title: “DGE of Venetoclax and CITE/Hash”
author: “David Mentrup”
Start date: “02-06-2020”
Current version date: “05 July, 2020”
output:
html_document: default
word_document: default
pdf_document: default
fig_width: 20
fig_height: 8
?

1 Preparations and Data

1.0 Required Packages

```
library(dplyr)
```

```
Attaching package: 'dplyr'
```

```
The following objects are masked from 'package:stats':

    filter, lag
```

```
The following objects are masked from 'package:base':

    intersect, setdiff, setequal, union
```

```
library(Seurat)
library(ggplot2)


packageVersion("dplyr")
```

```
[1] '0.8.99.9003'
```

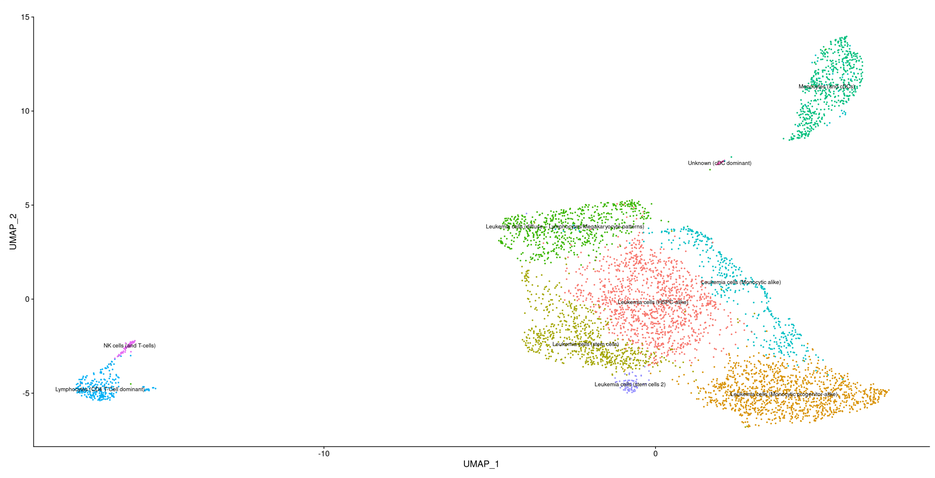
```
packageVersion("Seurat")
```

```
[1] '3.1.5'
```

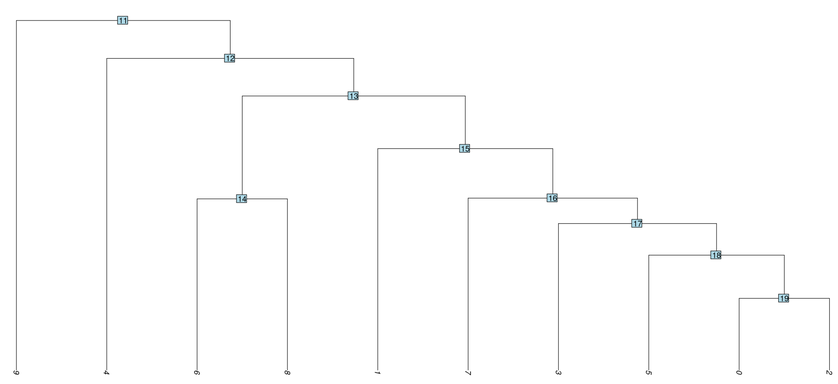
```
S01_CITE.combined <-  readRDS(file = "./Pat1_CITE_integrated.rds")
DefaultAssay(S01_CITE.combined)<- "RNA"
S01_CITE.combined<-SetIdent(S01_CITE.combined, value = S01_CITE.combined@meta.data$RNA_cluster)
S01_noCITE <-  readRDS(file = "./Pat1_noCITE_p.rds")
DefaultAssay(S01_noCITE)<- "RNA"
```

```
DimPlot(S01_CITE.combined, reduction = "umap", group.by="celltype", label = TRUE, label.size=3, pt.size = 0.5) + NoLegend()
```

```
Warning: Using `as.character()` on a quosure is deprecated as of rlang 0.3.0.
Please use `as_label()` or `as_name()` instead.
This warning is displayed once per session.
```




```
S01_CITE.combined <- BuildClusterTree(S01_CITE.combined, dims = 1:25)
PlotClusterTree(S01_CITE.combined)
```




```
output <- "~/HeterogenetyAML/ProjectData_CITE/DGE_Ven_Cluster/"
S01_CITE.combined$celltype.condition <- paste(S01_CITE.combined$celltype, S01_CITE.combined$Sample, sep="_")
Idents(S01_CITE.combined) <- "celltype.condition"
for (i in levels(S01_CITE.combined@meta.data$celltype)){ #or however many clusters you have
try({
ident1 <- paste0(i,"_Pat1_R1_Ven_H2-N")
ident2 <- paste0(i,"_Pat1_R1_H1-N")
condition.diffgenes <- FindMarkers(S01_CITE.combined, ident.1 = ident1, ident.2=ident2, min.pct=0.25, logfc.threshold=0.25)
write.csv(condition.diffgenes, file=paste0(output,i,".csv"))
})
}
```

```
Warning in file(file, ifelse(append, "a", "w")): cannot open file '/home/
david.mentrup/HeterogenetyAML/ProjectData_CITE/DGE_Ven_Cluster/Leukemia cells
(diffuse – Lymphocytes/Megakaryocyte patterns).csv': No such file or directory
```

```
Error in file(file, ifelse(append, "a", "w")) :
  cannot open the connection
```

```
Idents(S01_CITE.combined) <- "Sample"
condition.diffgenes <- FindMarkers(S01_CITE.combined, ident.1 = "Pat1_R1_Ven_H2-N", ident.2="Pat1_R1_H1-N", min.pct=0.25, lo
gfc.threshold=0.25)
write.csv(condition.diffgenes, file=paste0(output,"complete",".csv"))
```

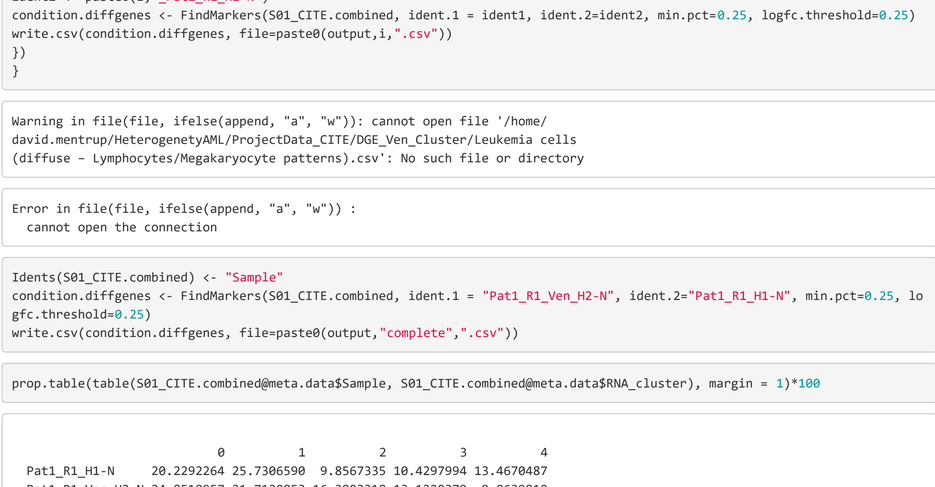
```
prop.table(table(S01_CITE.combined@meta.data$Sample, S01_CITE.combined@meta.data$RNA_cluster), margin = 1)*100
```

```
                          0         1          2         3         4
Pat1_R1_H1-N     20.2292264 25.7306590  9.8567335 10.4297994 13.4670487
Pat1_R1_Ven_H2-N 24.8518957 21.7120853 16.3803318 13.1220379  9.0639810

                          5         6          7         8         9
Pat1_R1_H1-N     10.2578797  6.0171920  2.1203438  1.4326648 0.4584527
Pat1_R1_Ven_H2-N  7.4348341  4.5023697  1.0959716  1.2440758 0.5924171
```

Downregulated in the Ven sample (all cell types, but none specific)

```
VlnPlot(S01_CITE.combined, assay= "RNA",features = c( "FCER1G", "NCF1", "ALOX5AP", "S100A10"), ncol = 2, pt.size = 0.1, grou
p.by = "RNA_cluster", split.by ="Sample", split.plot = TRUE)
```

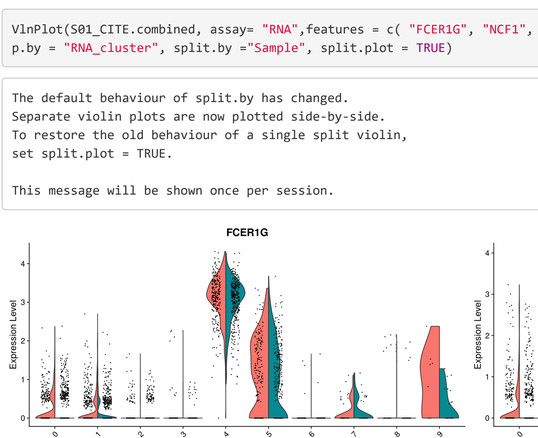
```
The default behaviour of split.by has changed.
Separate violin plots are now plotted side-by-side.
To restore the old behaviour of a single split violin,
set split.plot = TRUE.

This message will be shown once per session.
```



–> All 4 genes are overexpressed in cluster 4 (monocytes) and 5, which are overrepresented in the untreated sample -> DGE is therefore dur to the overrepresentation of those cells

Upregulated in the Ven sample (all cell types)

```
VlnPlot(S01_CITE.combined, assay= "RNA",features = c( "SOX4"), ncol = 2, pt.size = 0.1, group.by = "RNA_cluster", split.by
="Sample", split.plot = TRUE)
```



–> Overreresented in cluster 2 which is dominant in the Venetoclax treated sample (maybe there is a insignificant overexpression in the clusters 0-3 as well, needs to be checked)