QC and preliminary scRNA-seq analysis on healthy vs. AML samples.**
  1. Download expression matrices ("dem.txt") and metadata ("anno.txt") for below samples using the "custom" link from GEO accession GSE116256.
    - i. Healthy samples: "BM3", "BM4", "BM5-34p", "BM3-34p38n"
    - ii. AML samples: "AML419A-D0", "AML707B-D0", "AML916", "AML921A-D0"
  2. Import data to R and use Seurat to normalize, merge, and cluster data to generate UMAP plots. Label UMAP plots by:
    - i. cell type
    - ii. patient
    - iii. malignance status

***Describe your results and any interesting findings. How do your cell clusters correlate to these annotations?***

2. **Perform differential gene expression on scRNA-seq data.**
  1. Use the FindMarkers function in Seurat to generate DGE matrices comparing:
    - i. Clusters
    - ii. Cell types within AML and healthy patients
    - iii. Malignance status within same cell types of AML patients
  2. Generate heatmaps for top DE genes from these analyses

***Describe your results and any interesting findings. Are there any genes that are commonly upregulated in malignant vs. healthy cells within the same cell type in AML cells?***

1. van Galen P, Hovestadt V, Wadsworth II MH, Hughes TK, Griffin GK, Battaglia S, Verga JA, Stephansky J, Pastika TJ, Lombardi Story J, Pinkus GS, Pozdnyakova O, Galinsky I, Stone RM, Graubert TA, Shalek AK, Aster JC, Lane AA, Bernstein BE. Single-Cell RNA-Seq Reveals AML Hierarchies Relevant to Disease Progression and Immunity. Cell. 2019 Mar 7;176(6):1265-1281.e24. doi: 10.1016/j.cell.2019.01.031. Epub 2019 Feb 28. PMID: 30827681; PMCID: PMC6515904.