Stat M254 Homework 3

Due June 1 @ 11:59PM

```
library(Seurat)
library(ggplot2)
library(fastglmpca)
library(ggpubr)
library(motifcluster)
library(cluster)
library(factoextra)
library(mclust)
library(tightClust)
```

In this homework, you should use a new PBMC dataset with the given cell type labels stored as the Seurat object (v5) `PBMC_w_labels.rds`. This dataset has nine diFerent cell types.

Problem 1

Data preparation. Perform the following steps in Seurat with default parameters: (1) log1pCP10k, (2) highly variable gene selection, (3) scaling data, and (4) PCA.

```
pbmc <- readRDS("data/PBMC_w_labels.rds")
```

```
pbmc <- NormalizeData(pbmc,
                      normalization.method = "LogNormalize",
                      scale.factor = 10000)
```

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

```
pbmc <- ScaleData(pbmc)
```

```
pbmc <- RunPCA(pbmc,
               npcs = 100)
```

Problem 2

Implement the following four clustering algorithms on the PCA results (the first 50 PCs). Also, plot cells using their scores of the first two PCs and color them according to five sets of labels. Present these in five separate figures: four showing the cluster labels in the four clustering results and one showing the true cell type labels.

(1) kmeans [Rfunction]: Set k to 9.
(2) kmeans++ [Rfunction]: Set k to 9
(3) Hierarchical clustering (complete linkage; Euclidean distance for the dissimilarity) [Rfunction]: Cut the dendrogram to obtain 9 clusters.
(4) Seurat clustering: Choose an appropriate `resolution` parameter in FindCluster() to obtain 9 clusters.

```
pca_embeddings <- Embeddings(pbmc[["pca"]])[, 1:50]
```

```
kmeans_res <- kmeans(pca_embeddings, centers = 9)
```

```
kmeanspp_res <- kmeanspp(pca_embeddings, k = 9)
```

```
hclust_res <- hclust(dist(pca_embeddings, method = "euclidean"),
                     method = "complete")
hclust_res <- cutree(hclust_res, k = 9)
```

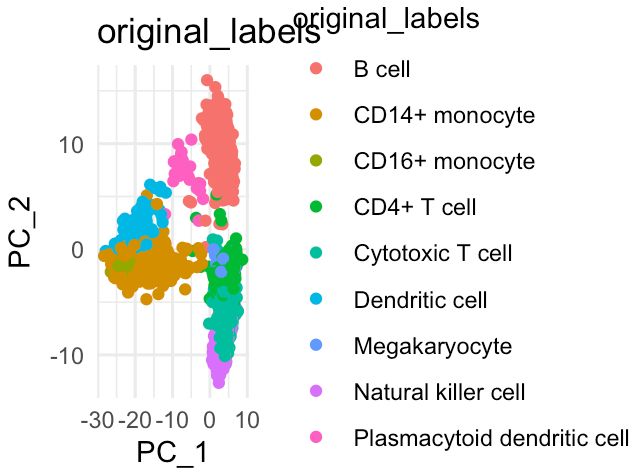
```
pbmc <- FindNeighbors(pbmc, dims = 1:50)
pbmc <- FindClusters(pbmc, resolution = 0.23)
```

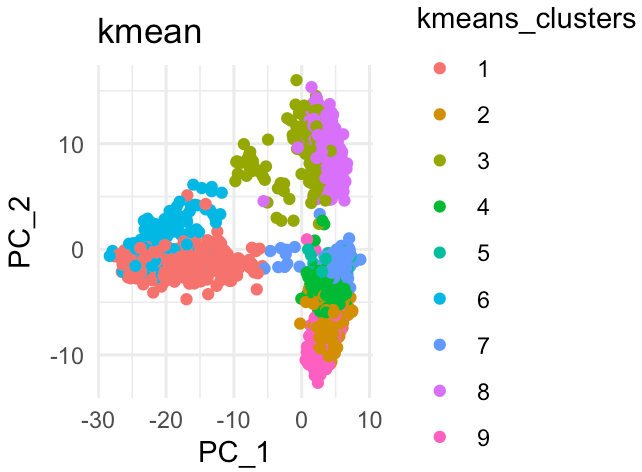
```
Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck

Number of nodes: 3362
Number of edges: 223918

Running Louvain algorithm...
Maximum modularity in 10 random starts: 0.9399
Number of communities: 9
Elapsed time: 0 seconds
```

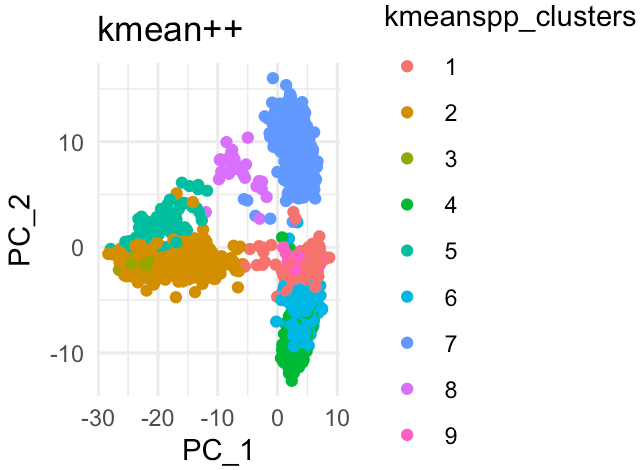
```
original_labels <- pbmc@meta.data$celltype

PC1 <- pca_embeddings[, 1]
PC2 <- pca_embeddings[, 2]
kmeans_clusters <- kmeans_res$cluster
kmeanspp_clusters <- kmeanspp_res$cluster
# Create a data frame with PC1, PC2, and cluster labels
df <- data.frame(PC_1 = PC1, PC_2 = PC2, kmeans_clusters = as.factor(kmeans_clust
```

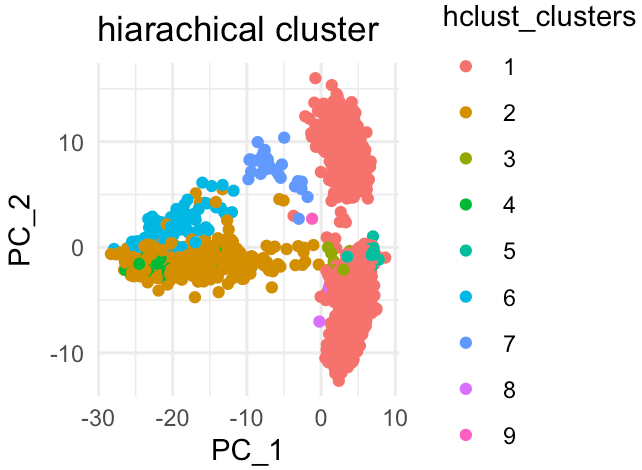
```
g0 <- ggplot(df, aes(x = PC_1, y = PC_2, color = original_labels)) +
  geom_point() +
  labs(title = "original_labels",
       x = "PC_1",
       y = "PC_2",
       color = "original_labels") +
  theme_minimal()

g1 <- ggplot(df, aes(x = PC_1, y = PC_2, color = kmeans_clusters)) +
  geom_point() +
  labs(title = "kmean",
       x = "PC_1",
       y = "PC_2",
       color = "kmeans_clusters") +
  theme_minimal()

g2 <- ggplot(df, aes(x = PC_1, y = PC_2, color = kmeanspp_clusters)) +
  geom_point() +
  labs(title = "kmean++",
       x = "PC_1",
       y = "PC_2",
       color = "kmeanspp_clusters") +
  theme_minimal()

g3 <-ggplot(df, aes(x = PC_1, y = PC_2, color = hclust_clusters)) +
  geom_point() +
  labs(title = "hiarachical cluster",
       x = "PC_1",
       y = "PC_2",
       color = "hclust_clusters") +
  theme_minimal()
```

```
ggarrange(g0, g1, g2, g3, ncol = 2, nrow = 2)
```

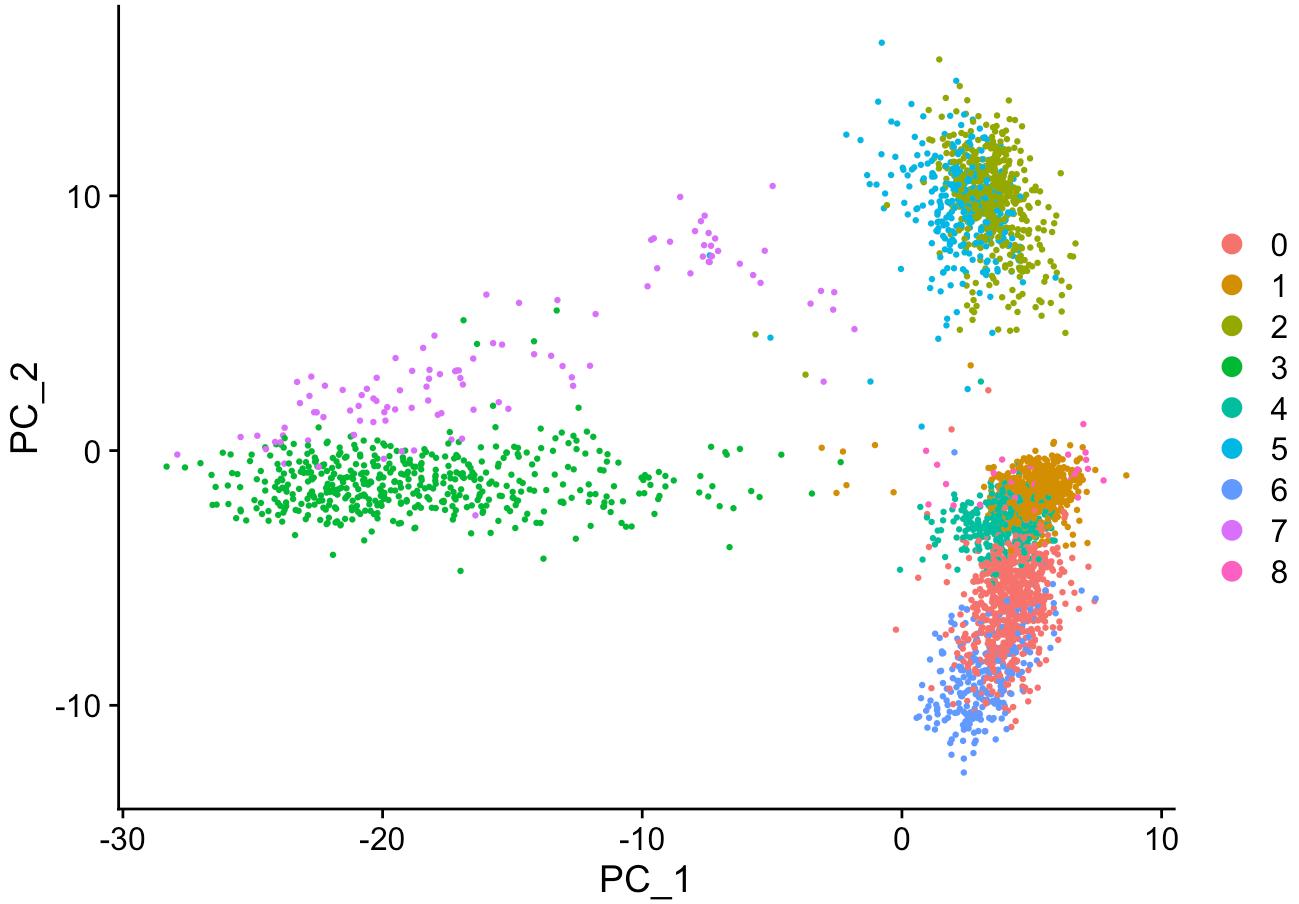









```
DimPlot(pbmc, reduction = "pca")
```



Problem 3

Evaluate and compare the four clustering results using:

(1) Silhouette score [Rfunction] (use the Euclidean distance).

(2) Adjusted rand index (ARI) [Rfunction]. Please refer to this introduction to ARI and provide a brief explanation of it.

Which clustering method has the best performance based on each metric? Can you explain the different conclusions?

```
# silhouette
kmean_sil <- silhouette(kmeans_clusters, dist(pca_embeddings, method = "euclidear

kmeanpp_sil <- silhouette(kmeanspp_clusters, dist(pca_embeddings, method = "eucli

hclust_sil <- silhouette(hclust_res, dist(pca_embeddings, method = "euclidean"))

seurat_sil <- silhouette(as.numeric(Idents(pbmc)), dist(pca_embeddings, method =
```

```
summary(kmean_sil)$avg.width
```

```
[1] 0.1519552
```

```
summary(kmeanpp_sil)$avg.width
```

```
[1] 0.2065472
```

```
summary(hclust_sil)$avg.width
```

```
[1] 0.3325924
```

```
summary(seurat_sil)$avg.width
```

```
[1] 0.1373643
```

```
# ARI
adjustedRandIndex(original_labels, kmeans_clusters)
```

```
[1] 0.640469
```

```
adjustedRandIndex(original_labels, kmeanspp_clusters)
```

```
[1] 0.9153481
```

```
adjustedRandIndex(original_labels, hclust_res)
```

```
[1] 0.2202939
```

```
adjustedRandIndex(original_labels, as.numeric(Idents(pbmc)))
```

```
[1] 0.6865867
```

By evaluating the average of `silhouette` score for each cluster method, hierarchical clustering has the highest score, which means it has the best performance in terns of this measure. By evaluating the `ARI` score, the `kmeans++` method has the highest score, which means it has the best performance in terms of this measure.

The difference is because the `silhouette` score is measures how similar each point is to its own cluster compared to other clusters, while the `ARI` score measures the similarity between the clustering result and a ground truth (reference) classification. It highlights the difference of focus between 2 methods.

Problem 4

Use the following nine combinations for parameters of the Seurat clustering algorithm. How does each parameter influence the number of clusters?

k.param (FindNeighbors)	prune.SNN (FindNeighbors)	resolution (FindClusters)
10	default	default
20	default	default
50	default	default
default	1/5	default
default	1/10	default
default	1/15	default
default	default	0.5
default	default	1
default	default	1.5

```
param <- c(10, 20, 50, 20, 20, 20, 20, 20, 20)
snn <-c(1/15, 1/15, 1/15, 1/7, 1/10, 1/15, 1/15, 1/15, 1/15)
resol <- c(0.8, 0.8, 0.8, 0.8, 0.8, 0.8, 0.5, 1, 1.5)

for (i in 1:9){
  pbmc <- FindNeighbors(pbmc, k.param = param[i], prune.SNN = snn[i])
  pbmc <- FindClusters(pbmc, resolution = resol[i])
}
```

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

Number of nodes: 3362
Number of edges: 49827

Running Louvain algorithm...
Maximum modularity in 10 random starts: 0.8882
Number of communities: 17
Elapsed time: 0 seconds
Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck

Number of nodes: 3362
Number of edges: 119362

Running Louvain algorithm...
Maximum modularity in 10 random starts: 0.8713
Number of communities: 12
Elapsed time: 0 seconds
Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck

Number of nodes: 3362
Number of edges: 275174
```

```
Running Louvain algorithm...
Maximum modularity in 10 random starts: 0.8512
Number of communities: 11
Elapsed time: 0 seconds
Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck

Number of nodes: 3362
Number of edges: 66450

Running Louvain algorithm...
Maximum modularity in 10 random starts: 0.8890
Number of communities: 16
Elapsed time: 0 seconds
Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck

Number of nodes: 3362
Number of edges: 88637

Running Louvain algorithm...
Maximum modularity in 10 random starts: 0.8801
Number of communities: 13
Elapsed time: 0 seconds
Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck

Number of nodes: 3362
Number of edges: 119362

Running Louvain algorithm...
Maximum modularity in 10 random starts: 0.8713
Number of communities: 12
Elapsed time: 0 seconds
Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck

Number of nodes: 3362
Number of edges: 119362

Running Louvain algorithm...
Maximum modularity in 10 random starts: 0.9061
Number of communities: 12
Elapsed time: 0 seconds
Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck

Number of nodes: 3362
Number of edges: 119362

Running Louvain algorithm...
Maximum modularity in 10 random starts: 0.8504
Number of communities: 14
Elapsed time: 0 seconds
Modularity Optimizer version 1.3.0 by Ludo Waltman and Nees Jan van Eck
```

```
Number of nodes: 3362
Number of edges: 119362

Running Louvain algorithm...
Maximum modularity in 10 random starts: 0.8080
Number of communities: 16
Elapsed time: 0 seconds
```

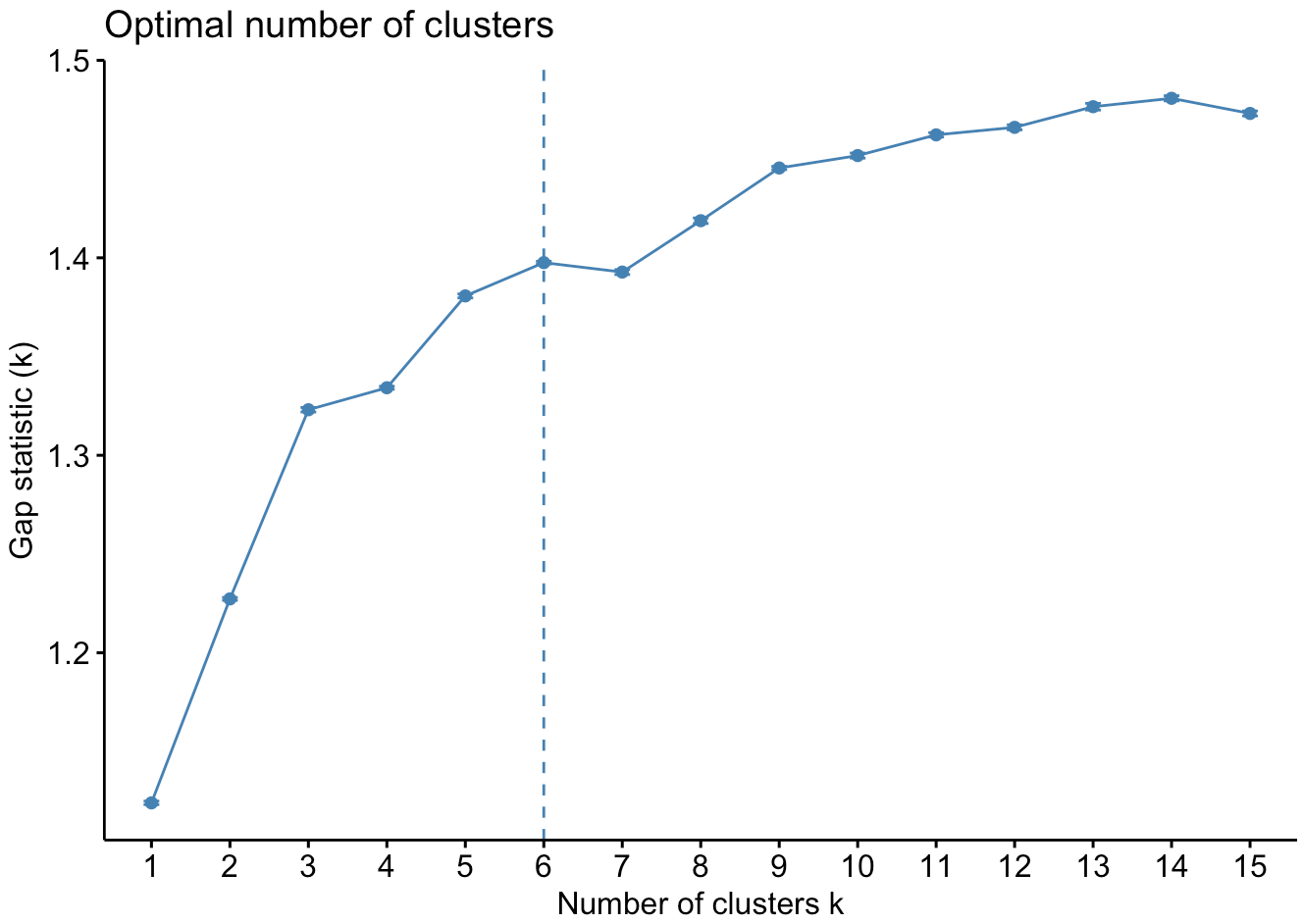
By observing the output, increasing `k.params` will decrease number of clusters. Decreasing `Prune.SNN` will also decrease number of clusters (If `Prune.SNN` is too large, we might get an error since too many of edge is set to 0). In this study, I use `1/7` instead of `1/5` since it gives me error with `1/5`. Increasing `resolution` will increase number of clusters.

Problem 5

Instead of setting k as nine in question 2, please calculate the Gap statistics for kmeans clustering, with k ranging from 1 to 15 [Rfunction]. What is the best k for kmeans based on Gap statistics?

```
set.seed(123)
z <- clusGap(pca_embeddings, FUN = kmeans, K.max = 15, B = 5)
```

```
fviz_gap_stat(z)
```



The best k for `kmean` is 6 based on gap statistic. Due to time constraint, the number of permutation is set to 5 which will not give consistent result because of standard error. If we increase the number of permutation, we will get more consistent result.

Problem 6

Perform the tight clustering on the PCA results (50 PCs) [Rfunction]. Set `target` =9, and `k.min` =15. What are the meanings of these two parameters? Plot cells using their scores of the first two PCs, coloring them according to the cluster labels from tight clustering results (use the gray color for cells labeled -1). Please explain why some cells are labeled as -1.

```
tight_clus <- tight.clust(pca_embeddings, target = 9, k.min = 15)
```

```
Number of points: 3362  Dimension: 50

Looking for tight cluster 1 ...
k = 15
k = 16
1 tight cluster(s) found!
Cluster size: 302   Remaining number of points: 3060
```

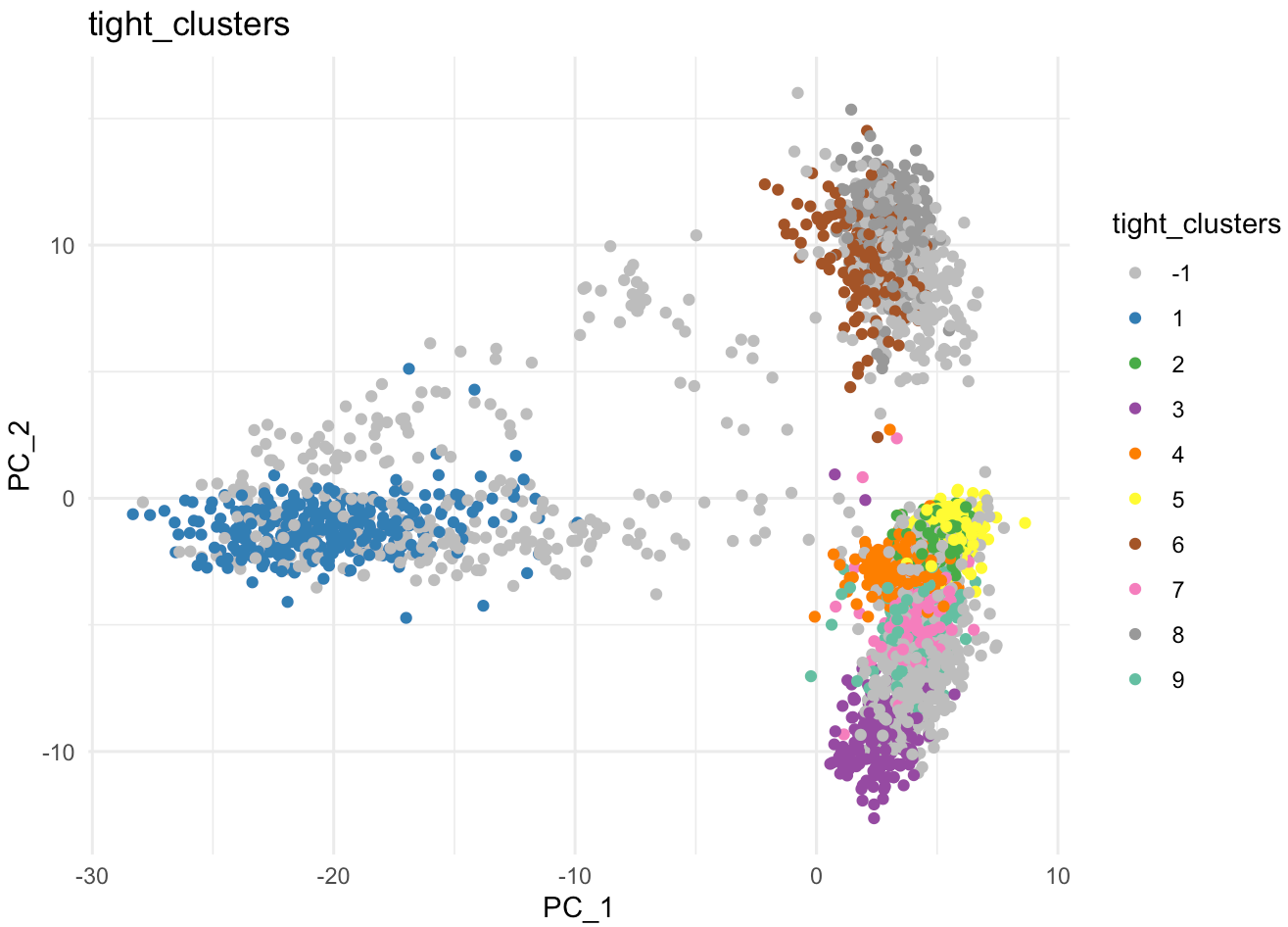
```
Looking for tight cluster 2 ...
k = 14
k = 15
2 tight cluster(s) found!
Cluster size: 253   Remaining number of points: 2807

Looking for tight cluster 3 ...
k = 13
k = 14
3 tight cluster(s) found!
Cluster size: 190   Remaining number of points: 2617

Looking for tight cluster 4 ...
k = 12
k = 13
4 tight cluster(s) found!
Cluster size: 298   Remaining number of points: 2319

Looking for tight cluster 5 ...
k = 11
k = 12
5 tight cluster(s) found!
Cluster size: 217   Remaining number of points: 2102

Looking for tight cluster 6 ...
k = 10
k = 11
6 tight cluster(s) found!
Cluster size: 199   Remaining number of points: 1903

Looking for tight cluster 7 ...
k = 9
k = 10
7 tight cluster(s) found!
Cluster size: 185   Remaining number of points: 1718

Looking for tight cluster 8 ...
k = 8
k = 9
8 tight cluster(s) found!
Cluster size: 337   Remaining number of points: 1381

Looking for tight cluster 9 ...
k = 7
k = 8
9 tight cluster(s) found!
Cluster size: 155   Remaining number of points: 1226
```

```
PC1 <- pca_embeddings[, 1]
PC2 <- pca_embeddings[, 2]
# Create a data frame with PC1, PC2, and cluster labels
df <- data.frame(PC_1 = PC1, PC_2 = PC2, tight_clusters = as.factor(tight_clus$cl
```

```
levels(df$tight_clusters)
```

```
[1] "-1" "1"  "2"  "3"  "4"  "5"  "6"  "7"  "8"  "9"
```

```
custom_colors <- c(
  "-1" = "gray",
  "1" = "#377EB8",
  "2" = "#4DAF4A",
  "3" = "#984EA3",
  "4" = "#FF7F00",
  "5" = "#FFFF33",
  "6" = "#A65628",
  "7" = "#F781BF",
  "8" = "#999999",
  "9" = "#66C2A5"
)

ggplot(df, aes(x = PC_1, y = PC_2, color = tight_clusters)) +
  geom_point() +
  labs(title = "tight_clusters",
       x = "PC_1",
       y = "PC_2",
       color = "tight_clusters") +
  scale_color_manual(values = custom_colors, na.translate = FALSE) +
  theme_minimal()
```



`target` is the total number of clusters we want. `k.min` is the a starting point for the iterations. The algorithm will stop when k is updated to be smaller than certain threhold. Some cells are labeled as -1 because they are not being assigned to any of the clusters, which is a characteristic of tight cluster.