Project-1(BRC)

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Note that the `echo = FALSE` parameter was added to the code chunk to prevent printing of the R code that generated the plot.

```
#Setting the working directory
setwd("~/Desktop/seurat-learning")

#Loading the necessary Libraries
#install.packages("tinytex")
#tinytex::install_tinytex()
library(harmony)
```

```
## Loading required package: Rcpp
```

```
library(ggplot2)
library(Seurat)
```

```
## Loading required package: SeuratObject
```

```
## Loading required package: sp
```

```
## 'SeuratObject' was built under R 4.4.0 but the current version is
## 4.4.2; it is recomended that you reinstall 'SeuratObject' as the ABI
## for R may have changed
```

```
## 'SeuratObject' was built with package 'Matrix' 1.7.0 but the current
## version is 1.7.2; it is recomended that you reinstall 'SeuratObject' as
## the ABI for 'Matrix' may have changed
```

```
##
## Attaching package: 'SeuratObject'
```

```
## The following objects are masked from 'package:base':
##
##     intersect, t
```

```
library(SeuratObject)
library(patchwork)
```

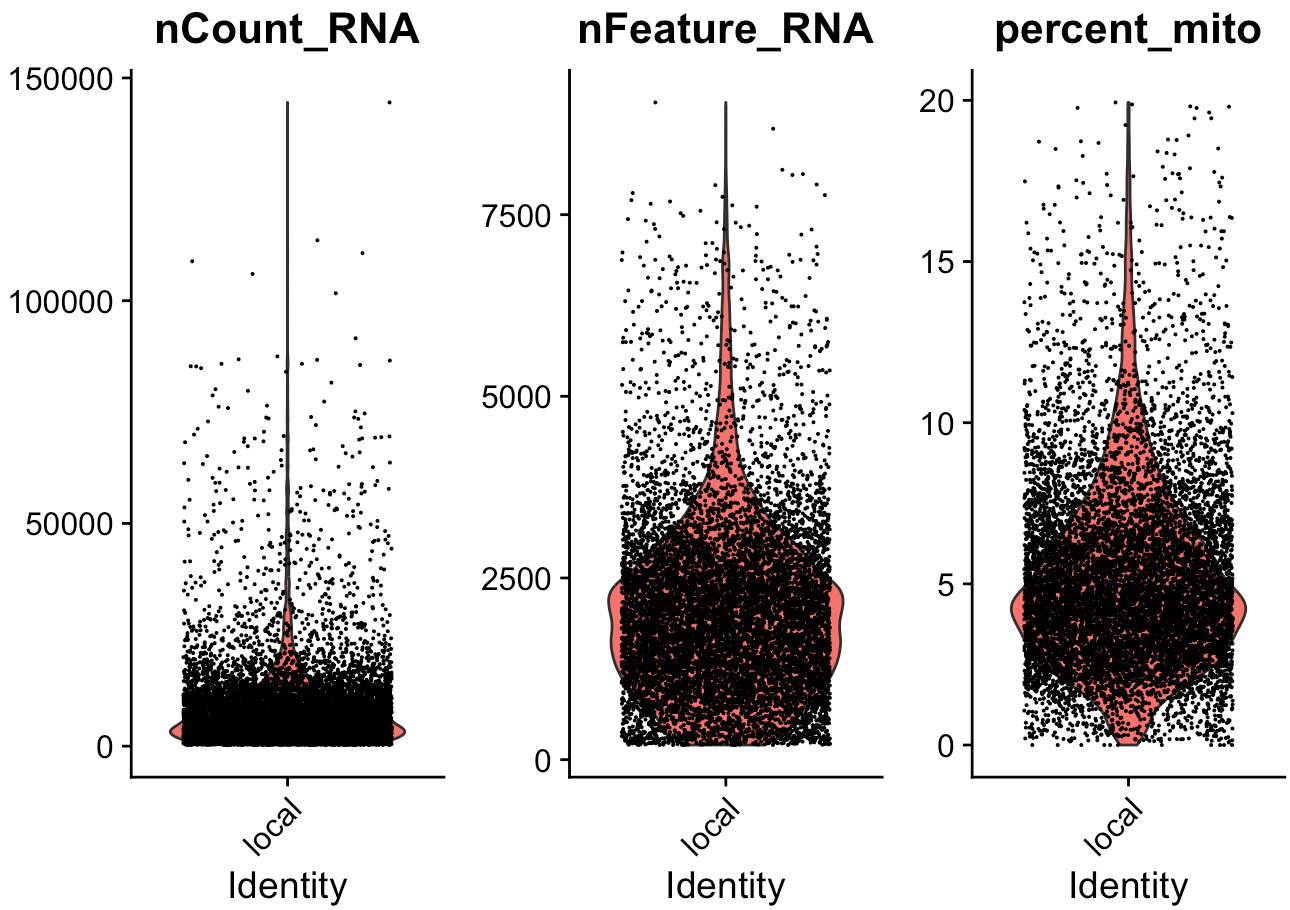
```
# Reading file
brc_file <- readRDS("breast_carcinoma.rds")
#Extracting metadata
metadata.brc <- brc_file@meta.data
```

```
#Calculating Mitochondrial percentage and adding it to metadata if not given
brc_file$mpercent <- PercentageFeatureSet(brc_file, pattern = "^MT")
metadata.brc$mpercent <- brc_file$mpercent
View(metadata.brc)
```

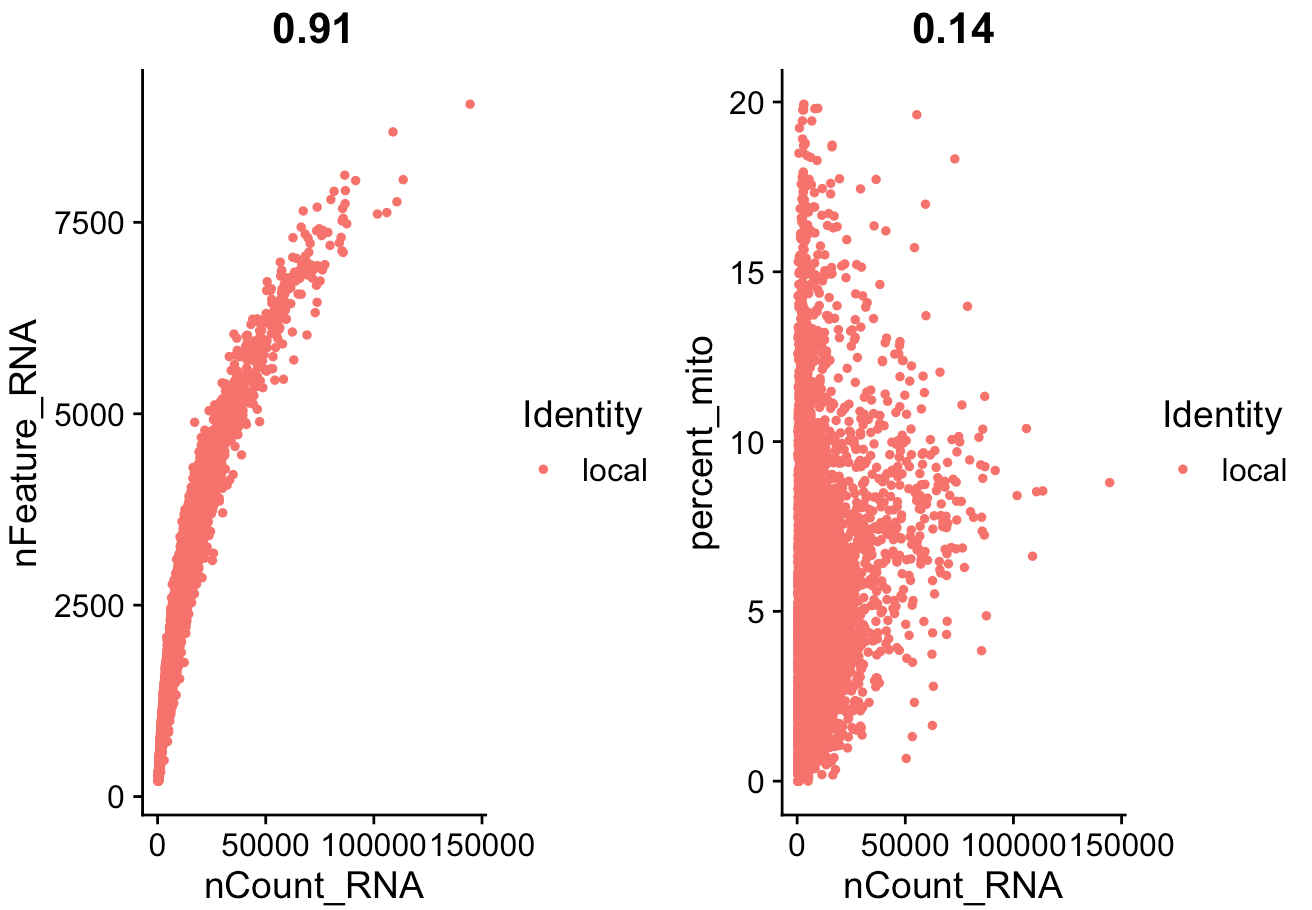
```
# In case nCount_RNA and n_Feature RNA not given:
#Extracting count matrix
count_matrix <- brc_file@assays$RNA@counts

#creating seurat object from count mmatrix
seurat_obj_cnt <- CreateSeuratObject(count_matrix)
new_seu_metadata <- seurat_obj_cnt@meta.data

#setting the variable
n_feature <- new_seu_metadata$nFeature_RNA
n_count <- new_seu_metadata$nCount_RNA
```

```
#Incorporating ncount and nfeature information into raw suerat file expecting it do not
have these features
brc_file$n_feature <- n_feature
brc_file$n_count <- n_count
brc_file$new_mito_percent <- PercentageFeatureSet(brc_file, pattern = "^MT-")

#Incorporating into metadata
metadata.brc$n_feature <- n_feature
metadata.brc$n_count <- n_count
metadata.brc$new_mito_percent <- PercentageFeatureSet(brc_file, pattern = "^MT-")
```

```
#Upstream Analysis#-----------------------------------------------------------------

# Visualize QC metrics as a violin plot
VlnPlot(brc_file, features = c("nCount_RNA", "nFeature_RNA", "percent_mito"), ncol = 3)
```

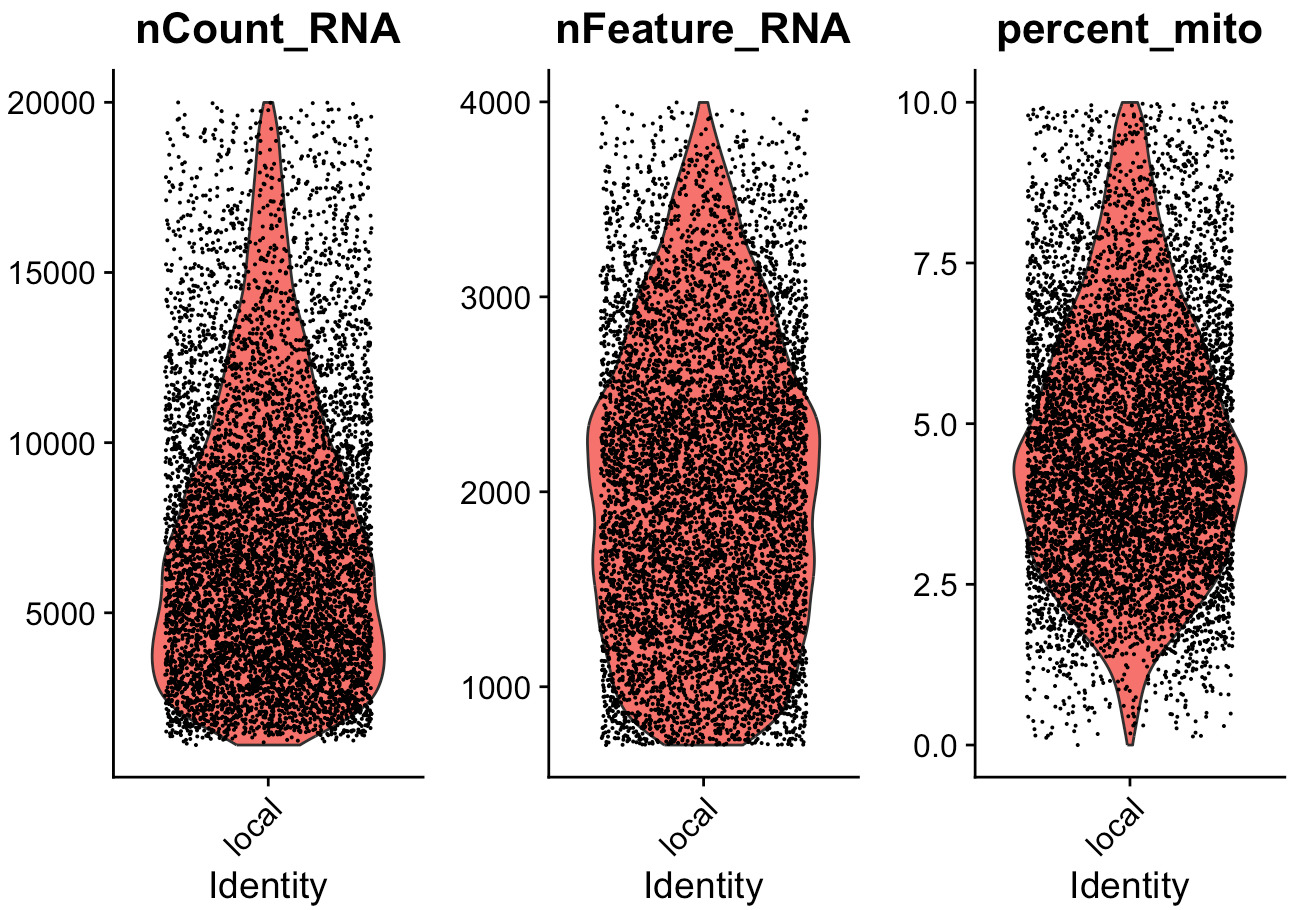



```
#A scatter plot visualizing the relationship between the number of detected features (nF
eature_RNA) and the total RNA count (nCount_RNA).
plot1 <- FeatureScatter(brc_file, feature1 = "nCount_RNA", feature2 = "nFeature_RNA")
plot2 <- FeatureScatter(brc_file, feature1 = "nCount_RNA", feature2 = "percent_mito")
plot1 + plot2
```

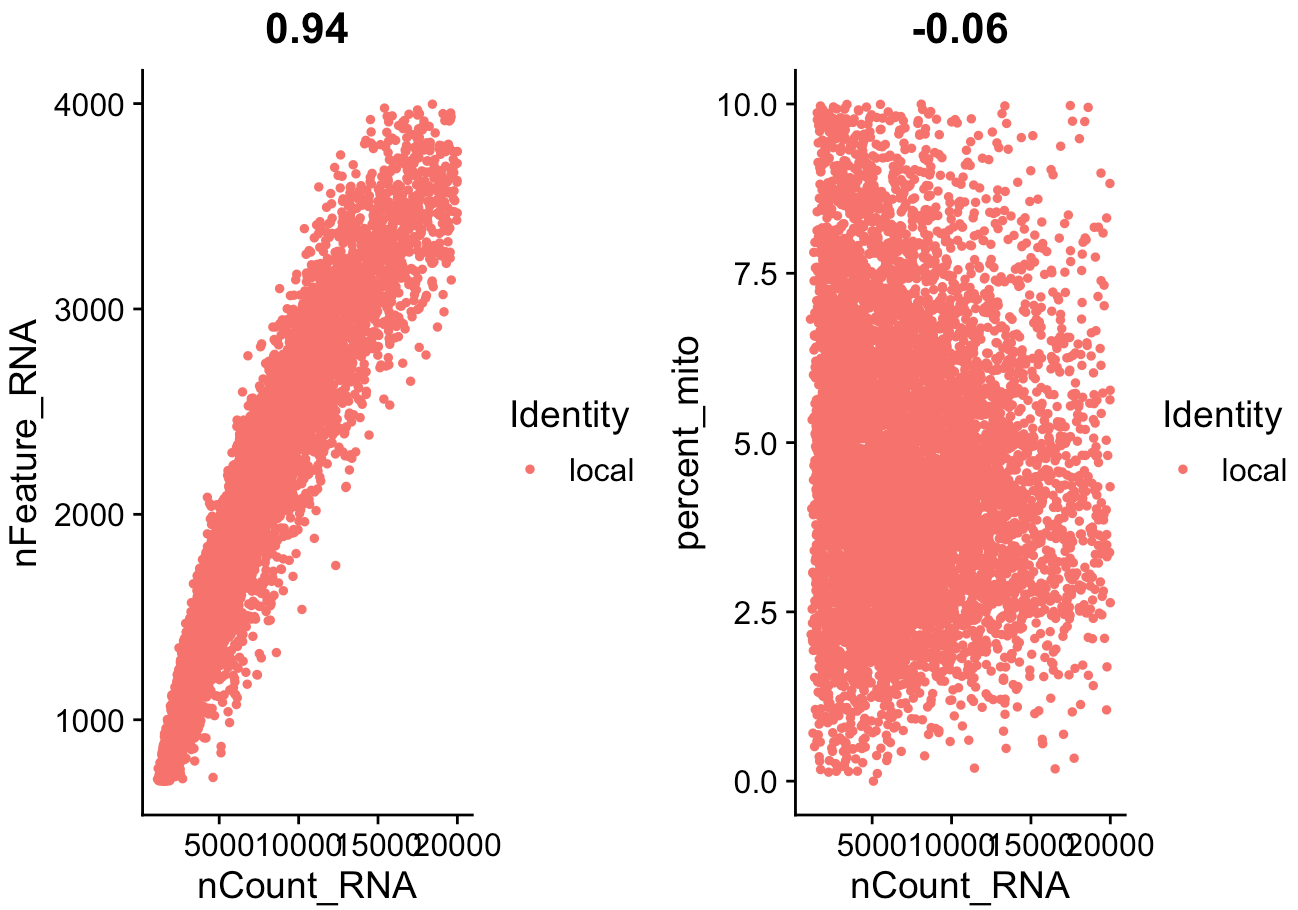



```
#Creating a filtered subset of the brc_file dataset (subset_brc), retaining cells with 7
00–4000 detected features (nFeature_RNA), 500–20,000 total RNA counts (nCount_RNA), and
less than 10% mitochondrial content (percent_mito)
subset_brc <- subset(brc_file, subset = nFeature_RNA <4000 & nFeature_RNA > 700 & nCount
_RNA < 20000 & nCount_RNA >500 & percent_mito <10)
View(subset_brc@meta.data)
```

```
#Violin plot displaying the distribution of filtered nCount RNA, nFeature RNA and mitoch
ondrial percentage across cells
plot1 = VlnPlot(subset_brc, features = c("nCount_RNA", "nFeature_RNA", "percent_mito"),
ncol = 3)
plot1
```




```
#Feature scatter plot
plot2 <- FeatureScatter(subset_brc, feature1 = "nCount_RNA", feature2 = "nFeature_RNA")
plot3 <- FeatureScatter(subset_brc, feature1 = "nCount_RNA", feature2 = "percent_mito")
plot2 + plot3
```




```
#Normalization to isolate the true biological variation from technical artifacts and acc
urately compare gene expression patterns
subset_brc <- NormalizeData(subset_brc, normalization.method = "LogNormalize", scale.fac
tor = 10000)
```

```
#Finding variable features
subset_brc <- FindVariableFeatures(subset_brc, selection.method = "vst", nfeatures = 200
0)
```

```
#Calculating the top10 variable genes
top10 <- head(VariableFeatures(subset_brc), 10)
top10
```

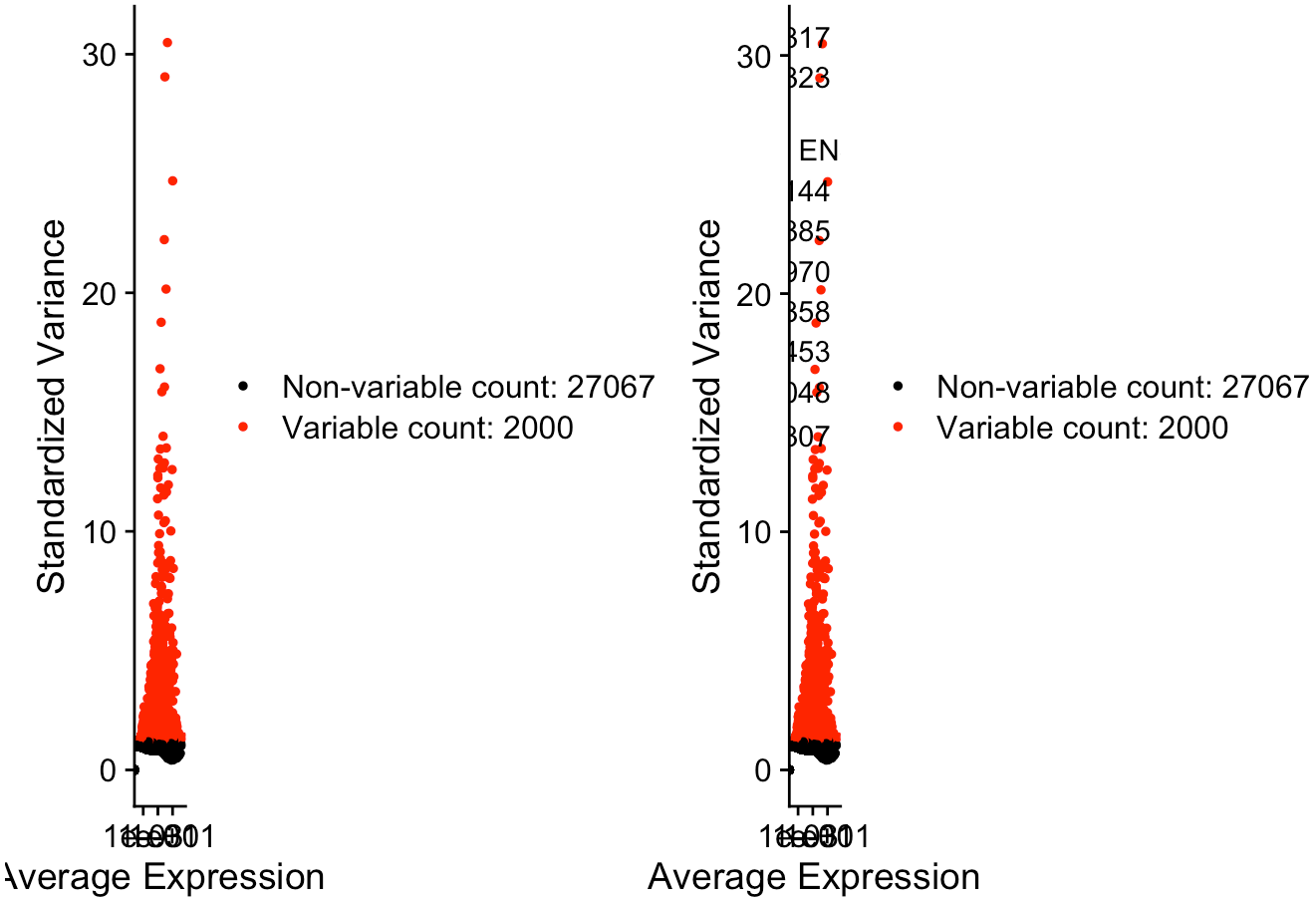
```
##  [1] "ENSG00000107317" "ENSG00000170323" "ENSG00000118785" "ENSG00000125144"
##  [5] "ENSG00000275385" "ENSG00000102970" "ENSG00000205358" "ENSG00000100453"
##  [9] "ENSG00000133048" "ENSG00000160307"
```

```
# Plot variable features without label and with label
plot1 <- VariableFeaturePlot(subset_brc)
plot2 <- LabelPoints(plot = plot1, points = top10, repel = TRUE)
```

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

```
# Display the plot
plot1 + plot2
```

```
## Warning in scale_x_log10(): log-10 transformation introduced infinite values.
## log-10 transformation introduced infinite values.
```




```
#Scaling the data to remove unwanted sources of variation from a single-cell dataset
all.genes <- rownames(subset_brc)
View(all.genes)
subset_brc <- ScaleData(subset_brc, features = all.genes)
```

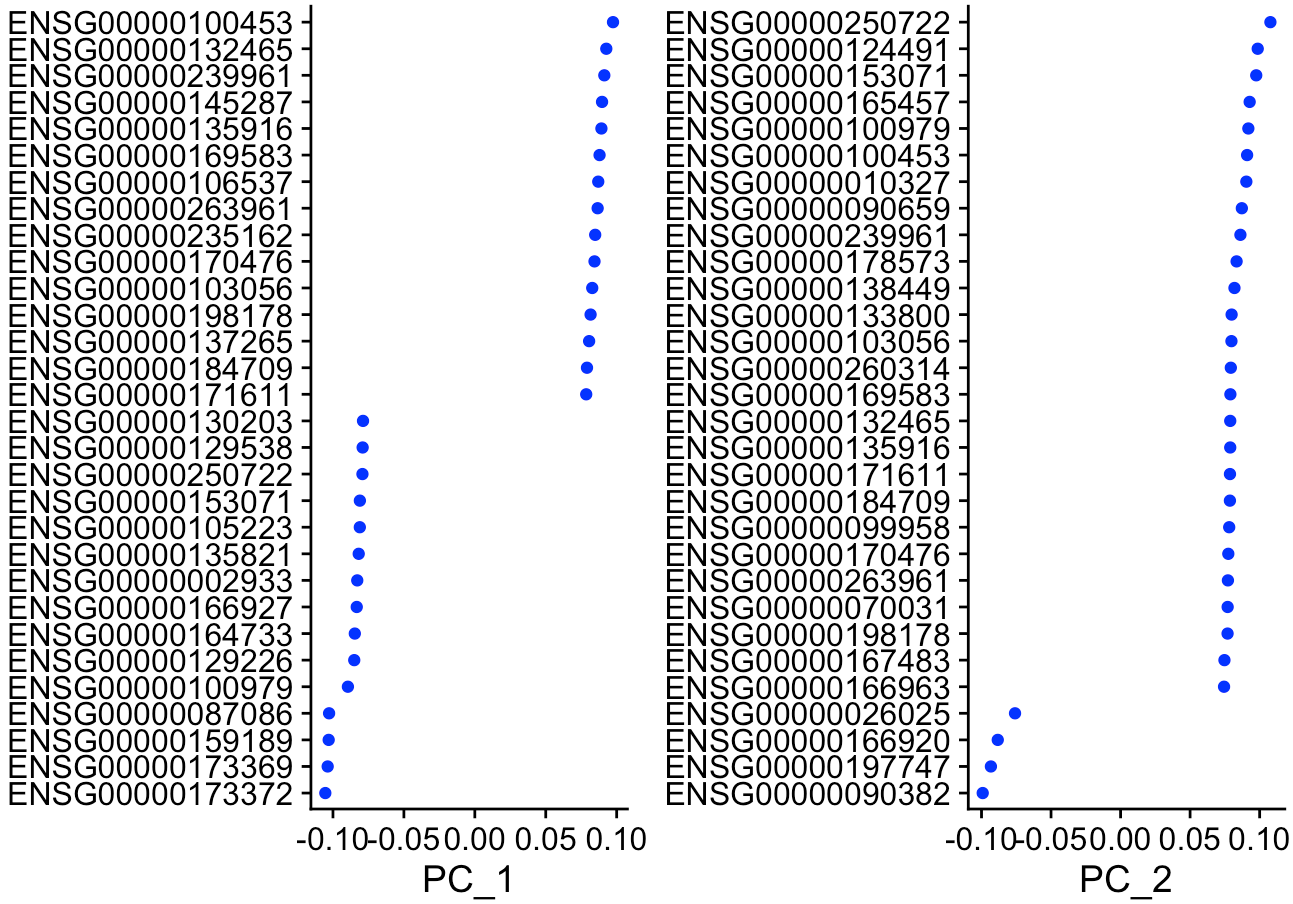
```
## Centering and scaling data matrix
```

```
#Perform linear dimensional reduction (PCA)
subset_brc <- RunPCA(subset_brc, features = VariableFeatures(object = subset_brc))
```

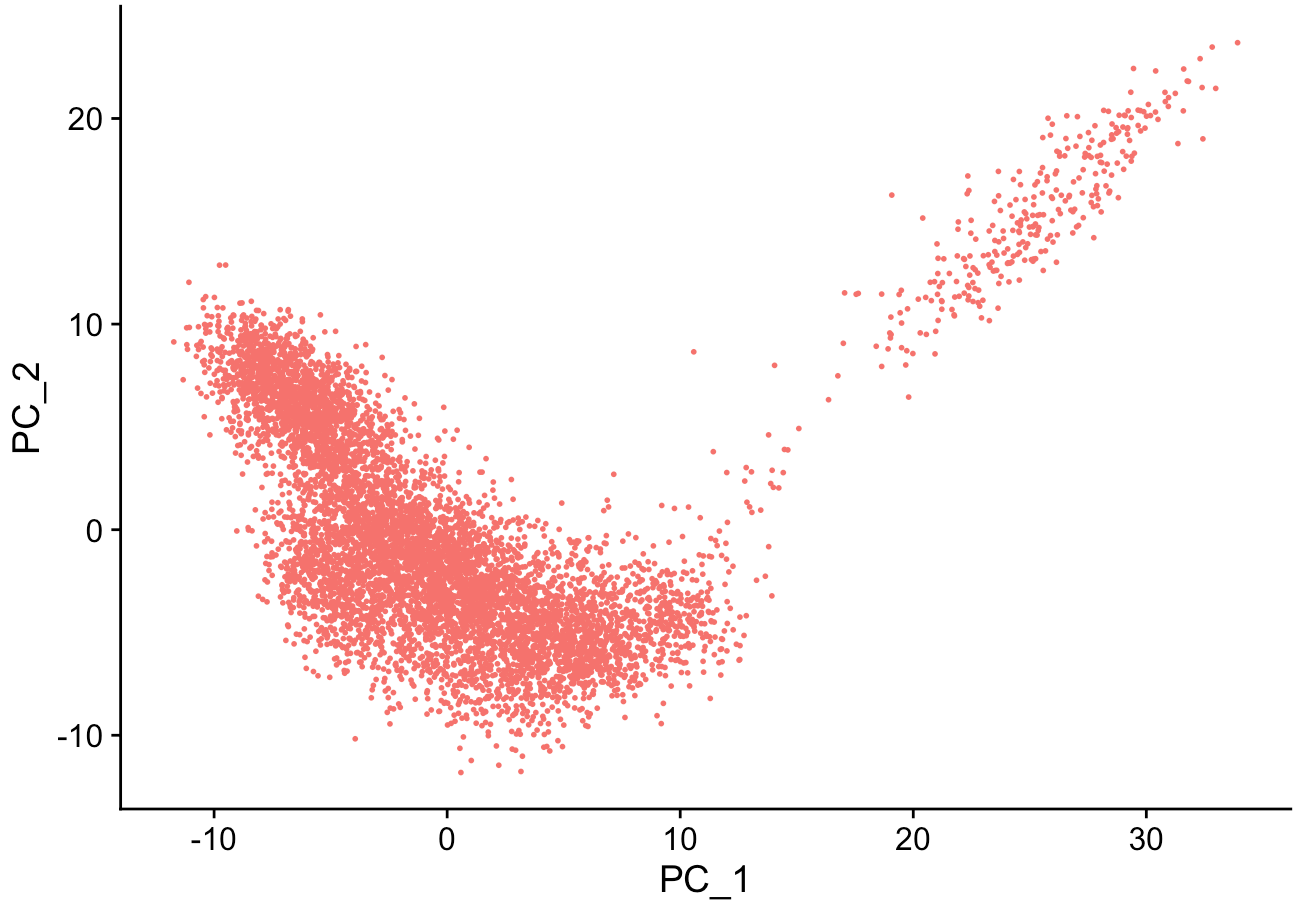
```
## PC_ 1
## Positive:  ENSG00000100453, ENSG00000132465, ENSG00000239961, ENSG00000145287, ENSG00
000135916, ENSG00000169583, ENSG00000106537, ENSG00000263961, ENSG00000235162, ENSG00000
170476
##     ENSG00000103056, ENSG00000198178, ENSG00000137265, ENSG00000184709, ENSG000001716
11, ENSG00000099958, ENSG00000227507, ENSG00000070031, ENSG00000186810, ENSG00000167483
##     ENSG00000185291, ENSG00000119866, ENSG00000105426, ENSG00000166963, ENSG000001636
87, ENSG00000076604, ENSG00000125869, ENSG00000204323, ENSG00000269404, ENSG00000161970
## Negative:  ENSG00000173372, ENSG00000173369, ENSG00000159189, ENSG00000087086, ENSG00
000100979, ENSG00000129226, ENSG00000164733, ENSG00000166927, ENSG00000002933, ENSG00000
135821
##     ENSG00000105223, ENSG00000153071, ENSG00000250722, ENSG00000129538, ENSG000001302
03, ENSG00000197746, ENSG00000106565, ENSG00000170458, ENSG00000010327, ENSG00000117984
##     ENSG00000165457, ENSG00000135404, ENSG00000143878, ENSG00000177575, ENSG000001100
79, ENSG00000165029, ENSG00000155659, ENSG00000130208, ENSG00000177606, ENSG00000260314
## PC_ 2
## Positive:  ENSG00000250722, ENSG00000124491, ENSG00000153071, ENSG00000165457, ENSG00
000100979, ENSG00000100453, ENSG00000010327, ENSG00000090659, ENSG00000239961, ENSG00000
178573
##     ENSG00000138449, ENSG00000133800, ENSG00000103056, ENSG00000260314, ENSG000001695
83, ENSG00000132465, ENSG00000135916, ENSG00000171611, ENSG00000184709, ENSG00000099958
##     ENSG00000170476, ENSG00000263961, ENSG00000070031, ENSG00000198178, ENSG000001674
83, ENSG00000166963, ENSG00000235162, ENSG00000196628, ENSG00000198467, ENSG00000100600
## Negative:  ENSG00000090382, ENSG00000197747, ENSG00000166920, ENSG00000026025, ENSG00
000100079, ENSG00000085265, ENSG00000197956, ENSG00000130592, ENSG00000169442, ENSG00000
143546
##     ENSG00000140105, ENSG00000135046, ENSG00000102265, ENSG00000140379, ENSG000000009
38, ENSG00000173391, ENSG00000196924, ENSG00000123689, ENSG00000182718, ENSG00000128383
##     ENSG00000115414, ENSG00000165140, ENSG00000204287, ENSG00000104951, ENSG000001808
17, ENSG00000117228, ENSG00000038427, ENSG00000216490, ENSG00000217555, ENSG00000010278
## PC_ 3
## Positive:  ENSG00000196735, ENSG00000198502, ENSG00000179344, ENSG00000196126, ENSG00
000042493, ENSG00000204287, ENSG00000231389, ENSG00000130203, ENSG00000117450, ENSG00000
223865
##     ENSG00000204525, ENSG00000206503, ENSG00000186818, ENSG00000132386, ENSG000000102
78, ENSG00000104951, ENSG00000130208, ENSG00000176046, ENSG00000136235, ENSG00000102575
##     ENSG00000138755, ENSG00000108679, ENSG00000115414, ENSG00000149131, ENSG000001098
61, ENSG00000117984, ENSG00000026751, ENSG00000109971, ENSG00000271503, ENSG00000008517
## Negative:  ENSG00000163220, ENSG00000038427, ENSG00000143546, ENSG00000125810, ENSG00
000137801, ENSG00000085265, ENSG00000163221, ENSG00000102265, ENSG00000245532, ENSG00000
113070
##     ENSG00000124882, ENSG00000059804, ENSG00000186407, ENSG00000018280, ENSG000001425
41, ENSG00000178726, ENSG00000196154, ENSG00000124491, ENSG00000112715, ENSG00000008988
##     ENSG00000172216, ENSG00000126759, ENSG00000182774, ENSG00000166441, ENSG000001474
54, ENSG00000125538, ENSG00000059728, ENSG00000183019, ENSG00000133800, ENSG00000224397
## PC_ 4
## Positive:  ENSG00000160932, ENSG00000119917, ENSG00000163220, ENSG00000168899, ENSG00
000117228, ENSG00000115415, ENSG00000243466, ENSG00000157601, ENSG00000126709, ENSG00000
121858
##     ENSG00000272398, ENSG00000149131, ENSG00000089127, ENSG00000185745, ENSG000001977
46, ENSG00000239951, ENSG00000241351, ENSG00000241755, ENSG00000206503, ENSG00000211659
##     ENSG00000134575, ENSG00000142089, ENSG00000154451, ENSG00000211653, ENSG000000254
```

```
34, ENSG00000109861, ENSG00000004468, ENSG00000068079, ENSG00000104951, ENSG00000165949
## Negative:  ENSG00000132002, ENSG00000204388, ENSG00000204389, ENSG00000120694, ENSG00
000112149, ENSG00000118515, ENSG00000151929, ENSG00000123358, ENSG00000123975, ENSG00000
080824
##     ENSG00000178381, ENSG00000188229, ENSG00000087074, ENSG00000162772, ENSG000001416
82, ENSG00000096384, ENSG00000144381, ENSG00000099860, ENSG00000086061, ENSG00000136826
##     ENSG00000115541, ENSG00000197989, ENSG00000090104, ENSG00000120129, ENSG000001694
29, ENSG00000275302, ENSG00000276070, ENSG00000170345, ENSG00000067082, ENSG00000276085
## PC_ 5
## Positive:  ENSG00000148773, ENSG00000176890, ENSG00000131747, ENSG00000171848, ENSG00
000175063, ENSG00000100162, ENSG00000088325, ENSG00000117724, ENSG00000089685, ENSG00000
137804
##     ENSG00000167900, ENSG00000075218, ENSG00000100526, ENSG00000145386, ENSG000001703
12, ENSG00000066279, ENSG00000117632, ENSG00000123219, ENSG00000127564, ENSG00000122952
##     ENSG00000092853, ENSG00000072571, ENSG00000146670, ENSG00000228716, ENSG000001116
65, ENSG00000138180, ENSG00000276043, ENSG00000117399, ENSG00000173207, ENSG00000178999
## Negative:  ENSG00000100906, ENSG00000291237, ENSG00000118503, ENSG00000144802, ENSG00
000137331, ENSG00000197766, ENSG00000119508, ENSG00000171174, ENSG00000125538, ENSG00000
081041
##     ENSG00000120129, ENSG00000128383, ENSG00000087074, ENSG00000162772, ENSG000001121
49, ENSG00000085265, ENSG00000090339, ENSG00000245532, ENSG00000130844, ENSG00000140379
##     ENSG00000116741, ENSG00000162711, ENSG00000158050, ENSG00000186818, ENSG000001855
07, ENSG00000124762, ENSG00000107372, ENSG00000255112, ENSG00000169508, ENSG00000173451
```

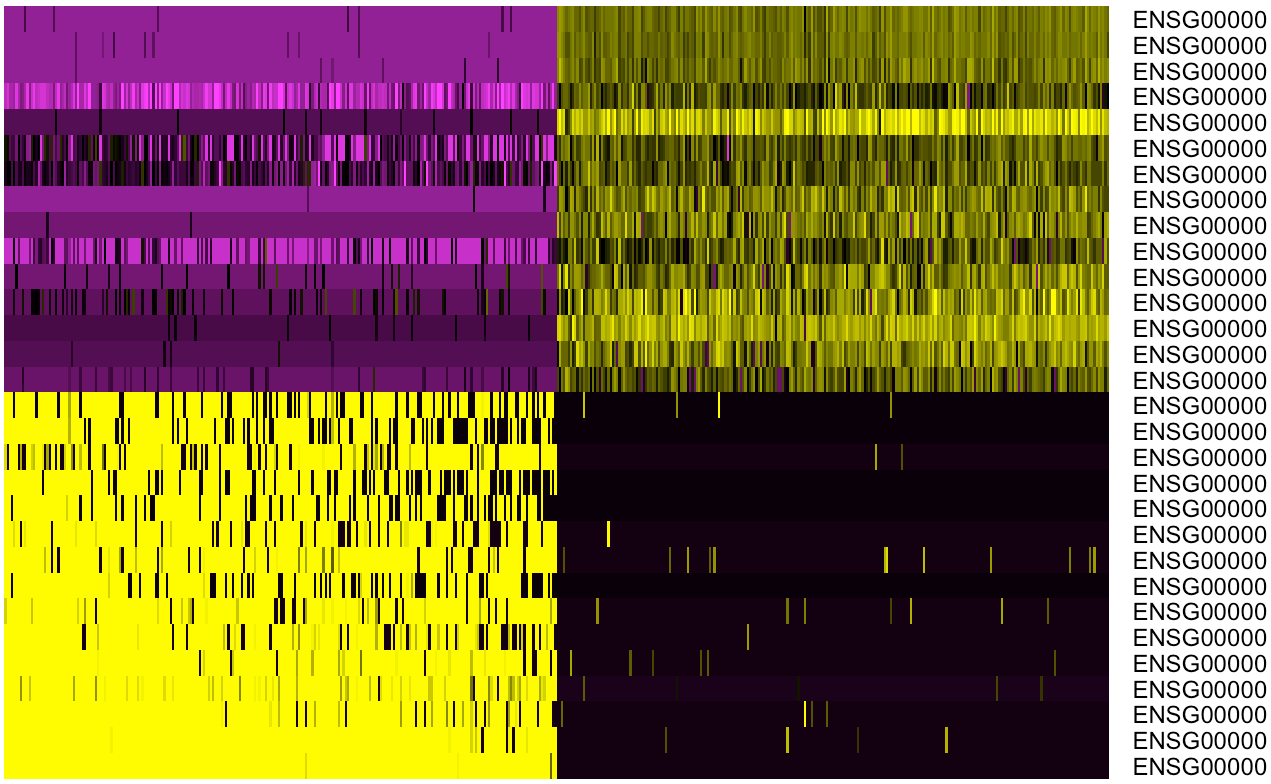
```
#Examine and visualize PCA results a few different ways
VizDimLoadings(subset_brc, dims = 1:2, reduction = "pca")
```



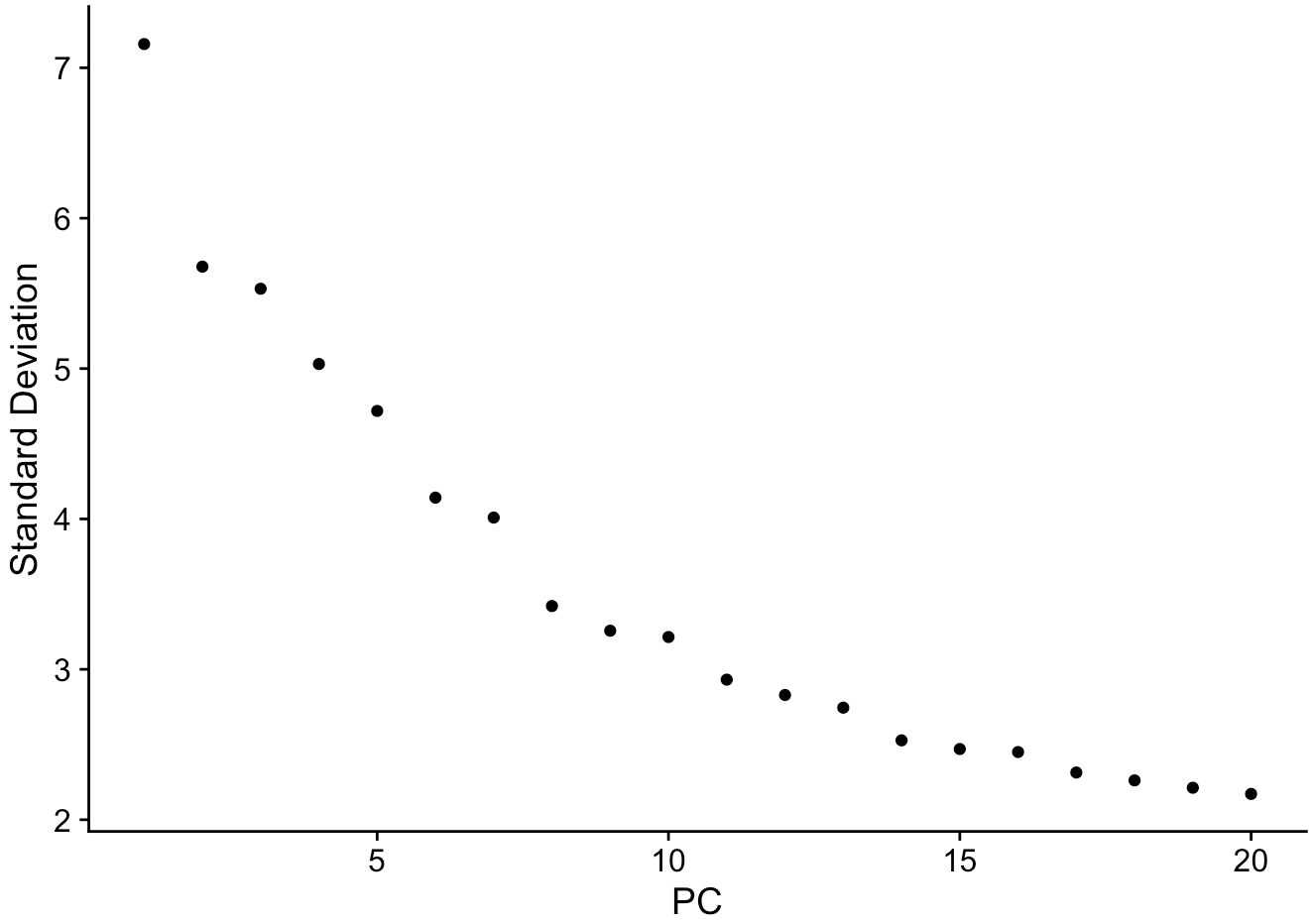

```
#Dimensional reduction plot, each point represents the cell
DimPlot(subset_brc, reduction = "pca") + NoLegend()
```




```
#Heatmap for easy exploration of the primary sources of heterogeneity
DimHeatmap(subset_brc, dim = 1, cells =500, balanced = TRUE)
```




```
#Determining the top PCs that represents the robust compression of the dataset
ElbowPlot(subset_brc)
```




```
#Clustering the cells
#First constructing a KNN graph based on the euclidean distance in PCA space, and refini
ng the edge weights between any two cells based on the shared overlap in their local nei
ghborhoods (Jaccard similarity).
subset_brc <- FindNeighbors(subset_brc, dims = 1:15) #dims = dimension,
```

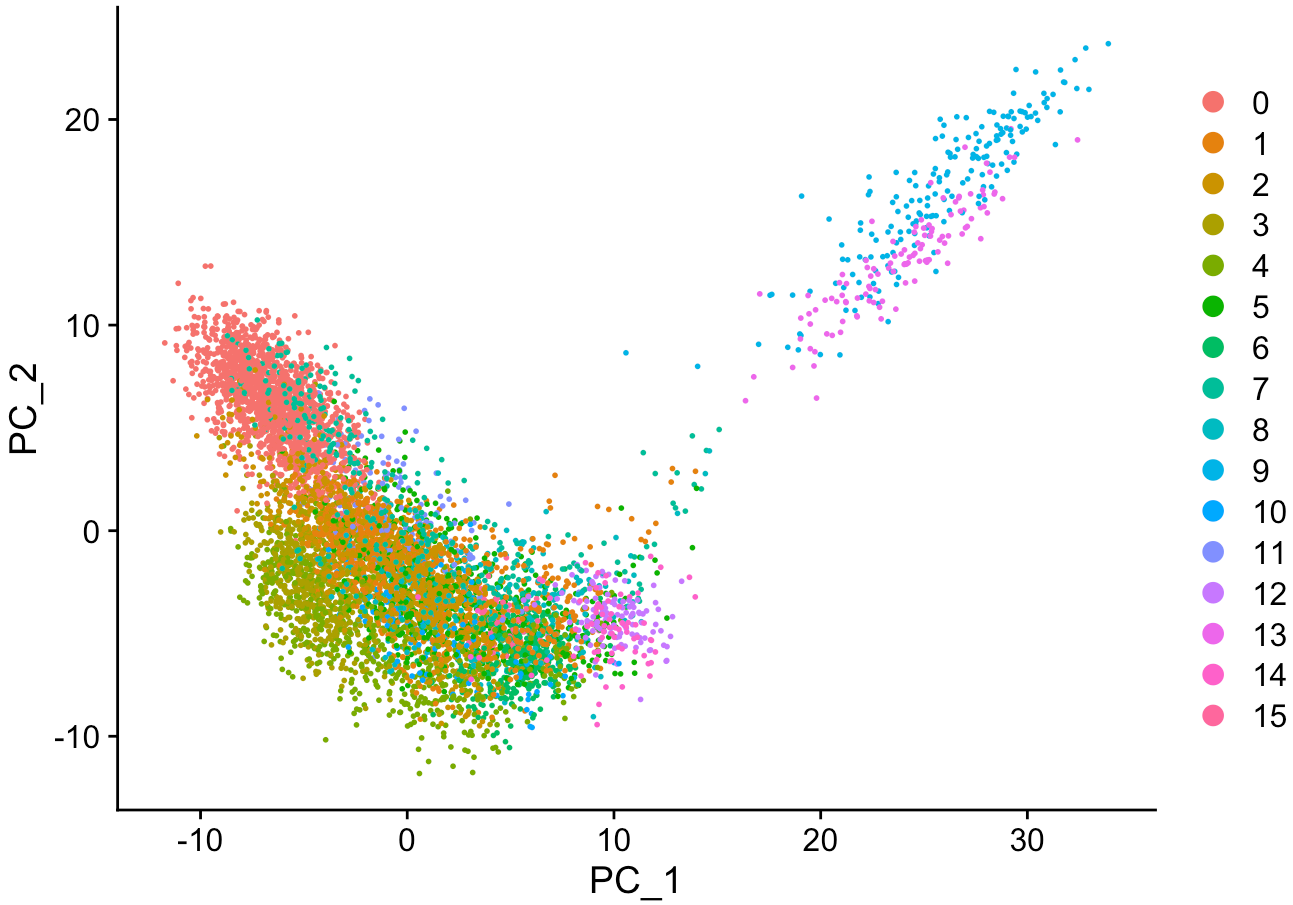
```
## Computing nearest neighbor graph
```

```
## Computing SNN
```

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

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 7488
## Number of edges: 245221
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.9060
## Number of communities: 16
## Elapsed time: 0 seconds
```

```
DimPlot(subset_brc, reduction = "pca")
```




```
#Uniform Manifold Approximation and Projection (UMAP), performed on the filtered dataset (subset_brc) using the first 15 principal components (dims = 1:15) from PCA.
subset_brc <- RunUMAP(subset_brc, dims = 1:15, reduction = "pca")
```

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

```
## 00:18:21 UMAP embedding parameters a = 0.9922 b = 1.112
```

```
## 00:18:21 Read 7488 rows and found 15 numeric columns
```

```
## 00:18:21 Using Annoy for neighbor search, n_neighbors = 30
```

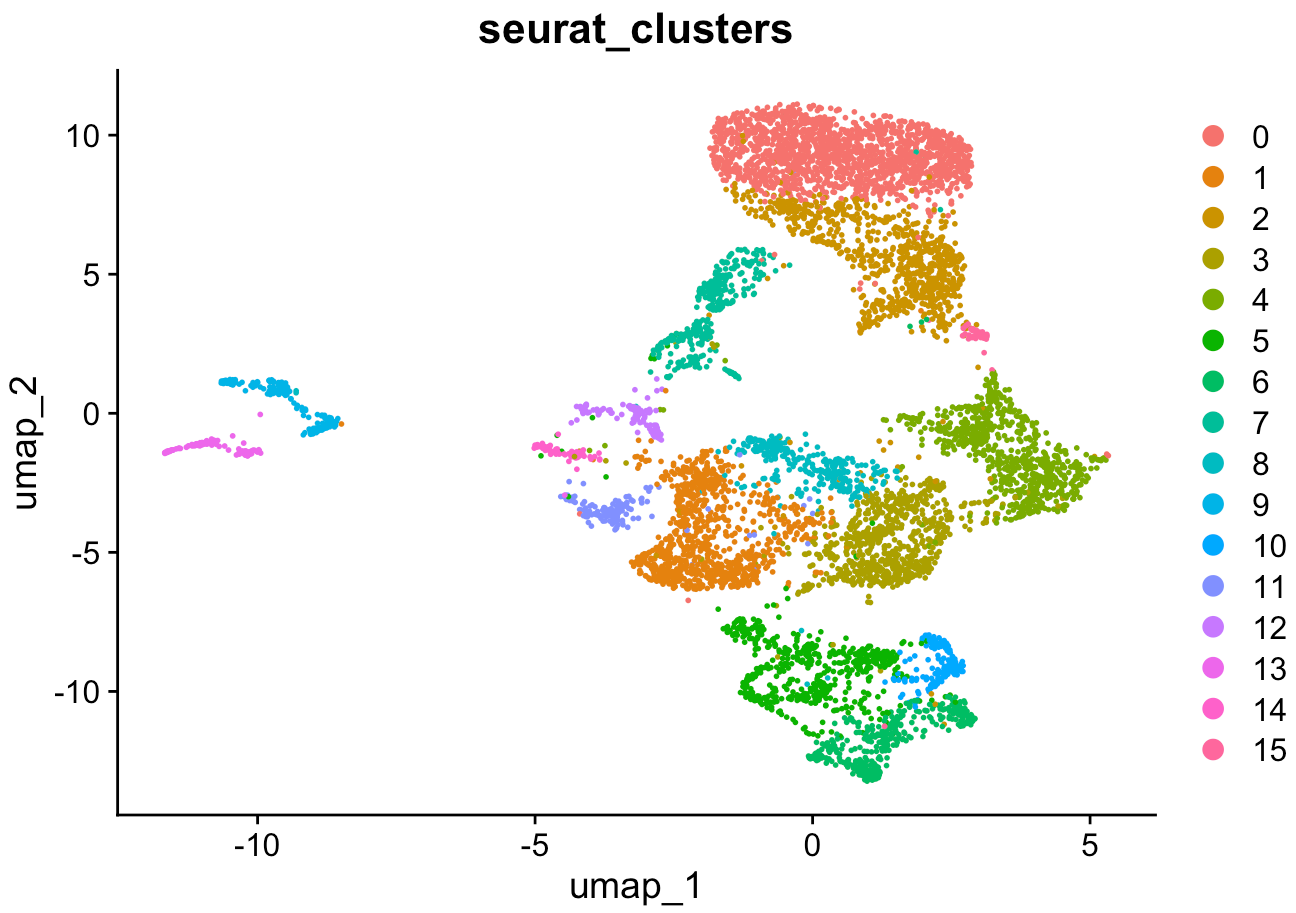
```
## 00:18:21 Building Annoy index with metric = cosine, n_trees = 50
```

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

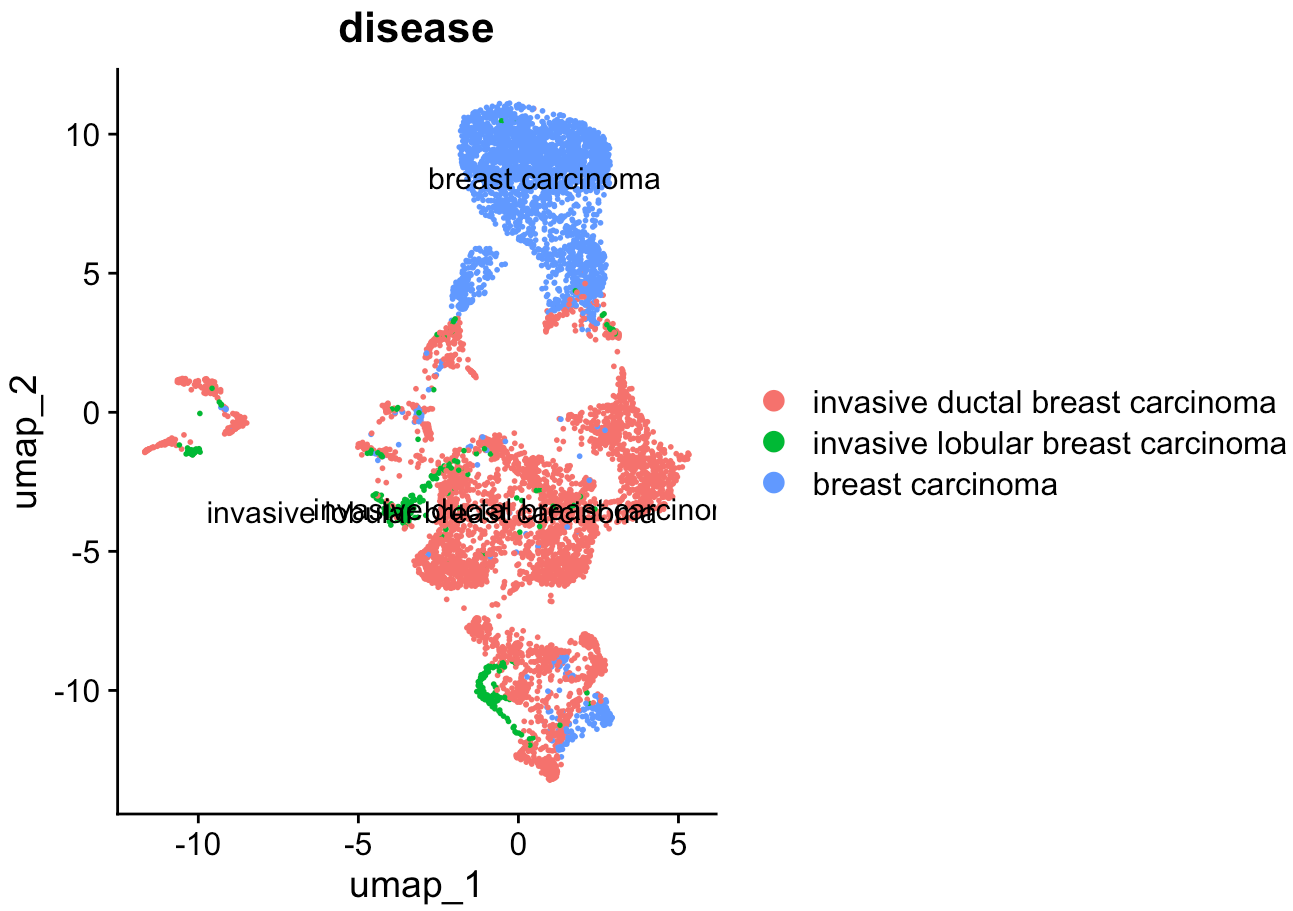
```
## [----|----|----|----|----|----|----|----|----|----|
```

```
## **************************************************|
## 00:18:22 Writing NN index file to temp file /var/folders/rq/8sjqg4hj3k99cfwf49v9_dlc0
000gn/T//Rtmp3m32Ul/file9dbf3d9d91dd
## 00:18:22 Searching Annoy index using 1 thread, search_k = 3000
## 00:18:24 Annoy recall = 100%
## 00:18:24 Commencing smooth kNN distance calibration using 1 thread with target n_neig
hbors = 30
## 00:18:25 Initializing from normalized Laplacian + noise (using RSpectra)
## 00:18:25 Commencing optimization for 500 epochs, with 305360 positive edges
## 00:18:25 Using rng type: pcg
## 00:18:32 Optimization finished
```

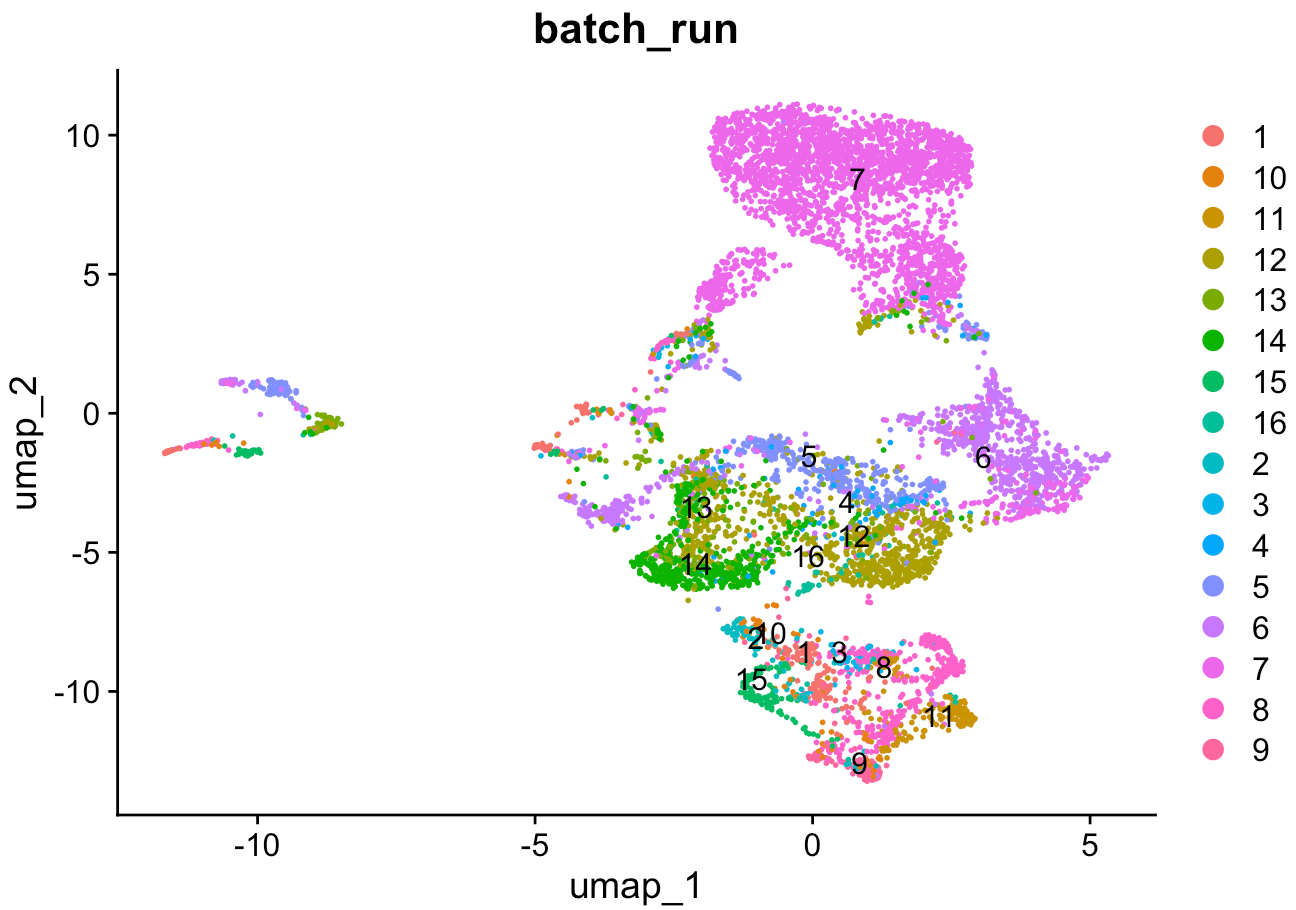
```
#Cell clustering based on seurat_clusters
DimPlot(subset_brc, reduction = "umap", group.by = "seurat_clusters")
```



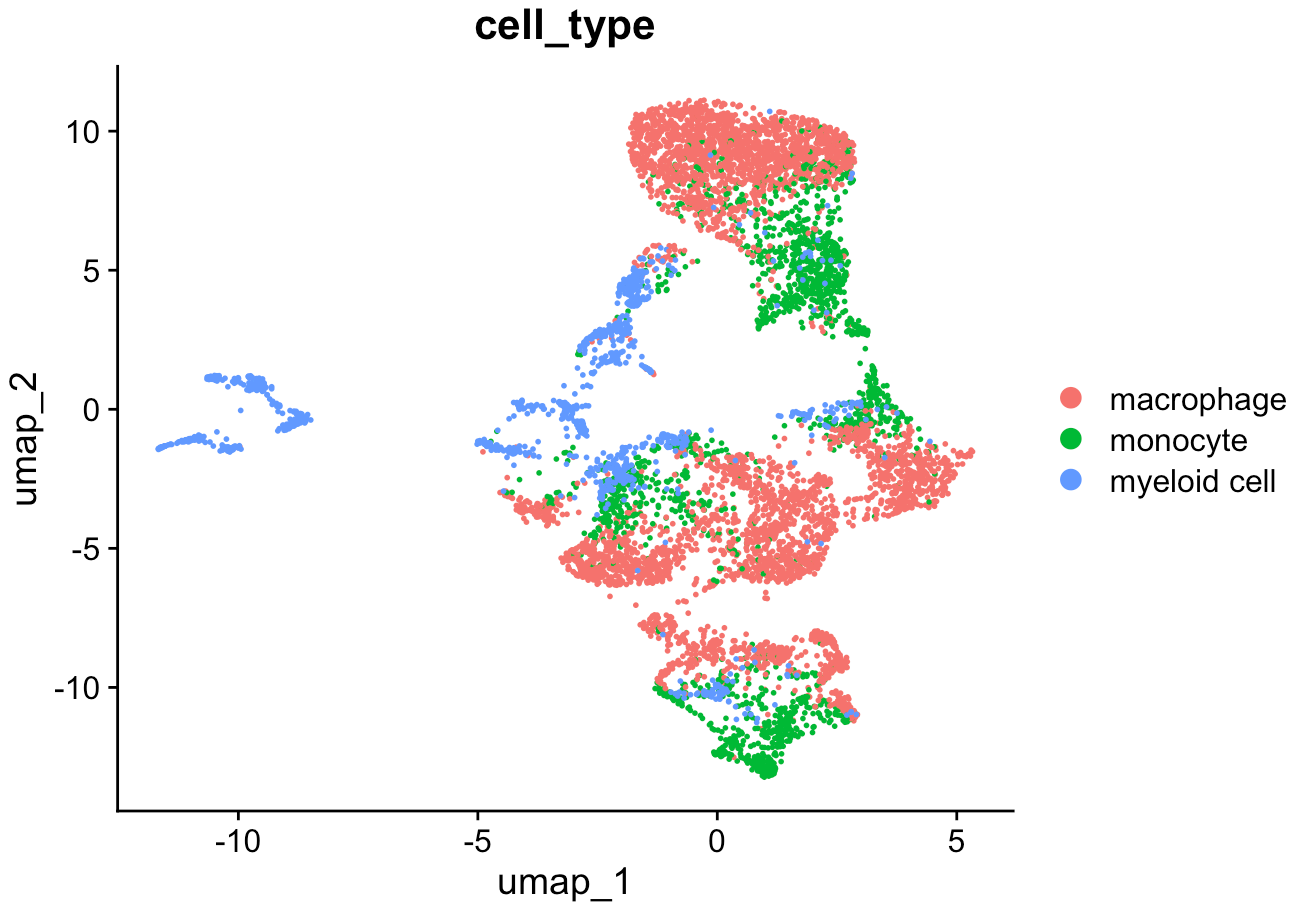
```
#Cell clustering based on disease
disease <- DimPlot(subset_brc, reduction = "umap", group.by = "disease" , label = "TRU
E")
disease
```




```
#Cell clustering based on batch run
batch <- DimPlot(subset_brc, reduction = "umap", group.by = "batch_run", label = "TRUE")
batch
```




```
#Cell clustering based on cell type
cell_type<- DimPlot(subset_brc, reduction = "umap", group.by = "cell_type")
cell_type
```




```
#Batch effect removal using harmony
subset_brc <- RunHarmony(
  object = subset_brc,
  group.by.vars = "batch_run",
  dims.use = 1:15 # Use the same dimensions you selected in FindNeighbors
)
```

```
## Transposing data matrix
```

```
## Initializing state using k-means centroids initialization
```

```
## Harmony 1/10
```

```
## Harmony 2/10
```

```
## Harmony 3/10
```

```
## Harmony 4/10
```

```
## Harmony 5/10
```

```
## Harmony 6/10
```

```
## Harmony 7/10
```

```
## Harmony converged after 7 iterations
```

```
# Re- running clustering using harmony
subset_brc <- subset_brc %>%
  RunUMAP(reduction = 'harmony',dims = 1:15) %>%
  FindNeighbors(reduction = 'harmony' , dims = 1:15) %>%
  FindClusters(resolution = 0.5)
```

```
## 00:18:39 UMAP embedding parameters a = 0.9922 b = 1.112
```

```
## 00:18:39 Read 7488 rows and found 15 numeric columns
```

```
## 00:18:39 Using Annoy for neighbor search, n_neighbors = 30
```

```
## 00:18:39 Building Annoy index with metric = cosine, n_trees = 50
```

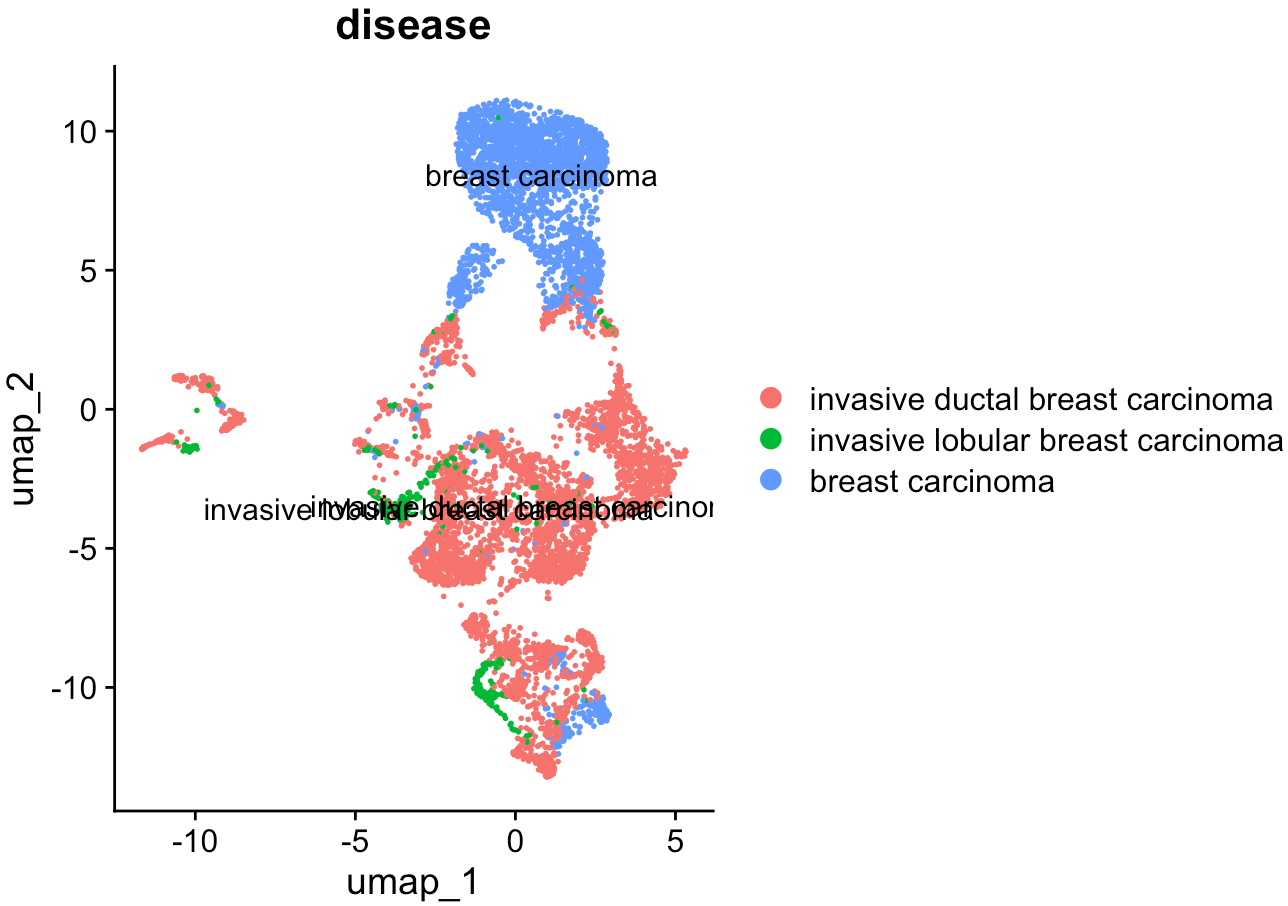
```
## 0%   10   20   30   40   50   60   70   80   90   100%
```

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

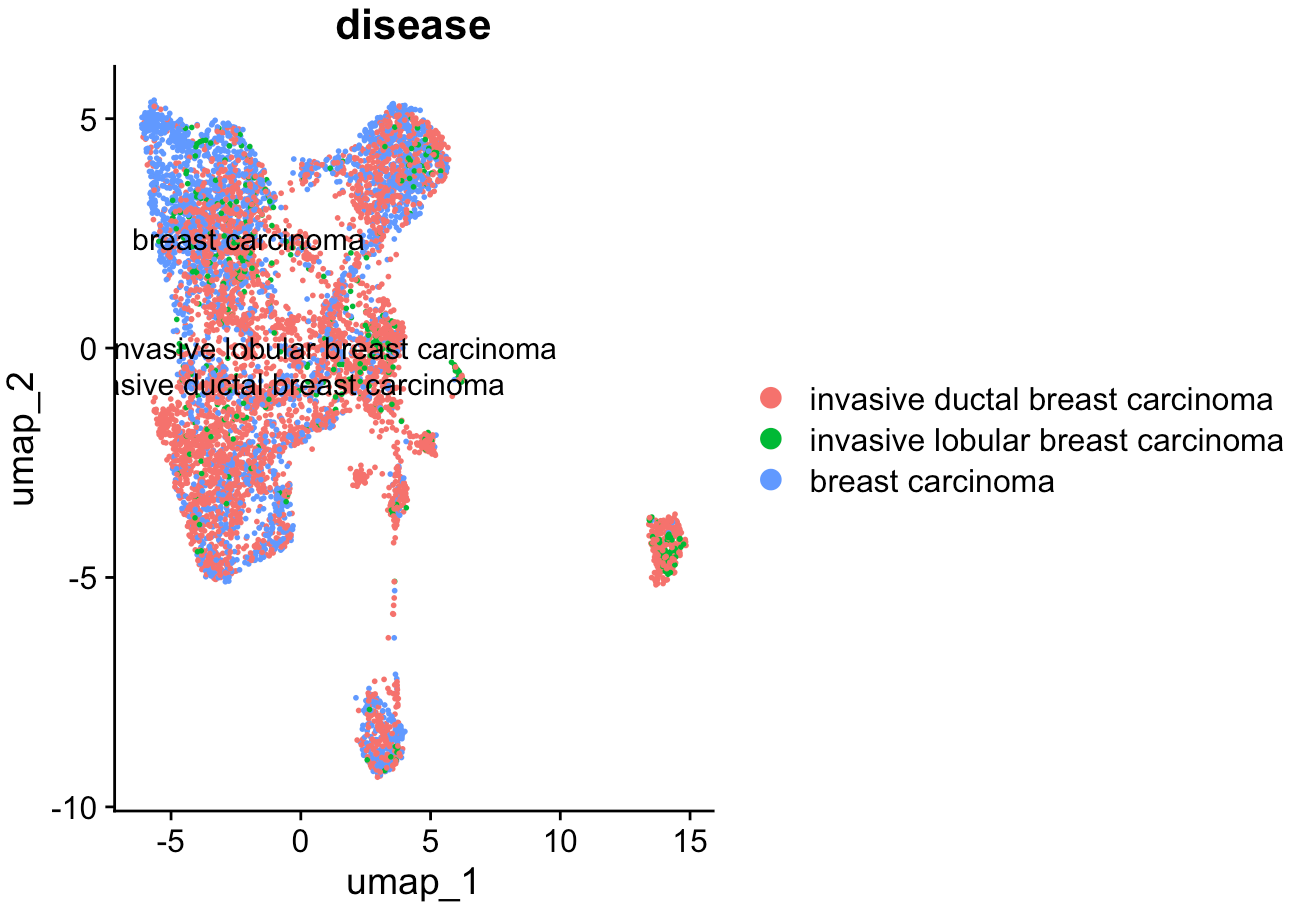
```
## **************************************************|
## 00:18:39 Writing NN index file to temp file /var/folders/rq/8sjqg4hj3k99cfwf49v9_dlc0
000gn/T//Rtmp3m32Ul/file9dbf11c90598
## 00:18:39 Searching Annoy index using 1 thread, search_k = 3000
## 00:18:41 Annoy recall = 100%
## 00:18:41 Commencing smooth kNN distance calibration using 1 thread with target n_neig
hbors = 30
## 00:18:42 Initializing from normalized Laplacian + noise (using RSpectra)
## 00:18:42 Commencing optimization for 500 epochs, with 311638 positive edges
## 00:18:42 Using rng type: pcg
## 00:18:49 Optimization finished
## Computing nearest neighbor graph
## Computing SNN
```

```
## Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
##
## Number of nodes: 7488
## Number of edges: 267661
##
## Running Louvain algorithm...
## Maximum modularity in 10 random starts: 0.8731
## Number of communities: 13
## Elapsed time: 0 seconds
```

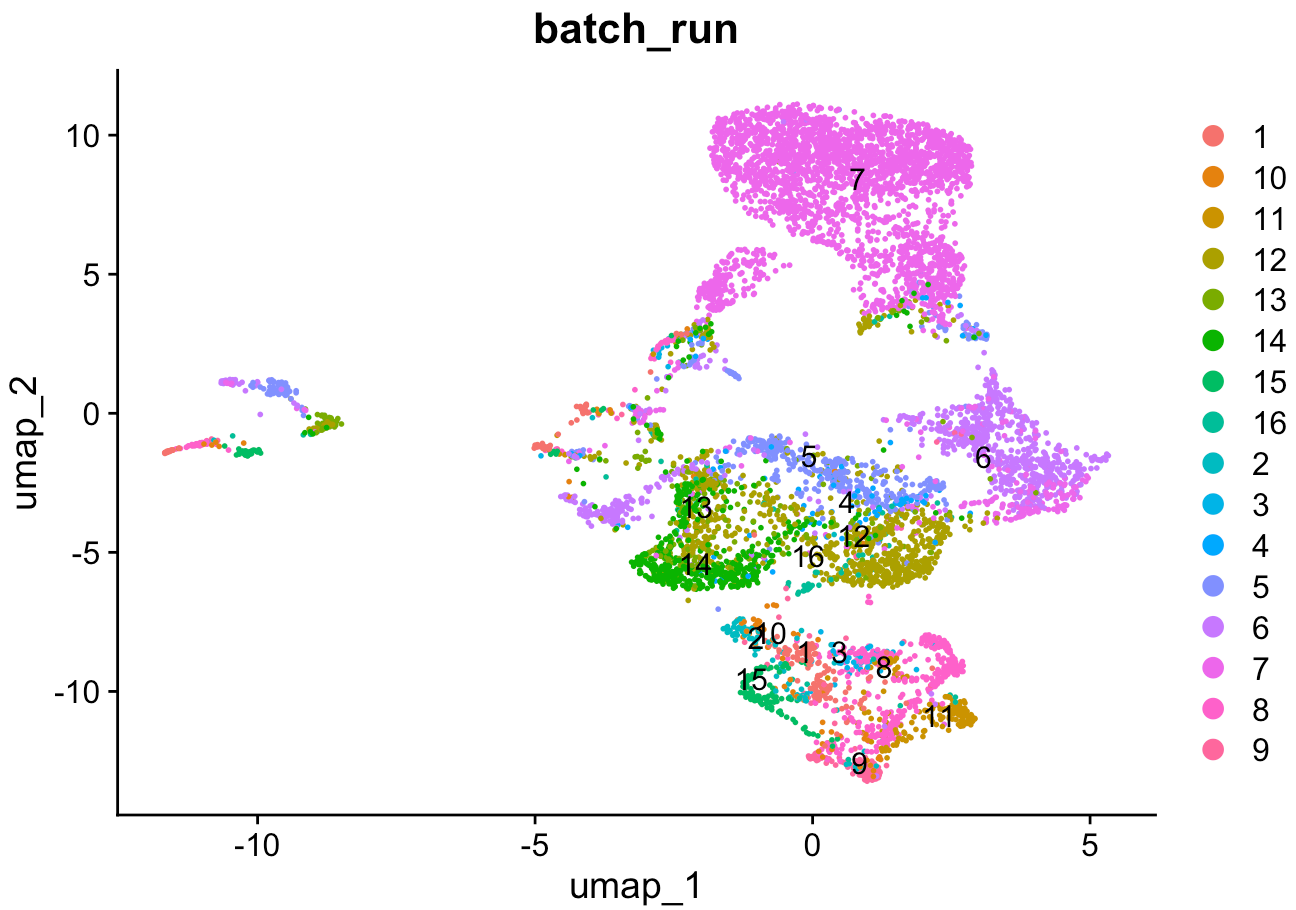
```
disease_after_harmony <- DimPlot(subset_brc, reduction = "umap", group.by = "disease" ,
label = "TRUE")
batch_after_harmony <- DimPlot(subset_brc, reduction = "umap", group.by = "batch_run", l
abel = "TRUE")
disease
```



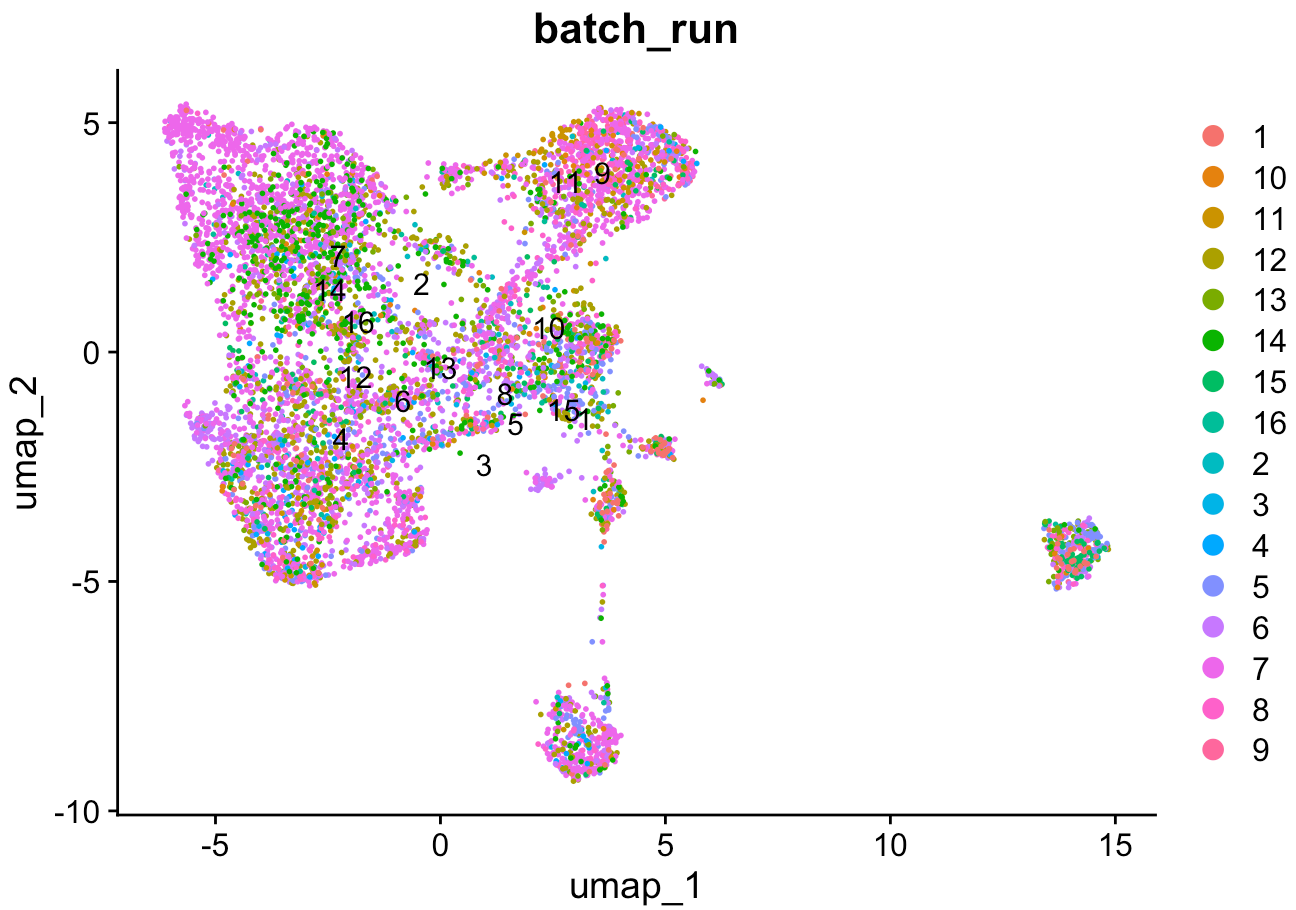

```
disease_after_harmony
```


disease
breast carcinoma
nvasive lobular breast carcinoma
sive ductal breast carcinoma
umap_2
umap_1
5
0
-5
-10
-5
0
5
10
15
invasive ductal breast carcinoma
invasive lobular breast carcinoma
breast carcinoma

```
batch
```




```
batch_after_harmony
```


batch_run
umap_2
umap_1
5
0
-5
-10
-5
0
5
10
15
1
10
11
12
13
14
15
16
2
3
4
5
6
7
8
9