## ##CITE-Seq 1 Script - Clonotype Analysis####

####Setup####

###Load required packages

library(Seurat)

library(ggplot2)

library(tidyverse)

library(patchwork)

library(Matrix)

library(RColorBrewer)

library(writexl)

library(ggridges)

library(clustree)

library(scRepertoire)

library(future)

library(alakazam)

library(immunarch)

library(airr)

library(biomaRt)

library(SeuratDisk)

library(SeuratData)

###Load Seurat object

experiment <- LoadH5Seurat("SeuratProject.h5Seurat")

###Individual plots

DefaultAssay(experiment) <- "RNA"

p1=DimPlot(experiment, label = TRUE,cols=colbig,reduction = "rna.umap", label.size = 2.5) + NoLegend()

p2=DimPlot(experiment, label = TRUE,cols=colbig,reduction = "adt.umap", label.size = 2.5) + NoLegend()

p3=DimPlot(experiment, label = TRUE,cols=colbig, reduction = "wnn.umap", label.size = 2.5) + NoLegend()

p1

p2

p3

DefaultAssay(experiment) <- "RNA"

DefaultAssay(experiment) <- "ADT"

####Simple plotting####

###Umap-wnn by mouse

plot\_mouse <- DimPlot(experiment, label = TRUE,reduction = "wnn.umap", label.size = 2.5, group.by = "orig.ident") + ggtitle("Coloured by mouse")

###Umap-wnn by sample

DimPlot(experiment, label = TRUE,cols=colbig, reduction = "wnn.umap", label.size = 2.5, split.by = "orig.ident", ncol = 2) + NoLegend()

###Umap-wnn by cell cycle stage

DimPlot(experiment, label = TRUE,reduction = "wnn.umap", label.size = 2.5, group.by = "Phase") + ggtitle("Coloured by cell cycle stage")

###Feature and violin plot

FeaturePlot(experiment, features = c("CXCR5"), reduction = "wnn.umap")

VlnPlot(experiment, feature = "IgM")

####Clonotype analysis####

###Data visualization

###Percent/total number of unique clonotypes

quantContig(combined, cloneCall = "gene+nt", scale = T) #percent of unique clonotypes of total size of the size of clonotyeps

quantContig(combined, cloneCall = "gene+nt", scale = F) #number of uniqe clonotypes

quantContig(combined, cloneCall = "gene+nt", scale = T, chain = "IGH") + ggtitle("IGH")#by IGH

quantContig(combined, cloneCall = "gene+nt", scale = F, chain = "IGH") + ggtitle("IGH")#by IGH

quantContig(combined, cloneCall = "gene+nt", scale = T, chain = "IGL") + ggtitle("IGL")#by IGL

quantContig(combined, cloneCall = "gene+nt", scale = F, chain = "IGL") + ggtitle("IGL")#by IGL

###Abundance of clonotypes

Abundance\_clonotypes <- abundanceContig(combined, cloneCall = "gene", scale = F, exportTable = T)

Abundance\_clonotypes <- Abundance\_clonotypes %>%

arrange(desc(Abundance))

Abundance\_clonotypes

abundanceContig(combined, cloneCall = "gene", scale = T)

###Length of clonotypes

lengthContig(combined, cloneCall = "aa")

lengthContig(combined, cloneCall = "nt")

###Compare clonotypes

compareClonotypes(combined, samples = c("a", "b"), cloneCall = "aa", graph = "alluvial") #Computationally intense

###Visualise Gene Usage

vizGenes(combined, gene = "V", chain = "IGH", plot = "bar", order = "variance", scale = TRUE)

vizGenes(combined, gene = "V", chain = "IGL", plot = "bar", order = "variance", scale = TRUE)

vizGenes(combined, gene = "V", chain = "IGL", plot = "heatmap", scale = TRUE, order = "gene")

###Clonal overlap

clonalOverlap(combined, cloneCall = "gene+nt",

method = "morisita")

###Clonotype proportion

clonalProportion(combined, cloneCall = "gene")

clonalProportion(combined, cloneCall = "nt")

###Clonal Homeostasis

clonalHomeostasis(combined, cloneCall = "gene")

clonalHomeostasis(combined, cloneCall = "nt")