Seurat_Tutorial_1

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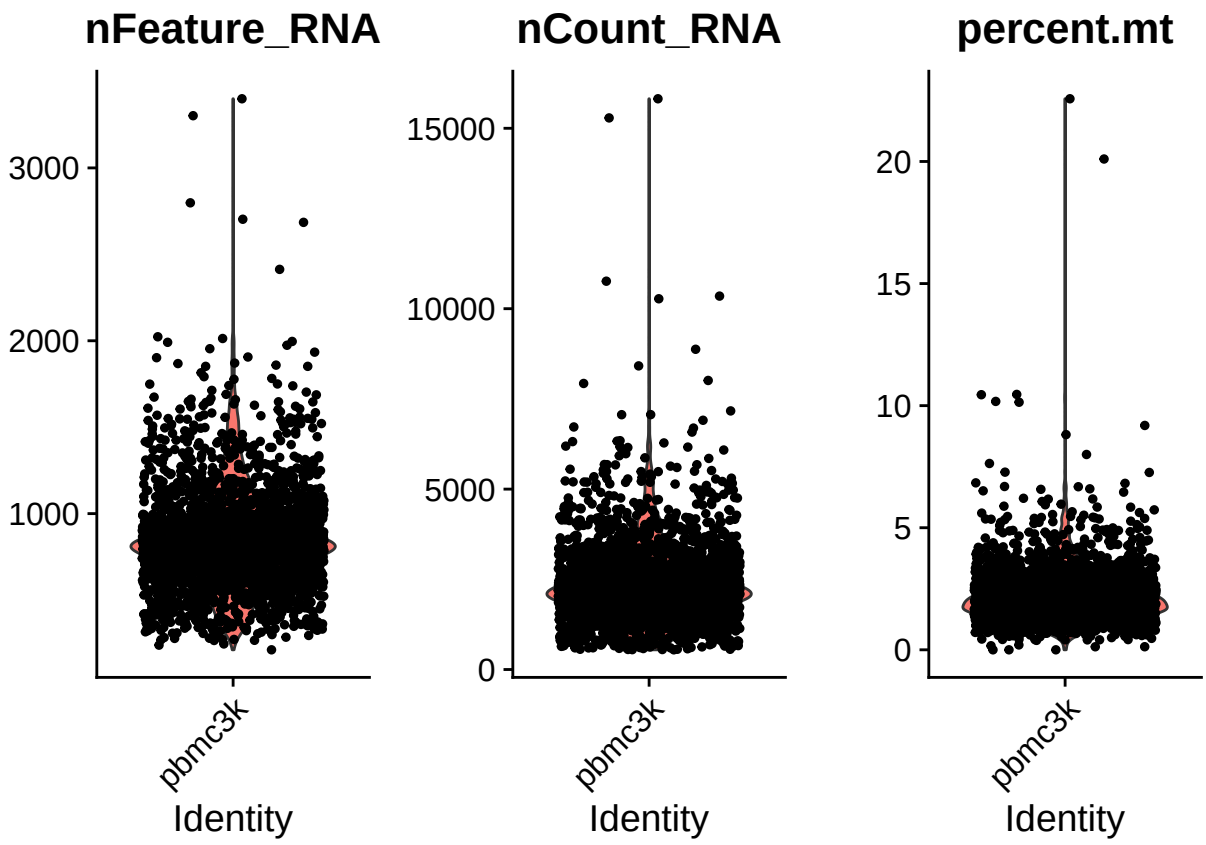
```
library(dplyr)
library(Seurat)

pbmc.data <- Read10X(data.dir = "/Users/siddarth/Desktop/datasets/filtered_gene_bc_matrices/hg19/")
pbmc <- CreateSeuratObject(counts = pbmc.data, project = "pbmc3k", min.cells = 3, min.features = 200)
```

```
## Warning: Feature names cannot have underscores ('_'), replacing with dashes
## ('-')
```

```
pbmc
```

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

```
#QC Visualizations

pbmc[["percent.mt"]] <- PercentageFeatureSet(pbmc, pattern = "^MT-")
VlnPlot(pbmc, features = c("nFeature_RNA", "nCount_RNA", "percent.mt"), ncol = 3)
```




```
#Subset
pbmc <- subset(pbmc, subset = nFeature_RNA > 200 & nFeature_RNA < 2500 & percent.mt < 5)

#Normalize by total expression, multiply by 10,000 and log transform
pbmc <- NormalizeData(pbmc, normalization.method = "LogNormalize", scale.factor = 10000)

#Following is normalization with default parameters
#pbmc <- NormalizeData(pbmc)
```

```
#Feature Selection
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 = TRUE)
```

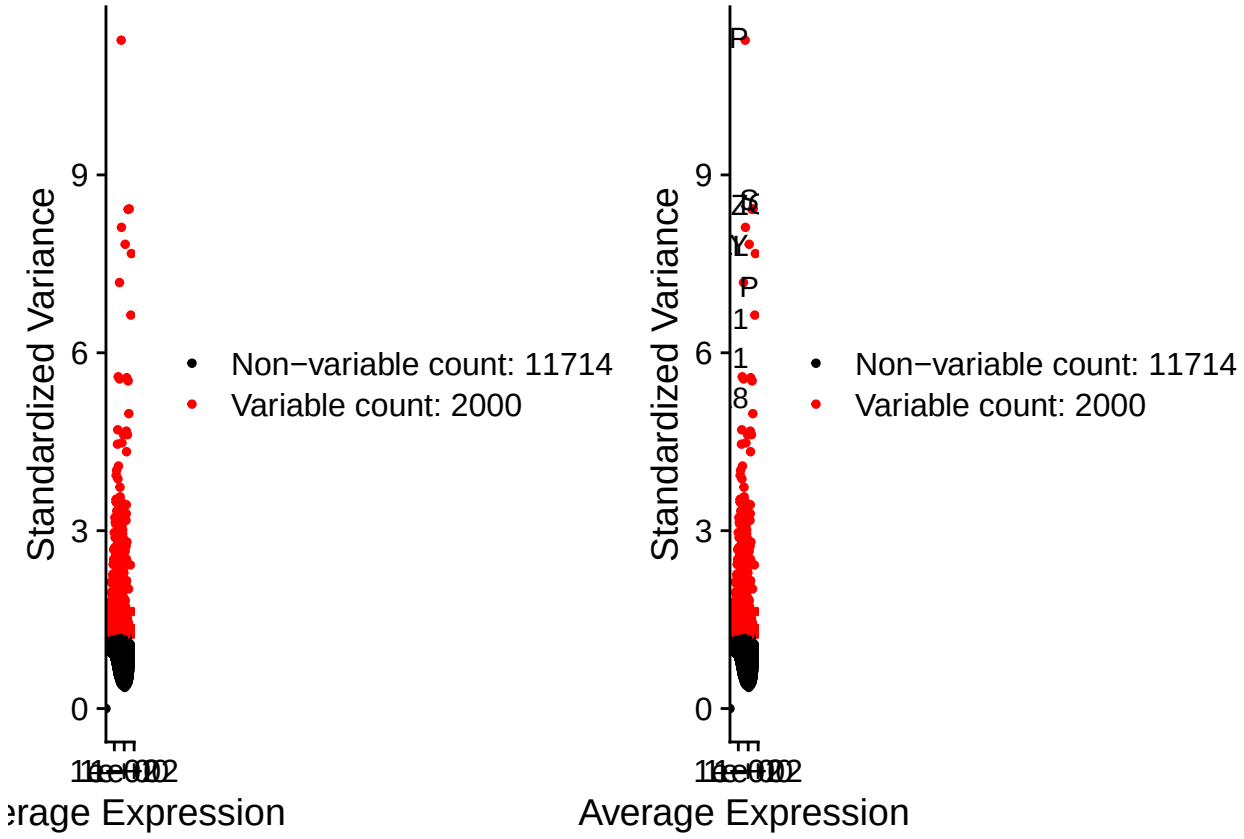
```
## Warning: Using `as.character()` on a quosure is deprecated as of rlang 0.3.0.
## Please use `as_label()` or `as_name()` instead.
## This warning is displayed once per session.
```

```
## When using repel, set xnudge and ynudge to 0 for optimal results
```

```
CombinePlots(plots = list(plot1, plot2))
```

```
## Warning: Transformation introduced infinite values in continuous x-axis
```

```
## Warning: Transformation introduced infinite values in continuous x-axis
```




```
#Scaling data
all.genes <- rownames(pbmc)
pbmc <- ScaleData(pbmc, features = all.genes)
```

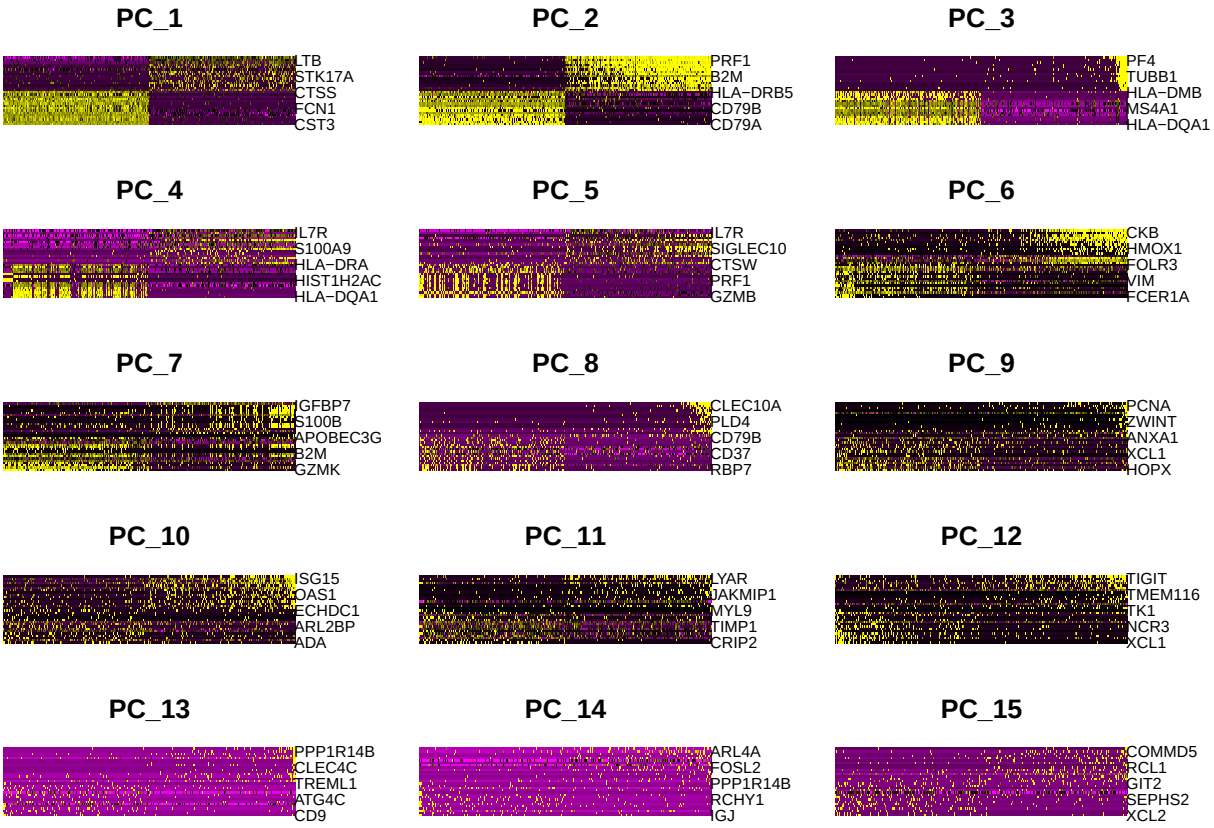
```
## Centering and scaling data matrix
```

```
pbmc <- RunPCA(pbmc, features = VariableFeatures(object = pbmc))
```

```
## PC_ 1
## Positive:  CST3, TYROBP, LST1, AIF1, FTL, FTH1, LYZ, FCN1, S100A9, TYMP
##     FCER1G, CFD, LGALS1, S100A8, CTSS, LGALS2, SERPINA1, IFITM3, SPI1, CFP
##     PSAP, IFI30, SAT1, COTL1, S100A11, NPC2, GRN, LGALS3, GSTP1, PYCARD
## Negative:  MALAT1, LTB, IL32, IL7R, CD2, B2M, ACAP1, CD27, STK17A, CTSW
##     CD247, GIMAP5, AQP3, CCL5, SELL, TRAF3IP3, GZMA, MAL, CST7, ITM2A
##     MYC, GIMAP7, HOPX, BEX2, LDLRAP1, GZMK, ETS1, ZAP70, TNFAIP8, RIC3
## PC_ 2
## Positive:  CD79A, MS4A1, TCL1A, HLA-DQA1, HLA-DQB1, HLA-DRA, LINC00926, CD79B, HLA-DRB1, CD74
```

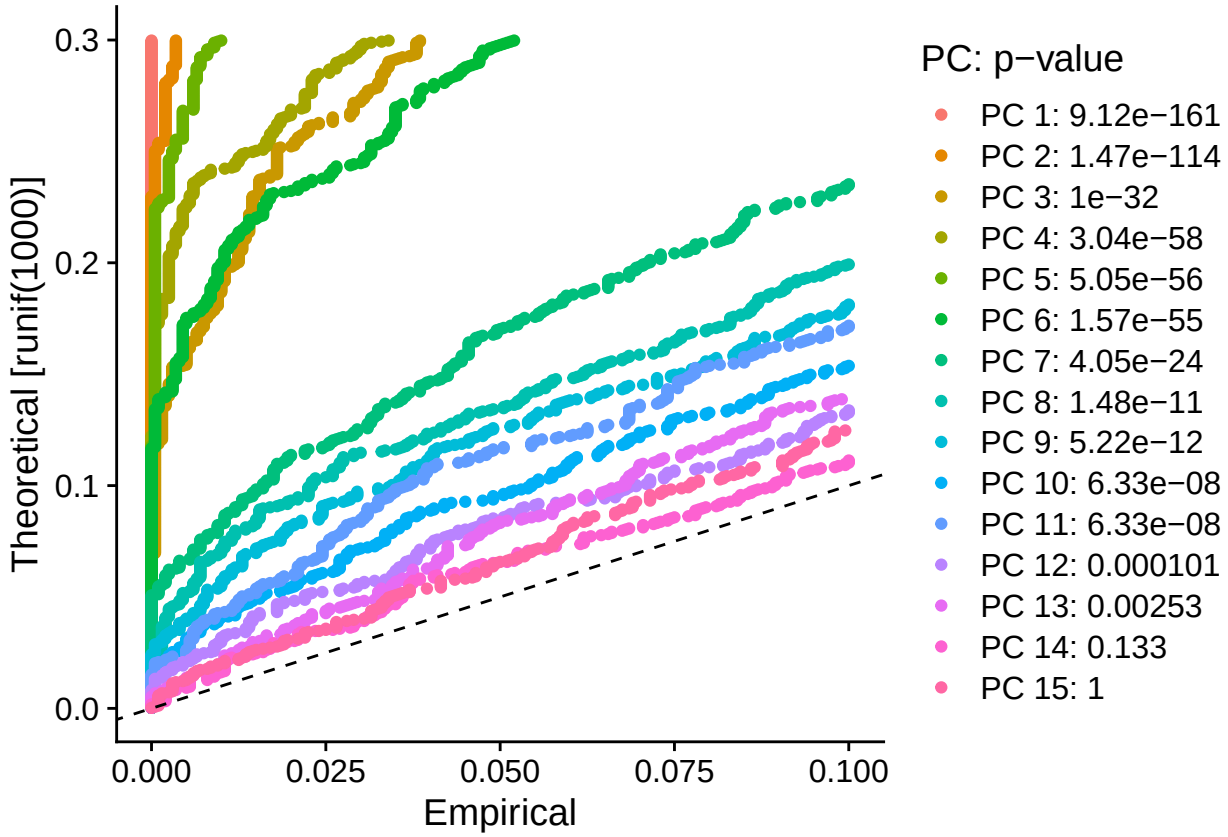
```
##     HLA-DMA, HLA-DPB1, HLA-DQA2, CD37, HLA-DRB5, HLA-DMB, HLA-DPA1, FCRLA, HVCN1, LTB
##     BLNK, P2RX5, IGLL5, IRF8, SWAP70, ARHGAP24, FCGR2B, SMIM14, PPP1R14A, C16orf74
## Negative:  NKG7, PRF1, CST7, GZMB, GZMA, FGFBP2, CTSW, GNLY, B2M, SPON2
##     CCL4, GZMH, FCGR3A, CCL5, CD247, XCL2, CLIC3, AKR1C3, SRGN, HOPX
##     TTC38, APMAP, CTSC, S100A4, IGFBP7, ANXA1, ID2, IL32, XCL1, RHOC
## PC_ 3
## Positive:  HLA-DQA1, CD79A, CD79B, HLA-DQB1, HLA-DPB1, HLA-DPA1, CD74, MS4A1, HLA-DRB1, HLA-DRA
##     HLA-DRB5, HLA-DQA2, TCL1A, LINC00926, HLA-DMB, HLA-DMA, CD37, HVCN1, FCRLA, IRF8
##     PLAC8, BLNK, MALAT1, SMIM14, PLD4, LAT2, IGLL5, P2RX5, SWAP70, FCGR2B
## Negative:  PPBP, PF4, SDPR, SPARC, GNG11, NRGN, GP9, RGS18, TUBB1, CLU
##     HIST1H2AC, AP001189.4, ITGA2B, CD9, TMEM40, PTCRA, CA2, ACRBP, MMD, TREML1
##     NGFRAP1, F13A1, SEPT5, RUFY1, TSC22D1, MPP1, CMTM5, RP11-367G6.3, MYL9, GP1BA
## PC_ 4
## Positive:  HLA-DQA1, CD79B, CD79A, MS4A1, HLA-DQB1, CD74, HLA-DPB1, HIST1H2AC, PF4, TCL1A
##     SDPR, HLA-DPA1, HLA-DRB1, HLA-DQA2, HLA-DRA, PPBP, LINC00926, GNG11, HLA-DRB5, SPARC
##     GP9, AP001189.4, CA2, PTCRA, CD9, NRGN, RGS18, GZMB, CLU, TUBB1
## Negative:  VIM, IL7R, S100A6, IL32, S100A8, S100A4, GIMAP7, S100A10, S100A9, MAL
##     AQP3, CD2, CD14, FYB, LGALS2, GIMAP4, ANXA1, CD27, FCN1, RBP7
##     LYZ, S100A11, GIMAP5, MS4A6A, S100A12, FOLR3, TRABD2A, AIF1, IL8, IFI6
## PC_ 5
## Positive:  GZMB, NKG7, S100A8, FGFBP2, GNLY, CCL4, CST7, PRF1, GZMA, SPON2
##     GZMH, S100A9, LGALS2, CCL3, CTSW, XCL2, CD14, CLIC3, S100A12, CCL5
##     RBP7, MS4A6A, GSTP1, FOLR3, IGFBP7, TYROBP, TTC38, AKR1C3, XCL1, HOPX
## Negative:  LTB, IL7R, CKB, VIM, MS4A7, AQP3, CYTIP, RP11-290F20.3, SIGLEC10, HMOX1
##     PTGES3, LILRB2, MAL, CD27, HN1, CD2, GDI2, ANXA5, CORO1B, TUBA1B
##     FAM110A, ATP1A1, TRADD, PPA1, CCDC109B, ABRACL, CTD-2006K23.1, WARS, VMO1, FYB
```

```
DimHeatmap(pbmc, dims = 1:15, cells = 500, balanced = TRUE)
```




```
#Takes a long time - can be used to find significant PCs for UMAP. Can be sort of determined from heatm
pbmc <- JackStraw(pbmc, num.replicate = 100)
pbmc <- ScoreJackStraw(pbmc, dims = 1:20)
JackStrawPlot(pbmc, dims = 1:15)
```

```
## Warning: Removed 23496 rows containing missing values (geom_point).
```




```
pbmc <- FindNeighbors(pbmc, dims = 1:10)
```

```
## Computing nearest neighbor graph
```

```
## Computing SNN
```

```
pbmc <- FindClusters(pbmc, resolution = 0.5)
```

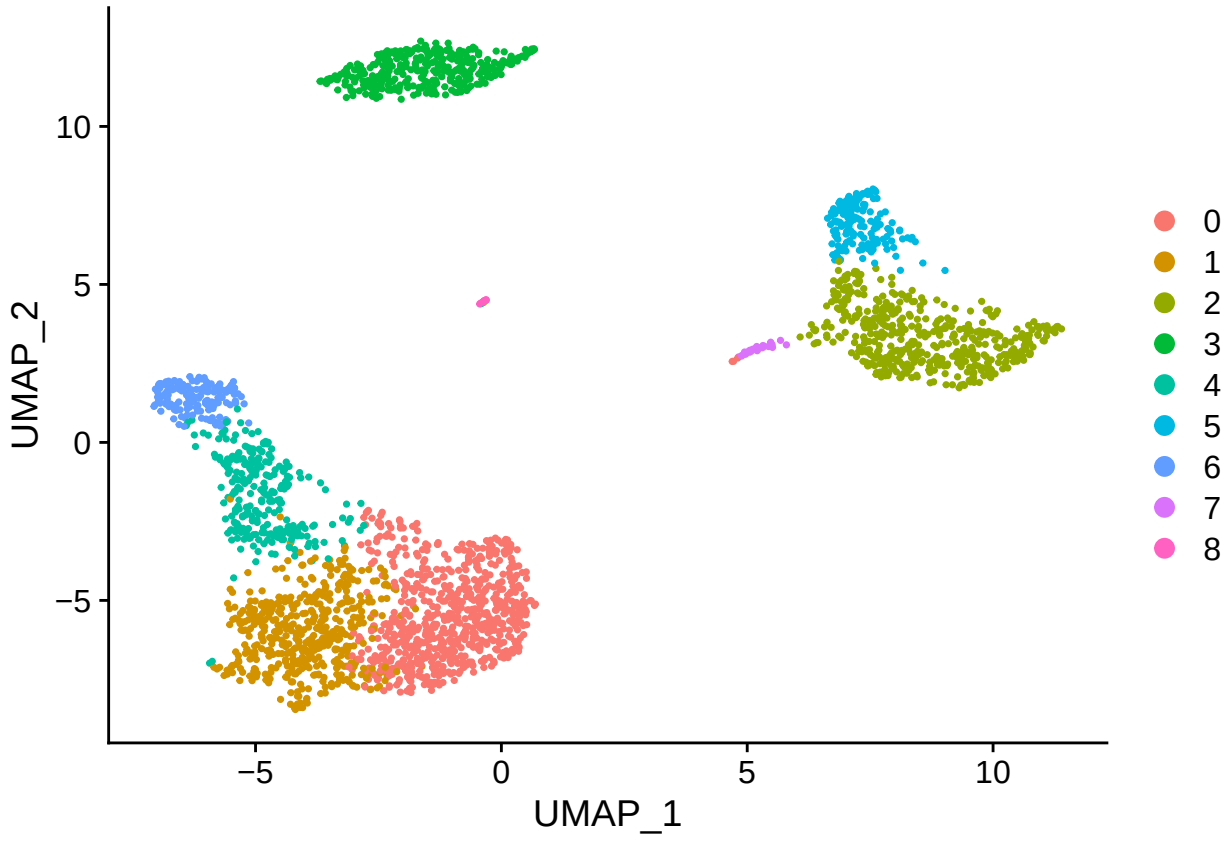
```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 2638
## Number of edges: 96033
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8720
## Number of communities: 9
## Elapsed time: 0 seconds
```

```
pbmc <- RunUMAP(pbmc, dims = 1:10)
```

```
## Warning: The default method for RunUMAP has changed from calling Python UMAP via reticulate to the R-
## To use Python UMAP via reticulate, set umap.method to 'umap-learn' and metric to 'correlation'
## This message will be shown once per session
```

```
## 18:02:06 UMAP embedding parameters a = 0.9922 b = 1.112

## 18:02:06 Read 2638 rows and found 10 numeric columns

## 18:02:06 Using Annoy for neighbor search, n_neighbors = 30

## 18:02:06 Building Annoy index with metric = cosine, n_trees = 50

## 0%   10   20   30   40   50   60   70   80   90   100%

## [----|----|----|----|----|----|----|----|----|----|

## **************************************************|
## 18:02:06 Writing NN index file to temp file /var/folders/h0/2bsm21213w587mz_25h_0kb00000gn/T//RtmpseI
## 18:02:06 Searching Annoy index using 1 thread, search_k = 3000
## 18:02:07 Annoy recall = 100%
## 18:02:07 Commencing smooth kNN distance calibration using 1 thread
## 18:02:08 Initializing from normalized Laplacian + noise
## 18:02:08 Commencing optimization for 500 epochs, with 105132 positive edges
## 18:02:14 Optimization finished
```

```r
# note that you can set `label = TRUE` or use the LabelClusters function to help label
# individual clusters
DimPlot(pbmc, reduction = "umap")
```



```
#Or even simpler, with Seurat v3, use scTransform function, which normalizes, scales (shifts) and featu

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pbmc
```

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## An object of class Seurat
## 13714 features across 2700 samples within 1 assay
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```
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VlnPlot(pbmc, features = c("nFeature_RNA", "nCount_RNA", "percent.mt"), ncol = 3)
```
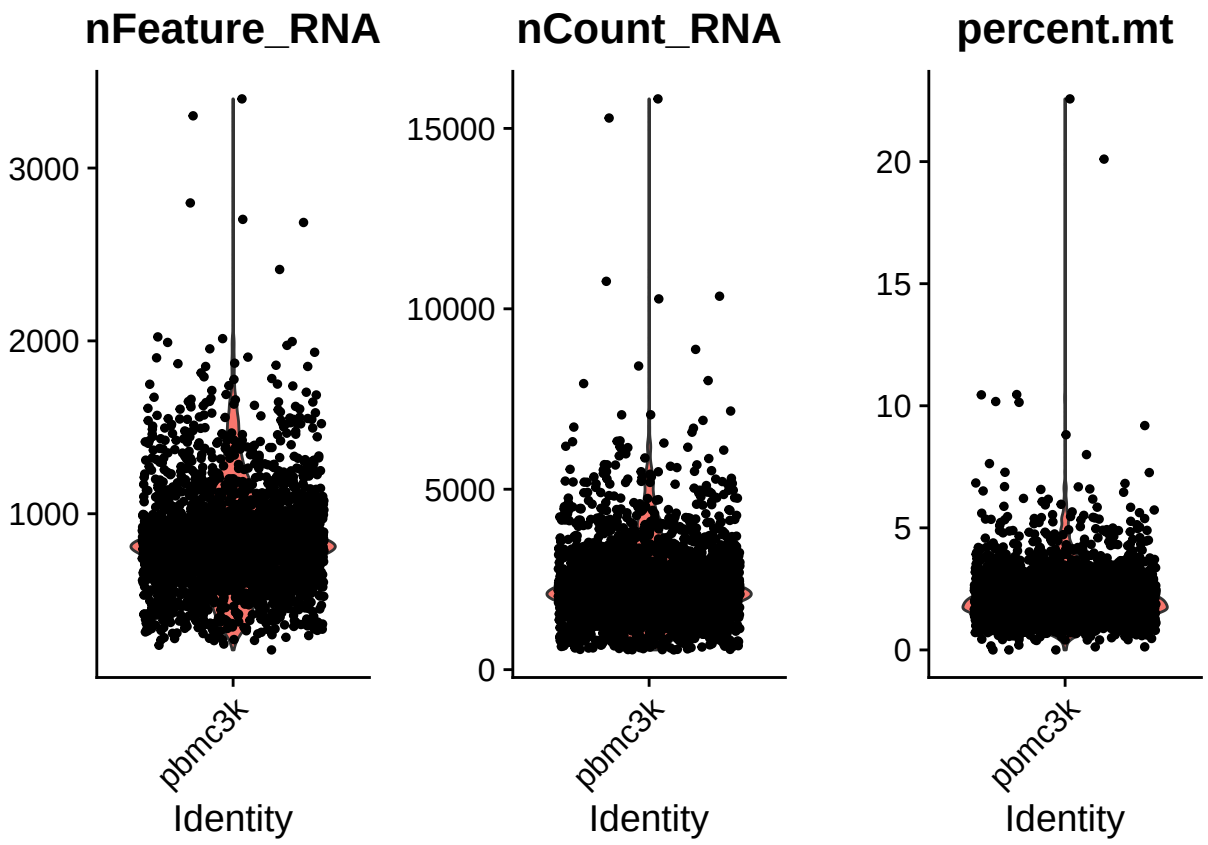
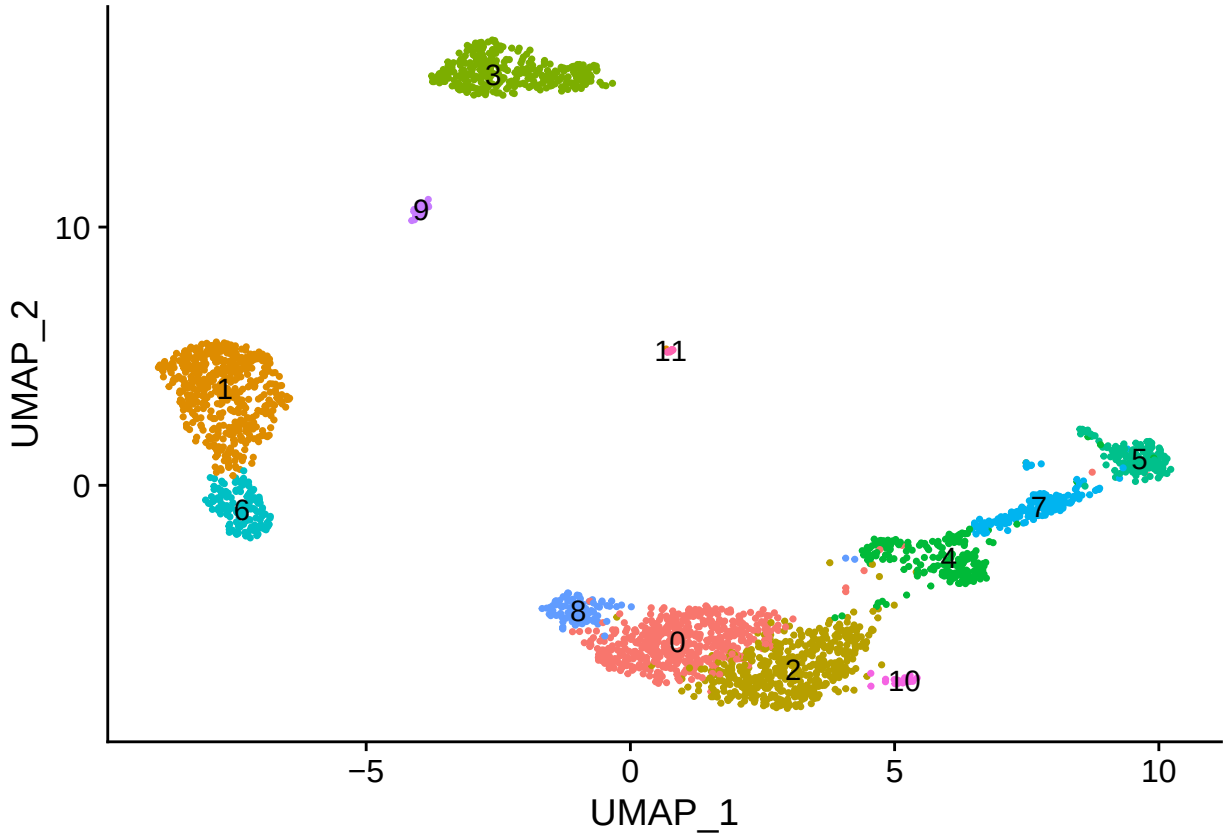




```
pbmc <- SCTransform(pbmc, vars.to.regress = "percent.mt", verbose = FALSE)

pbmc <- RunPCA(pbmc, verbose = FALSE)
pbmc <- RunUMAP(pbmc, dims = 1:30, verbose = FALSE)

pbmc <- FindNeighbors(pbmc, dims = 1:30, verbose = FALSE)
pbmc <- FindClusters(pbmc, verbose = FALSE)
DimPlot(pbmc, label = TRUE) + NoLegend()
```


UMAP_2
10
0
UMAP_1
−5
0
5
10
3
9
11
1
6
5
7
4
8
0
2
10