Serun singlecell data analysis notebook

Jan 02, 2020

Contents

A repository for keeping conda env and docker image file for running single cell analysis using Seurat

CHAPTER 1

List of example notebooks

1.1 Seurat - Guided Clustering Tutorial of 2,700 PBMCs

This notebook was created using the codes and documentations from the following Seurat tutorial: Seurat - Guided Clustering Tutorial. This notebook provides a basic overview of Seurat including the the following:

- QC and pre-processing
- Dimension reduction
- Clustering
- Differential expression

1.1.1 Downloading data from 10X Genomics

[1]:
```
system("cd /tmp;\
       wget -q http://cf.10xgenomics.com/samples/cell-exp/1.1.0/pbmc3k/pbmc3k_
↪filtered_gene_bc_matrices.tar.gz;\
       tar -xzf pbmc3k_filtered_gene_bc_matrices.tar.gz")
```

1.1.2 Setup the Seurat Object

[2]:
```
library(dplyr)
library(Seurat)
library(patchwork)

# Load the PBMC dataset
pbmc.data <- Read10X(data.dir = "/tmp/filtered_gene_bc_matrices/hg19/")
# Initialize the Seurat object with the raw (non-normalized data).
```

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```
pbmc <- CreateSeuratObject(counts = pbmc.data, project = "pbmc3k", min.cells = 3, min.
↪features = 200)
pbmc
```

```
An object of class Seurat
13714 features across 2700 samples within 1 assay
Active assay: RNA (13714 features, 0 variable features)
```

[3]:
```
# Lets examine a few genes in the first thirty cells
pbmc.data[c("CD3D", "TCL1A", "MS4A1"), 1:30]
```

```
3 x 30 sparse Matrix of class "dgCMatrix"

CD3D  4 . 10 . . 1 2 3 1 . . 2 7 1 . . 1 3 . 2  3 . . . . . 3 4 1 5
TCL1A . .  . . . . . 1 . . . . . . . . . . . .  . 1 . . . . . . .
MS4A1 . 6  . . . . . 1 1 1 . . . . . . . . . . 36 1 2 . . 2 . . . .
```

[4]:
```
dense.size <- object.size(as.matrix(pbmc.data))
dense.size
```

```
709591472 bytes
```

[5]:
```
sparse.size <- object.size(pbmc.data)
sparse.size
```

```
29905192 bytes
```

[6]:
```
dense.size/sparse.size
```

```
23.7 bytes
```

1.1.3 QC and selecting cells for further analysis

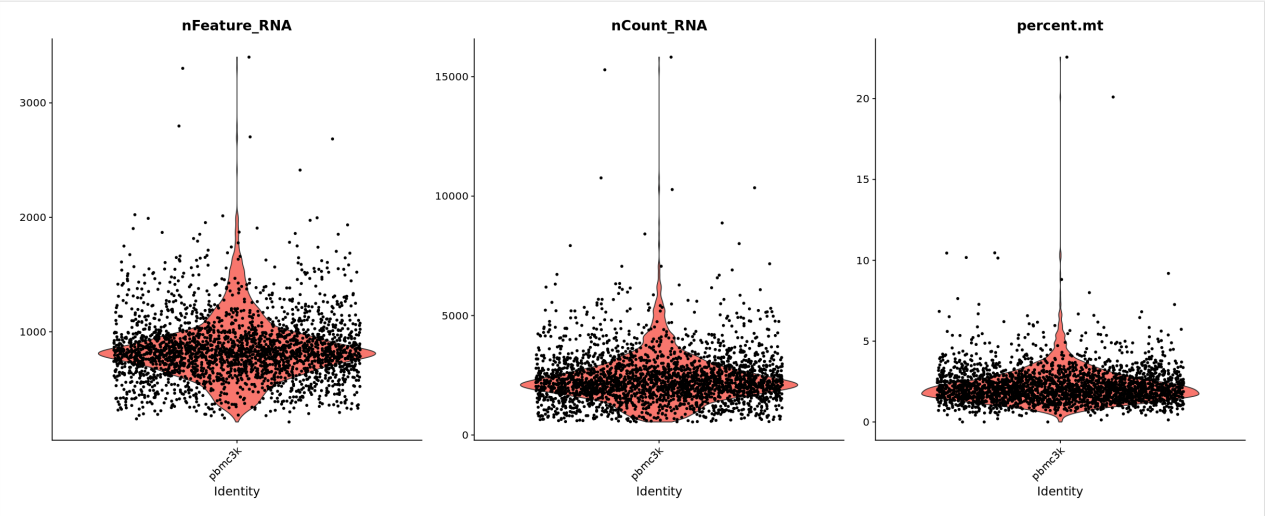
[7]:
```
# The [[ operator can add columns to object metadata. This is a great place to stash␣
↪QC stats
pbmc[["percent.mt"]] <- PercentageFeatureSet(pbmc, pattern = "^MT-")
```

[8]:
```
# Show QC metrics for the first 5 cells
head(pbmc@meta.data, 5)
```

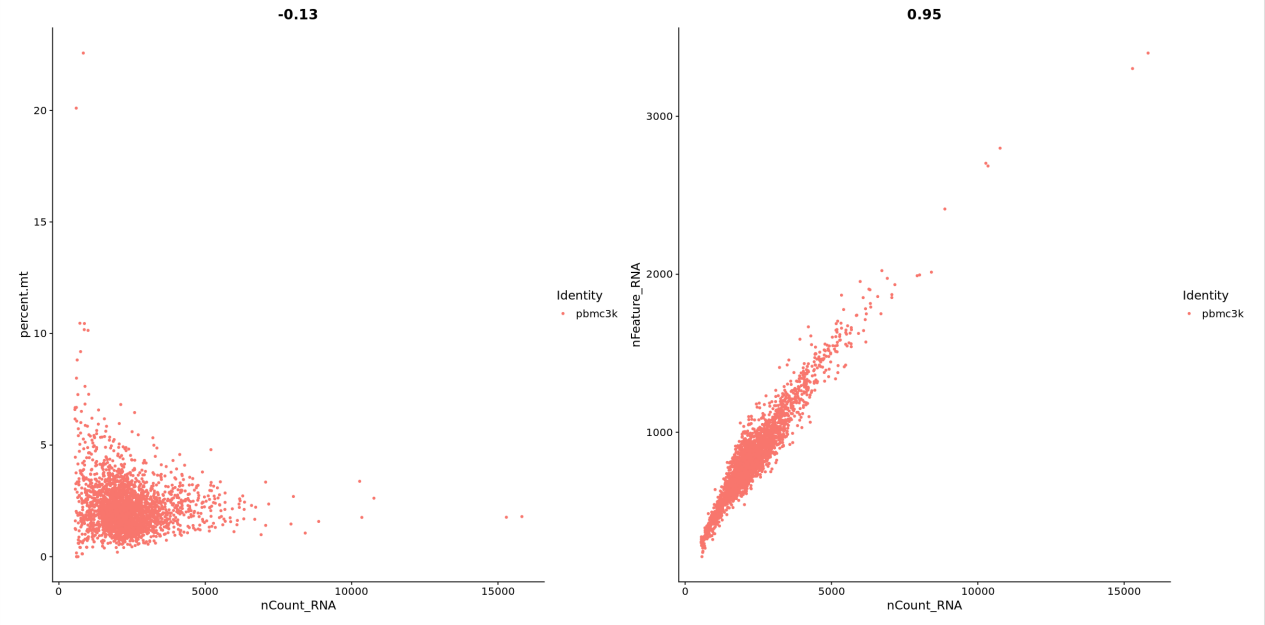
A data.frame: 5 × 4

	orig.ident <fct>	nCount_RNA <dbl>	nFeature_RNA <int>	percent.mt <dbl>
AAACATACAACCAC-1	pbmc3k	2419	779	3.0177759
AAACATTGAGCTAC-1	pbmc3k	4903	1352	3.7935958
AAACATTGATCAGC-1	pbmc3k	3147	1129	0.8897363
AAACCGTGCTTCCG-1	pbmc3k	2639	960	1.7430845
AAACCGTGTATGCG-1	pbmc3k	980	521	1.2244898

[9]:
```
# Visualize QC metrics as a violin plot
options(repr.plot.width=20, repr.plot.height=8)
VlnPlot(pbmc, features = c("nFeature_RNA", "nCount_RNA", "percent.mt"), ncol = 3)
```



[10]:
```
# FeatureScatter is typically used to visualize feature-feature relationships, but␣
↪can be used
# for anything calculated by the object, i.e. columns in object metadata, PC scores␣
↪etc.
options(repr.plot.width=20, repr.plot.height=10)
plot1 <- FeatureScatter(pbmc, feature1 = "nCount_RNA", feature2 = "percent.mt")
plot2 <- FeatureScatter(pbmc, feature1 = "nCount_RNA", feature2 = "nFeature_RNA")
plot1 + plot2
```



[11]:
```
pbmc <- subset(pbmc, subset = nFeature_RNA > 200 & nFeature_RNA < 2500 & percent.mt <␣
↪5)
```

1.1.4 Normalizing the data

[12]:
```
pbmc <- NormalizeData(pbmc, normalization.method = "LogNormalize", scale.factor =␣
↪10000)
```

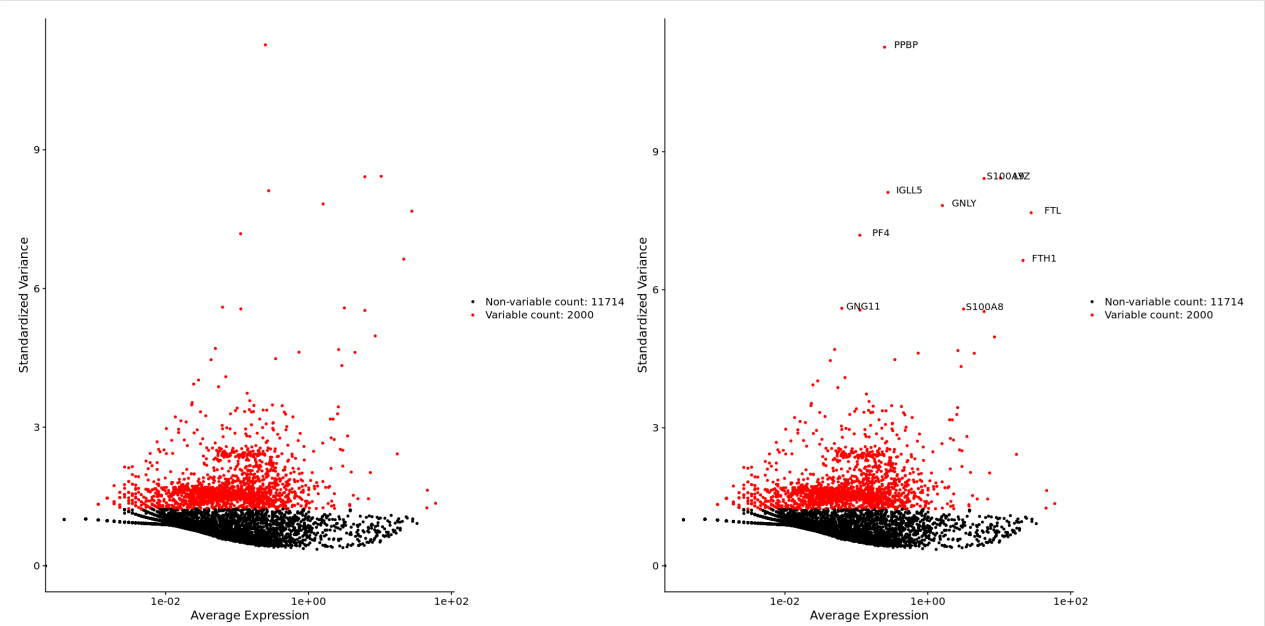
[13]:
```
pbmc <- NormalizeData(pbmc)
```

1.1.5 Identification of highly variable features (feature selection)

[14]:
```
pbmc <- FindVariableFeatures(pbmc, selection.method = "vst", nfeatures = 2000)

# Identify the 10 most highly variable genes
top10 <- head(VariableFeatures(pbmc), 10)

# plot variable features with and without labels
plot1 <- VariableFeaturePlot(pbmc)
plot2 <- LabelPoints(plot = plot1, points = top10, repel = FALSE)
plot1 + plot2
```



1.1.6 Scaling the data

[15]:
```
all.genes <- rownames(pbmc)
pbmc <- ScaleData(pbmc, features = all.genes)
```

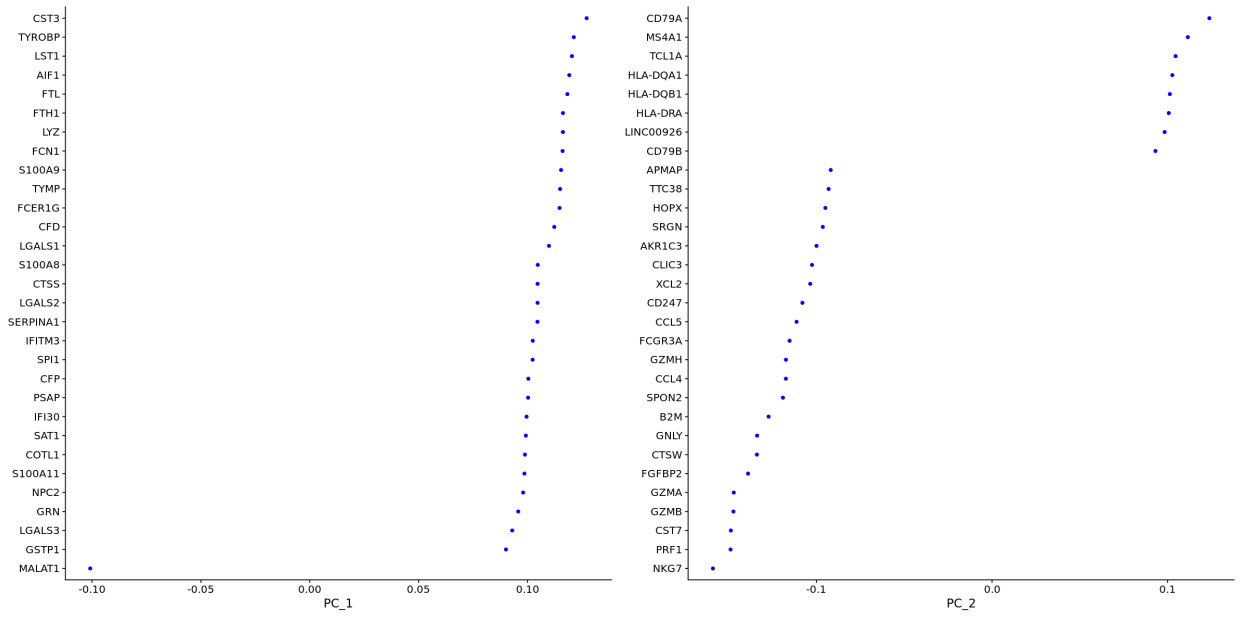
1.1.7 Perform linear dimensional reduction

[16]:
```
pbmc <- RunPCA(pbmc, features = VariableFeatures(object = pbmc))
```

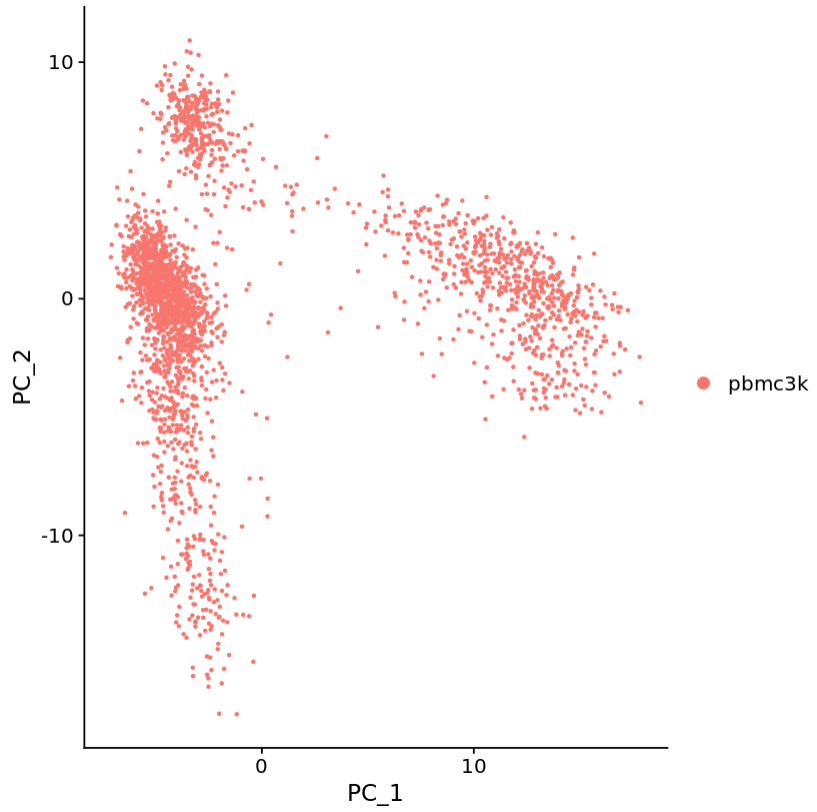
[17]:
```
# Examine and visualize PCA results a few different ways
print(pbmc[["pca"]], dims = 1:5, nfeatures = 5)
```

```
PC_ 1
Positive:  CST3, TYROBP, LST1, AIF1, FTL
Negative:  MALAT1, LTB, IL32, IL7R, CD2
PC_ 2
Positive:  CD79A, MS4A1, TCL1A, HLA-DQA1, HLA-DQB1
Negative:  NKG7, PRF1, CST7, GZMB, GZMA
PC_ 3
Positive:  HLA-DQA1, CD79A, CD79B, HLA-DQB1, HLA-DPB1
Negative:  PPBP, PF4, SDPR, SPARC, GNG11
PC_ 4
Positive:  HLA-DQA1, CD79B, CD79A, MS4A1, HLA-DQB1
Negative:  VIM, IL7R, S100A6, IL32, S100A8
PC_ 5
Positive:  GZMB, NKG7, S100A8, FGFBP2, GNLY
Negative:  LTB, IL7R, CKB, VIM, MS4A7
```

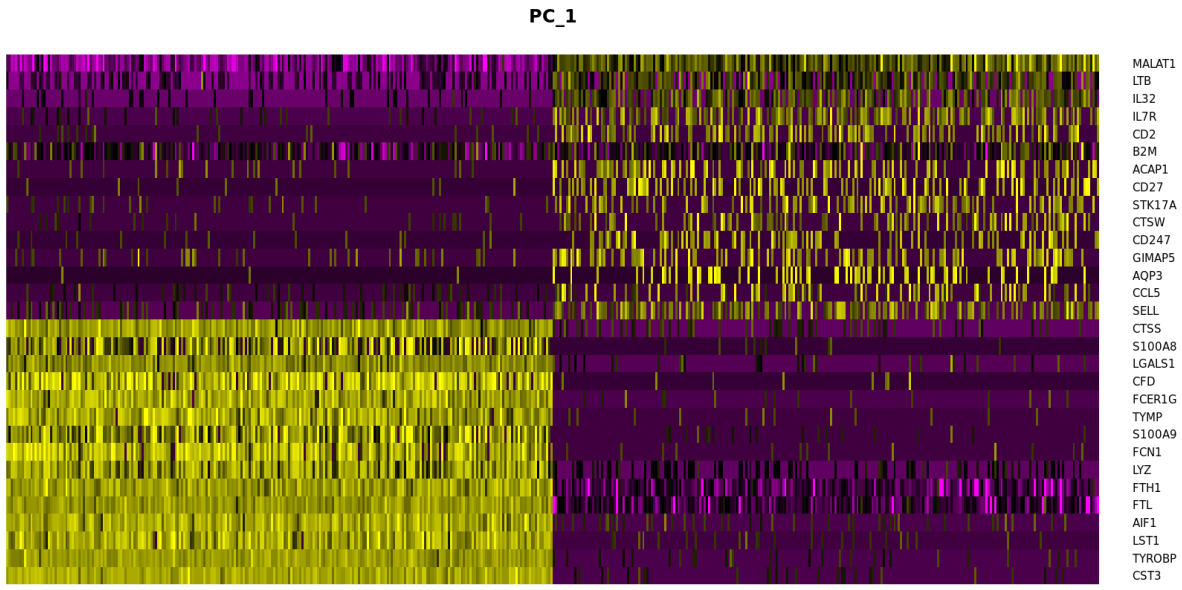
[18]:
```
VizDimLoadings(pbmc, dims = 1:2, reduction = "pca")
```



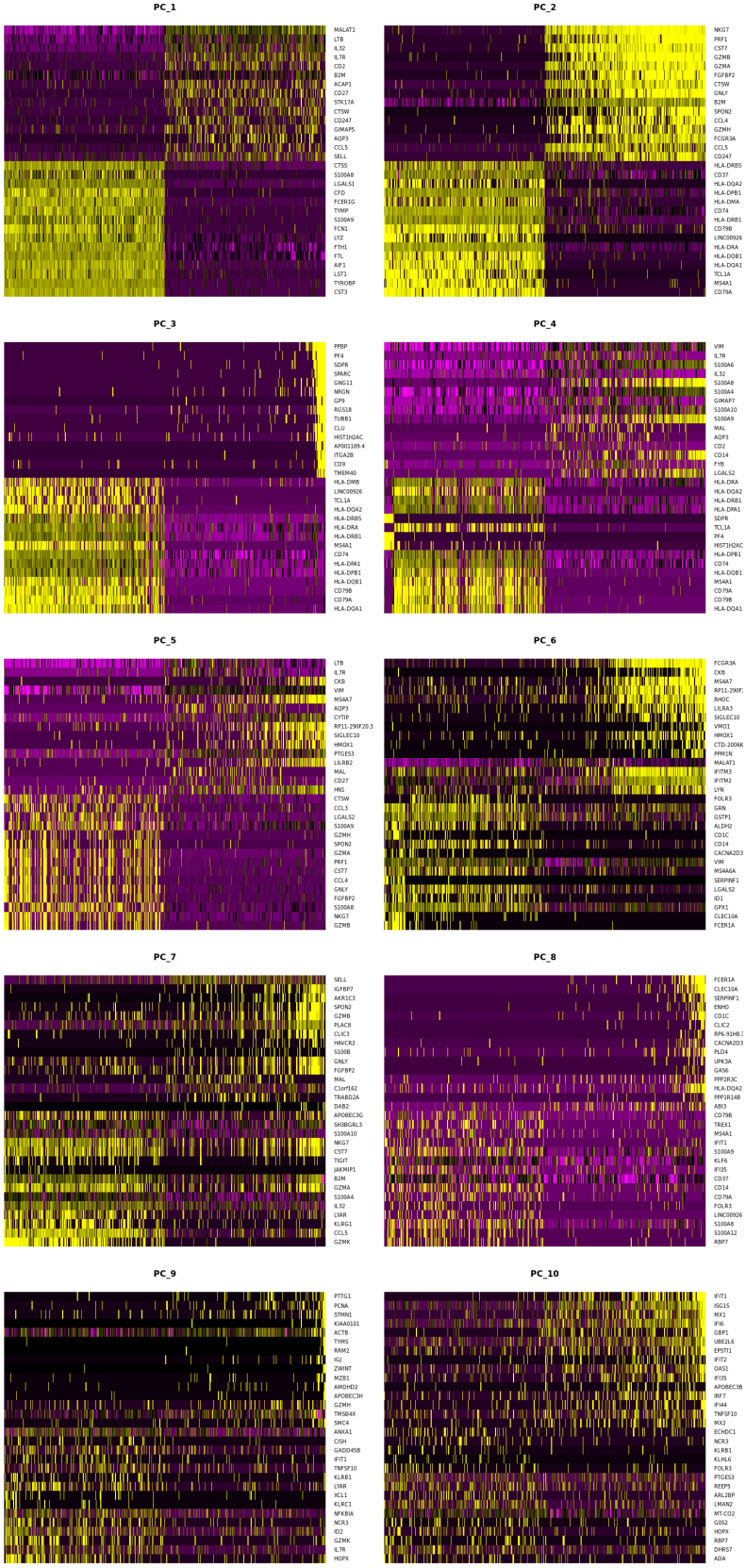
[19]:
```
options(repr.plot.width=7, repr.plot.height=7)
DimPlot(pbmc, reduction = "pca")
```

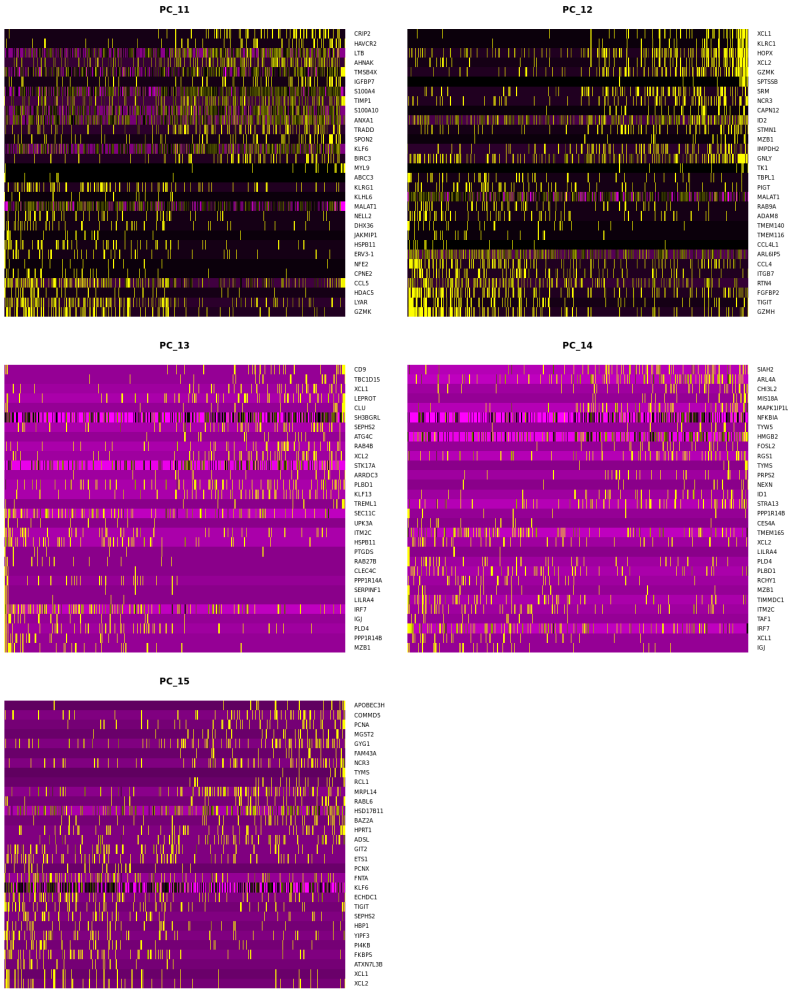


[20]:
```
options(repr.plot.width=14, repr.plot.height=7)
DimHeatmap(pbmc, dims = 1, cells = 500, balanced = TRUE)
```



[21]:
```
options(repr.plot.width=12, repr.plot.height=25)
DimHeatmap(pbmc, dims = 1:15, cells = 500, balanced = TRUE,ncol = 2)
```

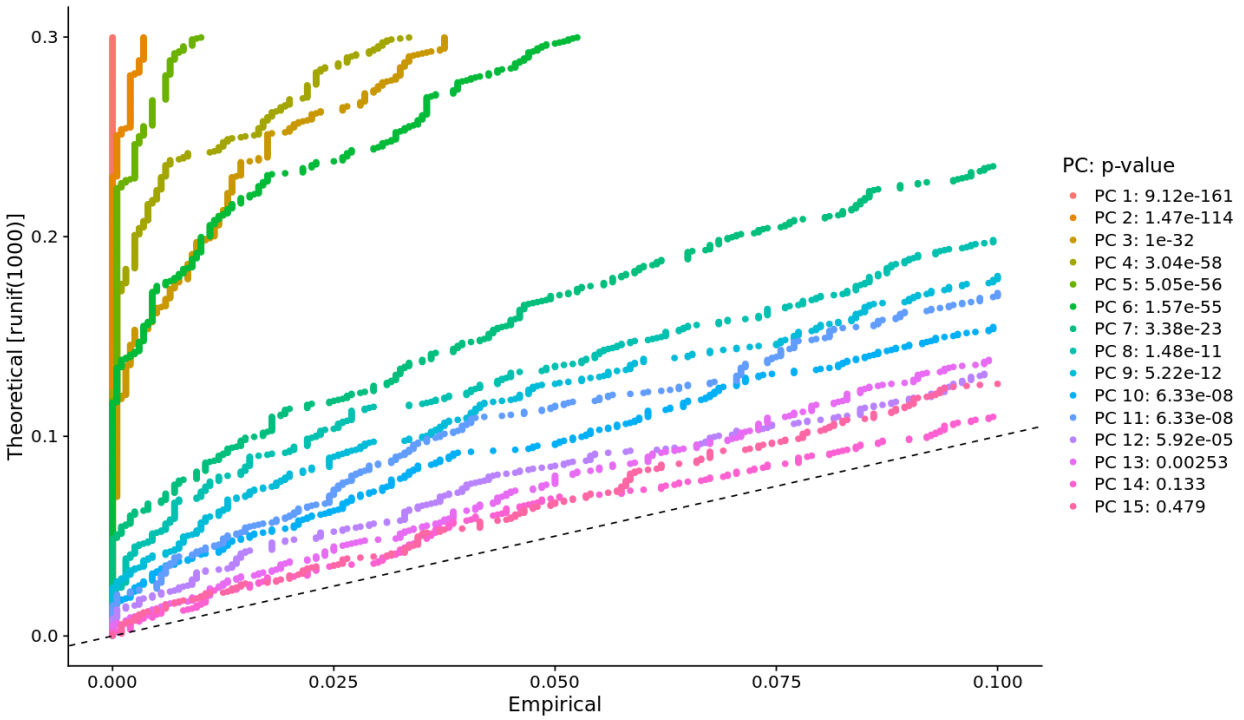

PC_1
MALAT1
LTB
IL32
IL7R
CD2
B2M
ACAP1
CD27
STK17A
CTSW
CD247
GIMAP5
AQP3
CCL5
SELL
CTSS
S100A8
LGALS1
CFD
FCER1G
TYMP
S100A9
FCN1
LYZ
FTH1
FTL
AIF1
LST1
TYROBP
CST3
PC_2
NKG7
PRF1
CST7
GZMB
GZMA
FGFBP2
CTSW
GNLY
B2M
SPON2
CCL4
GZMH
FCGR3A
CCL5
CD247
HLA-DRB5
CD37
HLA-DQA2
HLA-DPB1
HLA-DMA
CD74
HLA-DRB1
CD79B
LINC00926
HLA-DRA
HLA-DQB1
HLA-DQA1
TCL1A
MS4A1
CD79A
PC_3
PPBP
PF4
SDPR
SPARC
GNG11
NRGN
GP9
RGS18
TUBB1
CLU
HIST1H2AC
AP001189.4
ITGA2B
CD9
TMEM40
HLA-DMB
LINC00926
TCL1A
HLA-DQA2
HLA-DRB5
HLA-DRA
HLA-DRB1
MS4A1
CD74
HLA-DPA1
HLA-DPB1
HLA-DQB1
CD79B
CD79A
HLA-DQA1
PC_4
VIM
IL7R
S100A6
IL32
S100A8
S100A4
GIMAP7
S100A10
S100A9
MAL
AQP3
CD2
CD14
FYB
LGALS2
HLA-DRA
HLA-DQA2
HLA-DRB1
HLA-DPA1
SDPR
TCL1A
PF4
HIST1H2AC
HLA-DPB1
CD74
HLA-DQB1
MS4A1
CD79A
CD79B
HLA-DQA1
PC_5
LTB
IL7R
CKB
VIM
MS4A7
AQP3
CYTIP
RP11-290F20.3
SIGLEC10
HMOX1
PTGES3
LILRB2
MAL
CD27
HN1
CTSW
CCL3
LGALS2
S100A9
GZMH
SPON2
GZMA
PRF1
CST7
CCL4
GNLY
FGFBP2
S100A8
NKG7
GZMB
PC_6
FCGR3A
CKB
MS4A7
RP11-290F:
RHOC
LILRA3
SIGLEC10
VMO1
HMOX1
CTD-3006K
PPM1N
MALAT1
IFITM3
IFITM2
LYN
FOLR3
GRN
GSTP1
ALDH2
CD1C
CD14
CACNA2D3
VIM
MS4A6A
SERPINF1
LGALS2
ID1
GPX1
CLEC10A
FCER1A
PC_7
SELL
IGFBP7
AKR1C3
SPON2
GZMB
PLAC8
CLIC3
HAVCR2
S100B
GNLY
FGFBP2
MAL
C1orf162
TRABD2A
DAB2
APOBEC3G
SH3BGRL3
S100A10
NKG7
CST7
TIGIT
JAKMIP1
B2M
GZMA
S100A4
IL32
LYAR
KLRG1
CCL5
GZMK
PC_8
FCER1A
CLEC10A
SERPINF1
ENHO
CD1C
CLIC2
RPL-9348.1
CACNA2D3
PLD4
UPK3A
GAS6
PPP2R3C
HLA-DQA2
PPP1R14B
ABI3
CD79B
TREX1
MS4A1
IFIT1
S100A9
KLF6
IFI35
CD37
CD14
CD79A
FOLR3
LINC00926
S100A8
S100A12
RBP7
PC_9
PTTG1
PCNA
STMN1
KIAA0101
ACTB
TYMS
RRM2
IGJ
ZWINT
MZB1
AMDHD2
APOBEC3H
GZMH
TMSB4X
SMC4
ANXA1
CISH
GADD45B
IFIT1
TNFSF10
KLRB1
LYAR
XCL1
KLRC1
NFKBIA
NCK3
ID2
GZMK
IL7R
HOPX
PC_10
IFIT1
ISG15
MX1
IFI6
GBP1
UBE2L6
EPSTI1
IFIT2
OAS1
IFI35
APOBEC3B
IRF7
IFI44
TNFSF10
MX2
ECHDC1
NCK3
KLRB1
KLHL6
FOLR3
PTGES3
REEP5
ARL3RP
LMAN2
MT-CO2
GIS2
HOPX
RBP7
DHRS7
ADA


PC_11
CRIP2
HAVCR2
LTB
AHNAK
TMSB4X
IGFBP7
S100A4
TIMP1
S100A10
ANXA1
TRADD
SPON2
KLF6
BIRC3
MYL9
ABCC3
KLRG1
KLHL6
MALAT1
NELL2
DHX36
JAKMIP1
HSPB11
ERV3-1
NFE2
CPNE2
CCL5
HDAC5
LYAR
GZMK
PC_12
XCL1
KLRC1
HOPX
XCL2
GZMK
SPTSSB
SRM
NCR3
CAPN12
ID2
STMN1
MZB1
IMPDH2
GNLY
TK1
TBPL1
PIGT
MALAT1
RAB9A
ADAM8
TMEM140
TMEM116
CCL4L1
ARL6IP5
CCL4
ITGB7
RTN4
FGFBP2
TIGIT
GZMH
PC_13
CD9
TBC1D15
XCL1
LEPROT
CLU
SHOBGRL
SEPHS2
ATG4C
RAB4B
XCL2
STK17A
ARRDC3
PLBD1
KLF13
TREML1
SEC11C
UPK3A
ITM2C
HSPB11
PTGDS
RAB27B
CLEC4C
PPP1R14A
SERPINF1
LILRA4
IRF7
IGJ
PLD4
PPP1R14B
MZB1
PC_14
SIAH2
ARL4A
CH3L2
MS3BA
MAPK1IP1L
NFKBIA
TYW5
HMGB2
FOSL2
RGS1
TYMS
PRPS2
NEXN
ID3
STRA13
PPP1R14B
CES4A
TMEM165
XCL2
LILRA4
PLD4
PLBD1
RCHY1
MZB1
TIMMDC1
ITM2C
TAF1
IRF7
XCL1
IGJ
PC_15
APOBEC3H
COMMD5
PCNA
MGST2
GYG1
FAM43A
NCR3
TYMS
RCL1
MRPL14
RABL6
HSD17B11
BAZ2A
HPRT1
ADSL
GIT2
ETS1
PCNX
FNTA
KLF6
ECHDC1
TIGIT
SEPHS2
HBP1
YIPF3
PHKB
FKBP5
ATXN7L3B
XCL1
XCL2

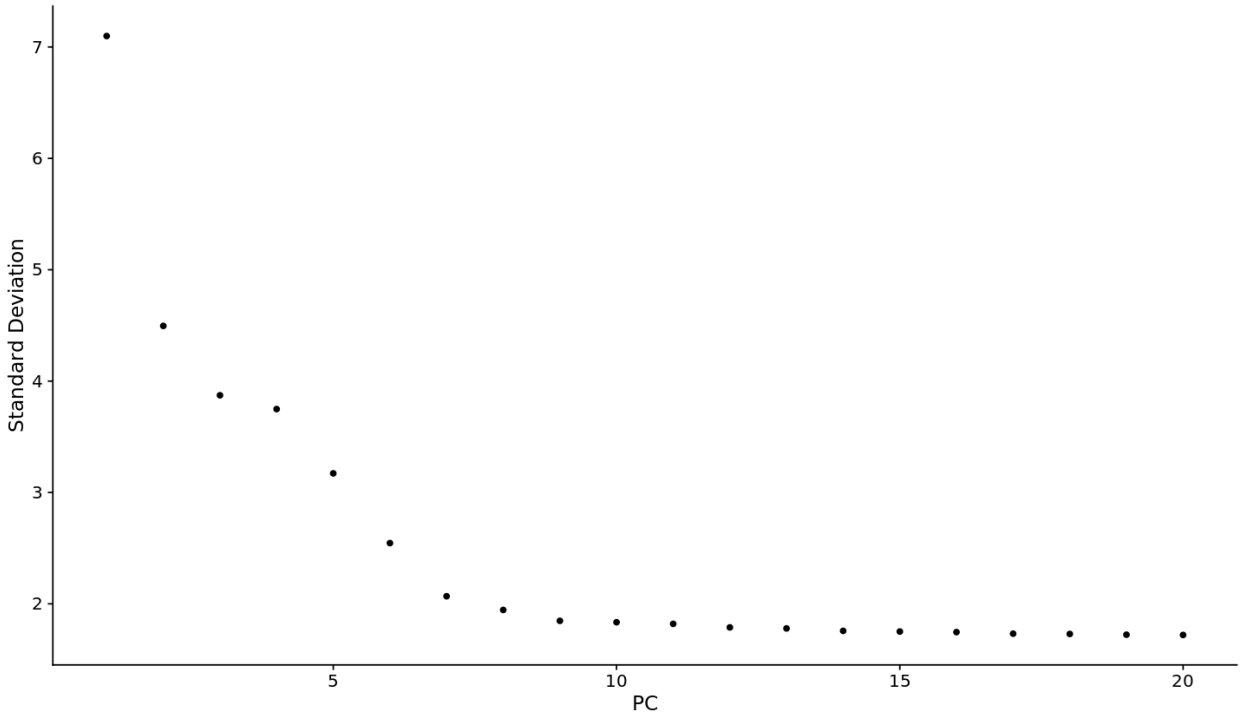
1.1.8 Determine the 'dimensionality' of the dataset

[22]:
```
# NOTE: This process can take a long time for big datasets, comment out for␣
↪expediency. More
# approximate techniques such as those implemented in ElbowPlot() can be used to␣
↪reduce
# computation time
pbmc <- JackStraw(pbmc, num.replicate = 100)
pbmc <- ScoreJackStraw(pbmc, dims = 1:20)
```

[23]:
```
options(repr.plot.width=12, repr.plot.height=7)
JackStrawPlot(pbmc, dims = 1:15)
```



[24]:
```
options(repr.plot.width=12, repr.plot.height=7)
ElbowPlot(pbmc)
```



1.1.9 Cluster the cells

[25]:
```
pbmc <- FindNeighbors(pbmc, dims = 1:10)
pbmc <- FindClusters(pbmc, resolution = 0.5)
```

```
Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck

Number of nodes: 2638
Number of edges: 96033

Running Louvain algorithm...
Maximum modularity in 10 random starts: 0.8720
Number of communities: 9
Elapsed time: 0 seconds
```

[26]:
```
# Look at cluster IDs of the first 5 cells
head(Idents(pbmc), 5)
```

1

3

1

2

6

Levels:

'0'

'1'

(continues on next page)

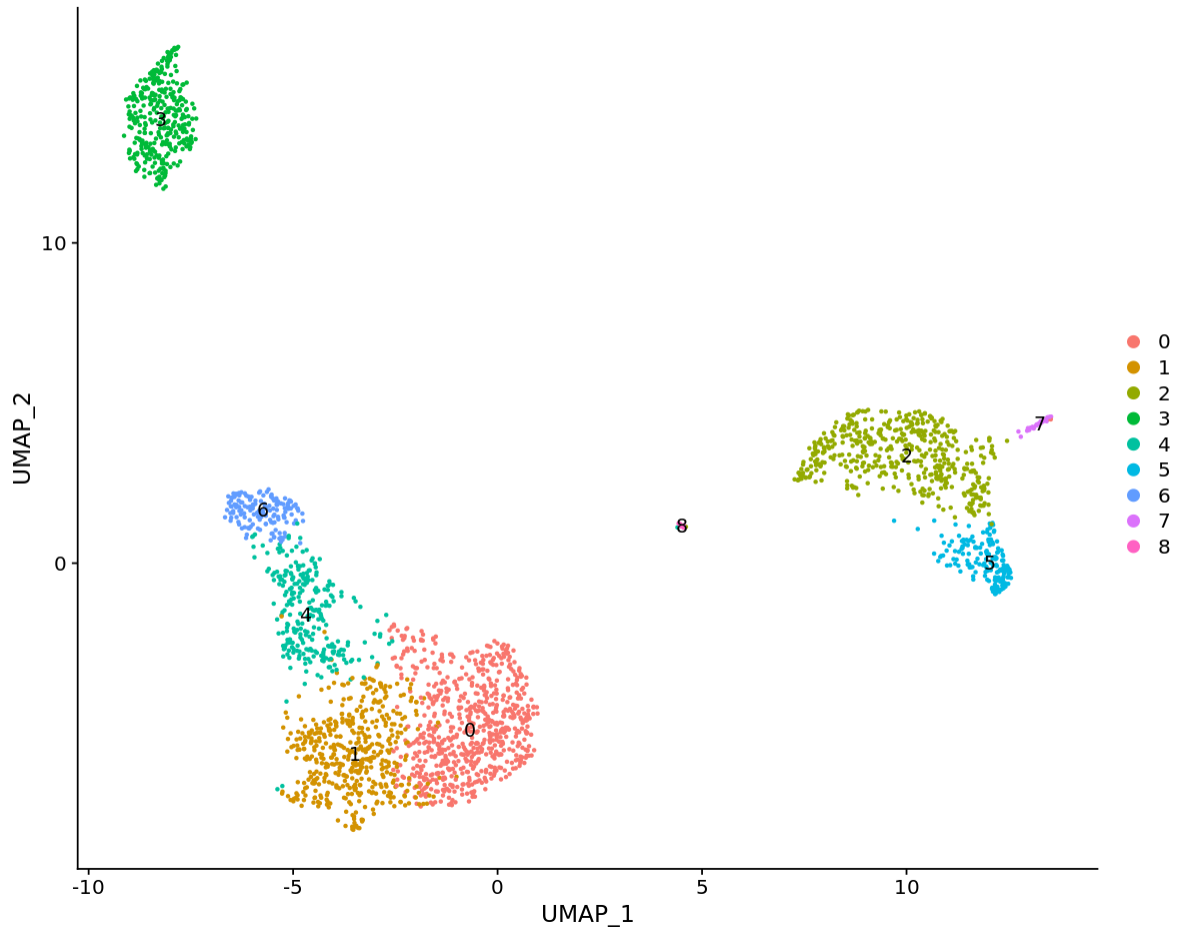
(continued from previous page)

```
'2'
'3'
'4'
'5'
'6'
'7'
'8'
```

1.1.10 Run non-linear dimensional reduction (UMAP/tSNE)

[27]:
```
# If you haven't installed UMAP, you can do so via reticulate::py_install(packages =
# 'umap-learn')
pbmc <- RunUMAP(pbmc, dims = 1:10)
```

[28]:
```
# note that you can set `label = TRUE` or use the LabelClusters function to help label
# individual clusters
options(repr.plot.width=10, repr.plot.height=8)
DimPlot(pbmc, reduction = "umap",label=TRUE)
```



1.1.11 Finding differentially expressed features (cluster biomarkers)

[29]:
```
# find all markers of cluster 1
cluster1.markers <- FindMarkers(pbmc, ident.1 = 1, min.pct = 0.25)
head(cluster1.markers, n = 5)
```

A data.frame: 5 × 5

	p_val <dbl>	avg_logFC <dbl>	pct.1 <dbl>	pct.2 <dbl>	p_val_adj <dbl>
IL32	1.894810e-92	0.8373872	0.948	0.464	2.598542e-88
LTB	7.953303e-89	0.8921170	0.981	0.642	1.090716e-84
CD3D	1.655937e-70	0.6436286	0.919	0.431	2.270951e-66
IL7R	3.688893e-68	0.8147082	0.747	0.325	5.058947e-64
LDHB	2.292819e-67	0.6253110	0.950	0.613	3.144372e-63

[30]:
```
# find all markers distinguishing cluster 5 from clusters 0 and 3
cluster5.markers <- FindMarkers(pbmc, ident.1 = 5, ident.2 = c(0, 3), min.pct = 0.25)
head(cluster5.markers, n = 5)
```

A data.frame: 5 × 5

	p_val <dbl>	avg_logFC <dbl>	pct.1 <dbl>	pct.2 <dbl>	p_val_adj <dbl>
FCGR3A	7.583625e-209	2.963144	0.975	0.037	1.040018e-204
IFITM3	2.500844e-199	2.698187	0.975	0.046	3.429657e-195
CFD	1.763722e-195	2.362381	0.938	0.037	2.418768e-191
CD68	4.612171e-192	2.087366	0.926	0.036	6.325132e-188
RP11-290F20.3	1.846215e-188	1.886288	0.840	0.016	2.531900e-184

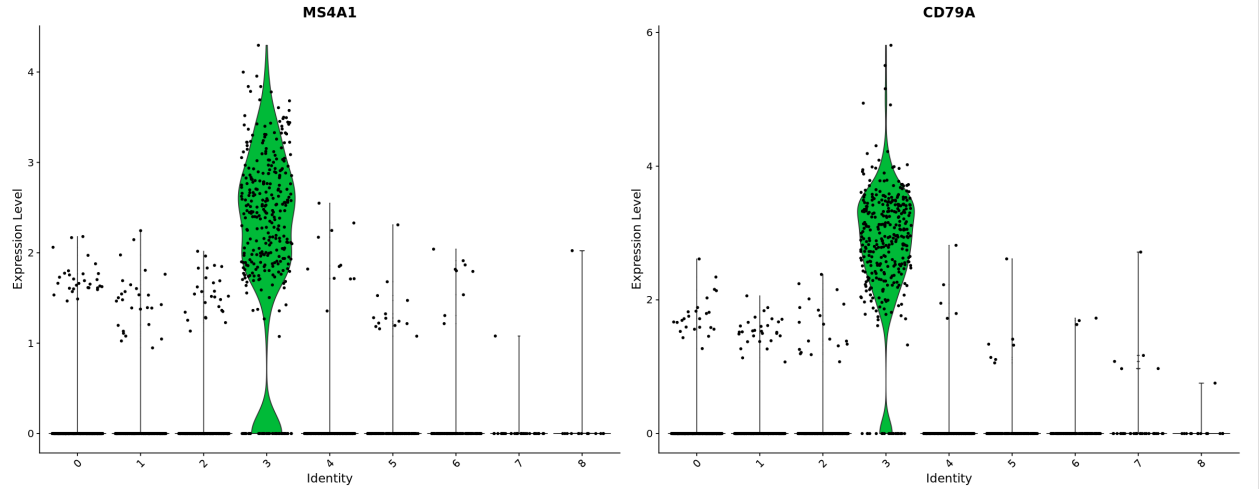
[31]:
```
# find markers for every cluster compared to all remaining cells, report only the␣
↪positive ones
pbmc.markers <- FindAllMarkers(pbmc, only.pos = TRUE, min.pct = 0.25, logfc.threshold␣
↪= 0.25);
pbmc.markers %>% group_by(cluster) %>% top_n(n = 2, wt = avg_logFC);
```

A grouped_df: 18 × 7

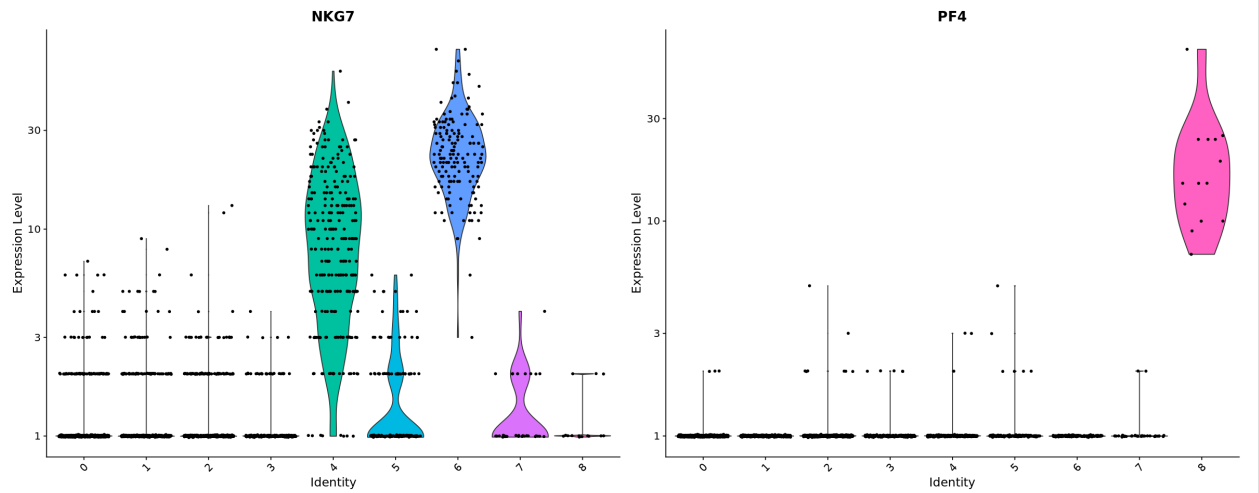
p_val <dbl>	avg_logFC <dbl>	pct.1 <dbl>	pct.2 <dbl>	p_val_adj <dbl>	cluster <fct>	gene <chr>
1.963031e-107	0.7300635	0.901	0.594	2.692101e-103	0	LDHB
1.606796e-82	0.9219135	0.436	0.110	2.203560e-78	0	CCR7
7.953303e-89	0.8921170	0.981	0.642	1.090716e-84	1	LTB
1.851623e-60	0.8586034	0.422	0.110	2.539316e-56	1	AQP3
0.000000e+00	3.8608733	0.996	0.215	0.000000e+00	2	S100A9
0.000000e+00	3.7966403	0.975	0.121	0.000000e+00	2	S100A8
0.000000e+00	2.9875833	0.936	0.041	0.000000e+00	3	CD79A
9.481783e-271	2.4894932	0.622	0.022	1.300332e-266	3	TCL1A
2.958181e-189	2.1220555	0.985	0.240	4.056849e-185	4	CCL5
2.568683e-158	2.0461687	0.587	0.059	3.522691e-154	4	GZMK
3.511192e-184	2.2954931	0.975	0.134	4.815249e-180	5	FCGR3A
2.025672e-125	2.1388125	1.000	0.315	2.778007e-121	5	LST1
7.949981e-269	3.3462278	0.961	0.068	1.090260e-264	6	GZMB
3.132281e-191	3.6898996	0.961	0.131	4.295609e-187	6	GNLY
1.480764e-220	2.6832771	0.812	0.011	2.030720e-216	7	FCER1A
1.665286e-21	1.9924275	1.000	0.513	2.283773e-17	7	HLA-DPB1
7.731180e-200	5.0207262	1.000	0.010	1.060254e-195	8	PF4
3.684548e-110	5.9443347	1.000	0.024	5.052989e-106	8	PPBP

[32]:
```
cluster1.markers <- FindMarkers(pbmc, ident.1 = 0, logfc.threshold = 0.25, test.use =
↪"roc", only.pos = TRUE)
```

[33]:
```
options(repr.plot.width=20, repr.plot.height=8)
VlnPlot(pbmc, features = c("MS4A1", "CD79A"))
```

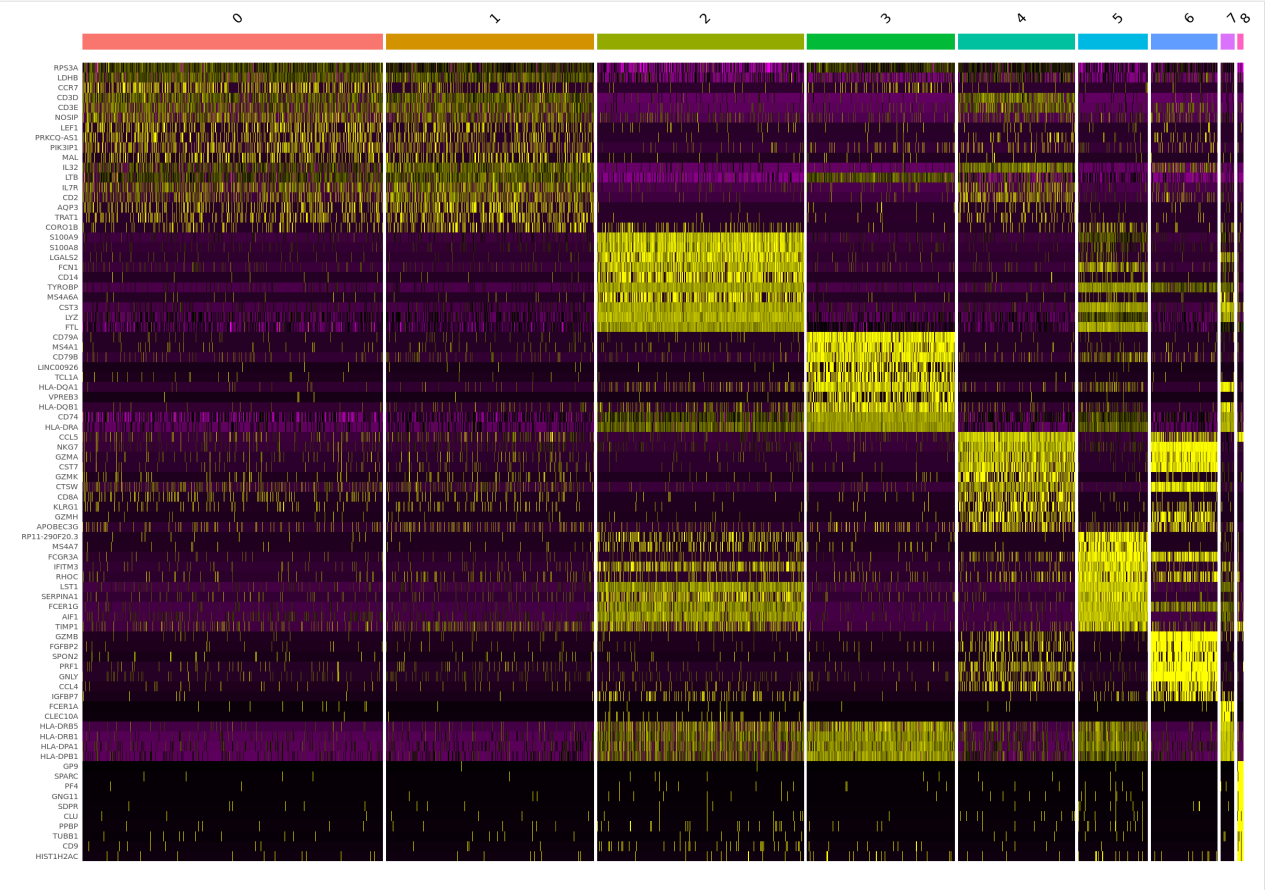


[34]:
```
# you can plot raw counts as well
VlnPlot(pbmc, features = c("NKG7", "PF4"), slot = "counts", log = TRUE)
```



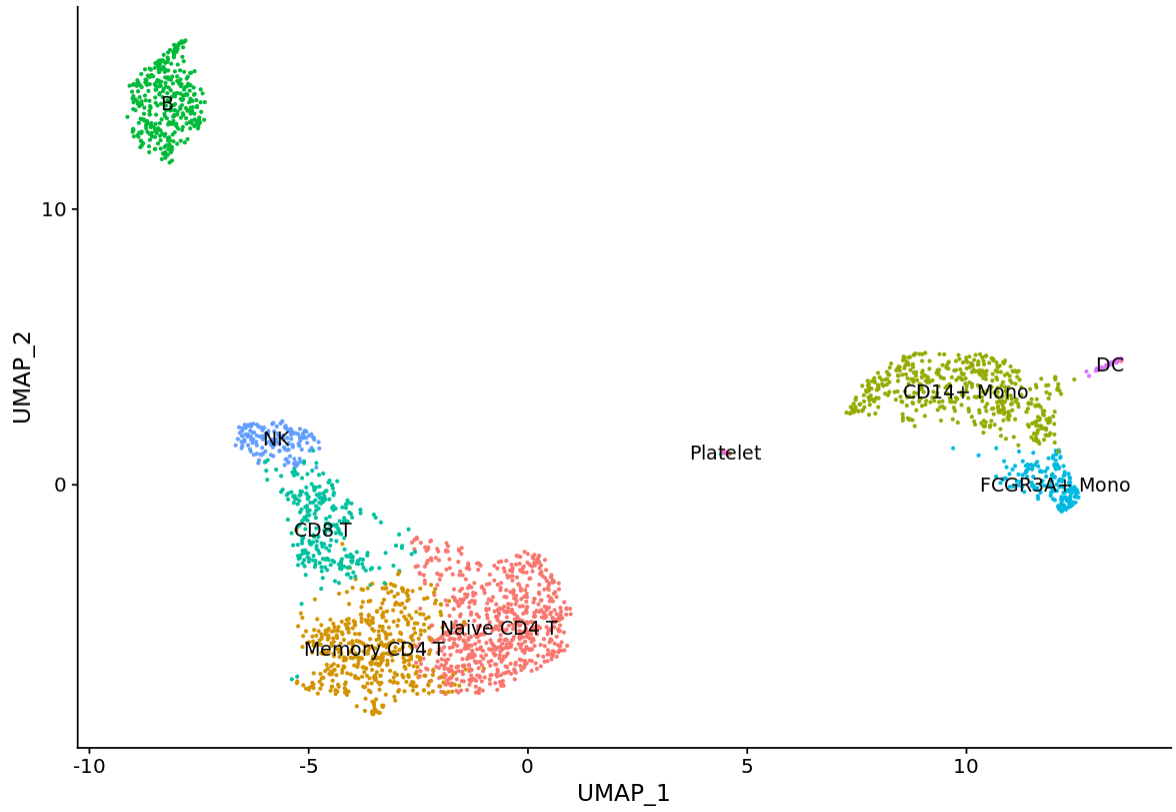
[35]:
```
options(repr.plot.width=20, repr.plot.height=20)
FeaturePlot(pbmc, features = c("MS4A1", "GNLY", "CD3E", "CD14", "FCER1A", "FCGR3A",
↪"LYZ", "PPBP",
    "CD8A"))
```

[36]:
```
options(repr.plot.width=20, repr.plot.height=14)
top10 <- pbmc.markers %>% group_by(cluster) %>% top_n(n = 10, wt = avg_logFC)
DoHeatmap(pbmc, features = top10$gene) + NoLegend()
```



1.1.12 Assigning cell type identity to clusters

[37]:
```
options(repr.plot.width=10, repr.plot.height=7)
new.cluster.ids <- c("Naive CD4 T", "Memory CD4 T", "CD14+ Mono", "B", "CD8 T",
↪"FCGR3A+ Mono",
    "NK", "DC", "Platelet")
names(new.cluster.ids) <- levels(pbmc)
pbmc <- RenameIdents(pbmc, new.cluster.ids)
DimPlot(pbmc, reduction = "umap", label = TRUE, pt.size = 0.5) + NoLegend()
```


B
NK
CD8 T
Naive CD4 T
Memory CD4 T
Platelet
CD14+ Mono
DC
FCGR3A+ Mono
UMAP_2
UMAP_1
10
0
-10
-5
0
5
10

[ ]: