Lab 7

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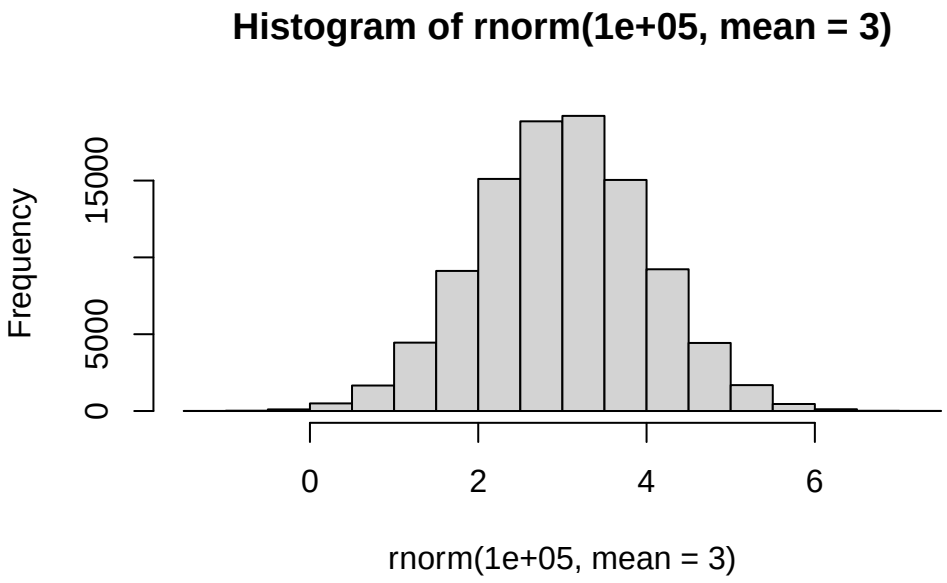
Clustering

K-means clustering

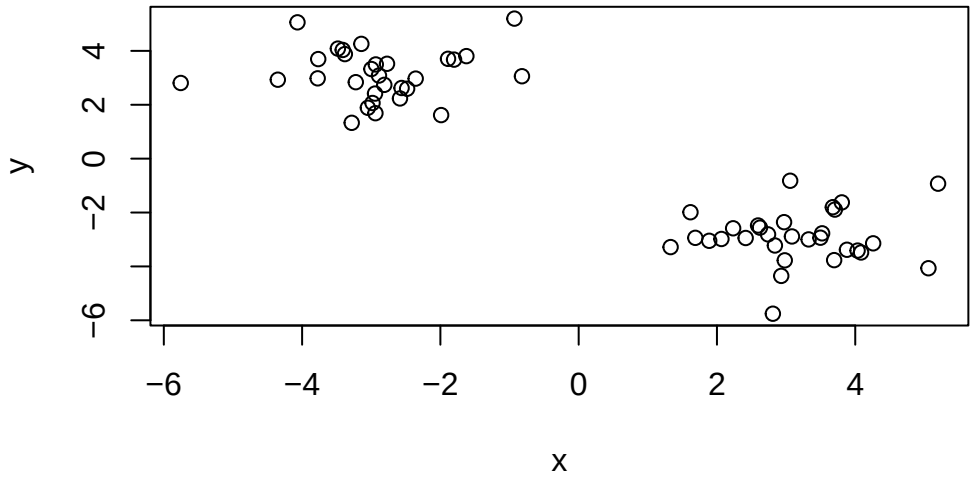
```
hist(rnorm(100000, mean=3))
```




```
tmp <- c(rnorm(30,3), rnorm(30,-3))
a <- cbind(x=tmp, y=rev(tmp))
head(a)
```

```
           x         y
[1,] 5.058695 -4.067132
[2,] 2.738686 -2.811005
[3,] 3.704893 -1.889931
[4,] 3.521197 -2.771627
[5,] 3.805522 -1.623905
[6,] 2.929767 -4.351384
```

```
plot(a)
```



K-means function: `kmeans()`

```
k <- kmeans(a, centers=2, nstart=20)
k
```

```
K-means clustering with 2 clusters of sizes 30, 30

Cluster means:
          x         y
1 -2.899911  3.120039
2  3.120039 -2.899911
```

```
Clustering vector:
 [1] 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 1 1 1 1 1 1 1
[39] 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1

Within cluster sum of squares by cluster:
[1] 53.80285 53.80285
 (between_SS / total_SS =  91.0 %)

Available components:

[1] "cluster"      "centers"      "totss"        "withinss"     "tot.withinss"
[6] "betweenss"    "size"         "iter"         "ifault"
```

```
k$size  #size of each cluster
```

```
[1] 30 30
```

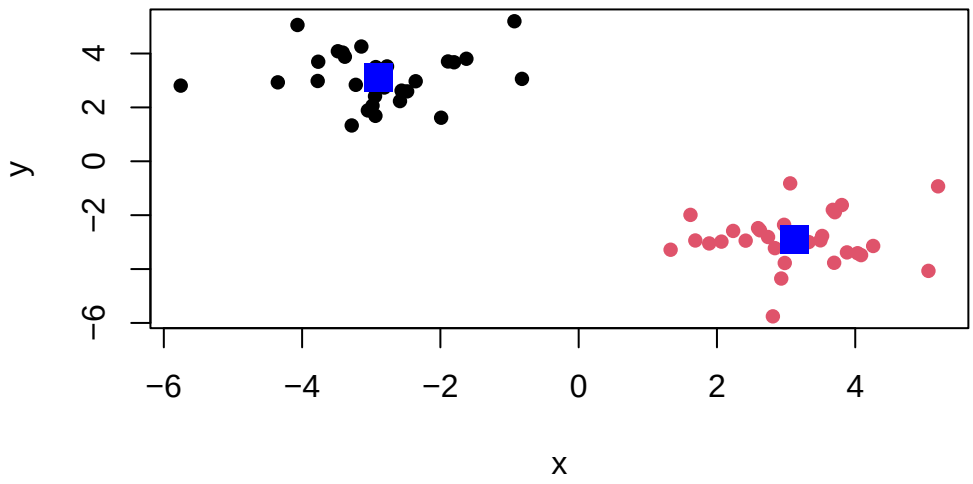
```
k$cluster #membership vectors
```

```
 [1] 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 1 1 1 1 1 1 1
[39] 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
```

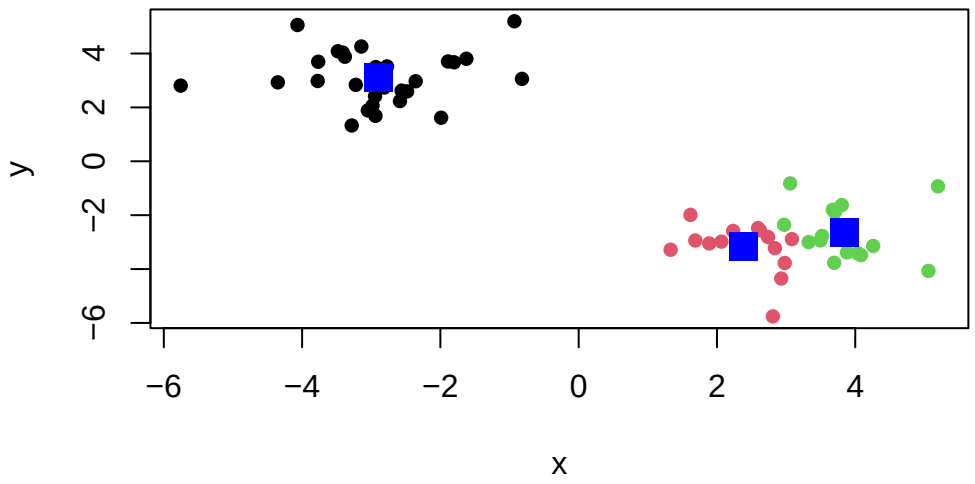
```
k$centers #center of cluster
```

```
          x         y
1 -2.899911  3.120039
2  3.120039 -2.899911
```

```
plot(a, col=k$cluster, pch=16)
points(k$centers, col="blue", pch=15, cex=2)
```




```
k3 <- kmeans(a, centers=3, nstart=20)
plot(a, col=k3$cluster, pch=16)
points(k3$centers, col="blue", pch=15, cex=2)
```



Hierarchical Clustering

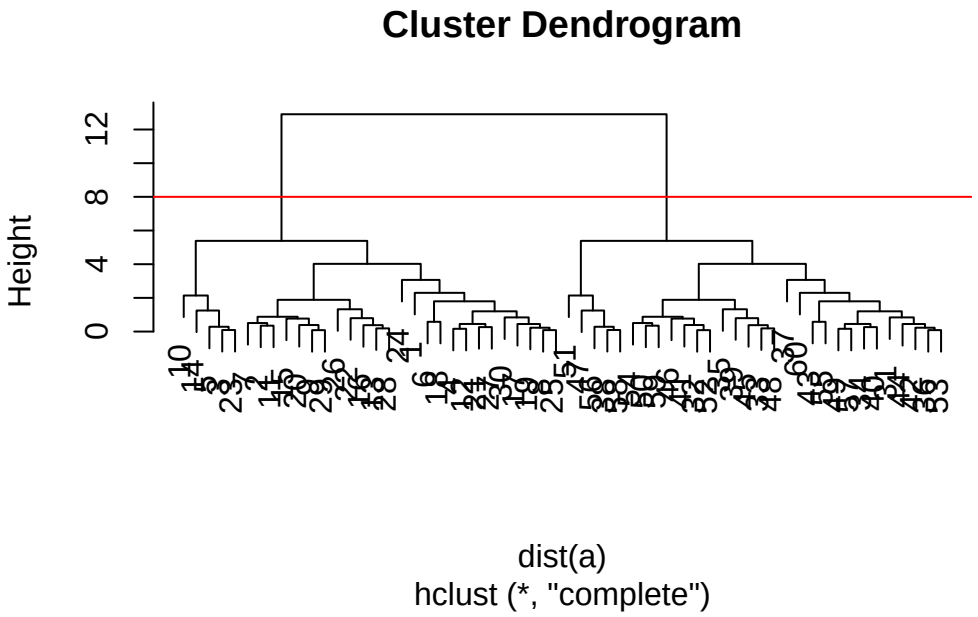
Reveal structure rather than imposing strcture (k-means)

Function (base R): `hclust()`, requires distance matrix as input

```
hc <- hclust(dist(a))
hc
```

```
Call:
hclust(d = dist(a))

Cluster method   : complete
Distance         : euclidean
Number of objects: 60
```

```
plot(hc)
abline(h=8, col="red")
```

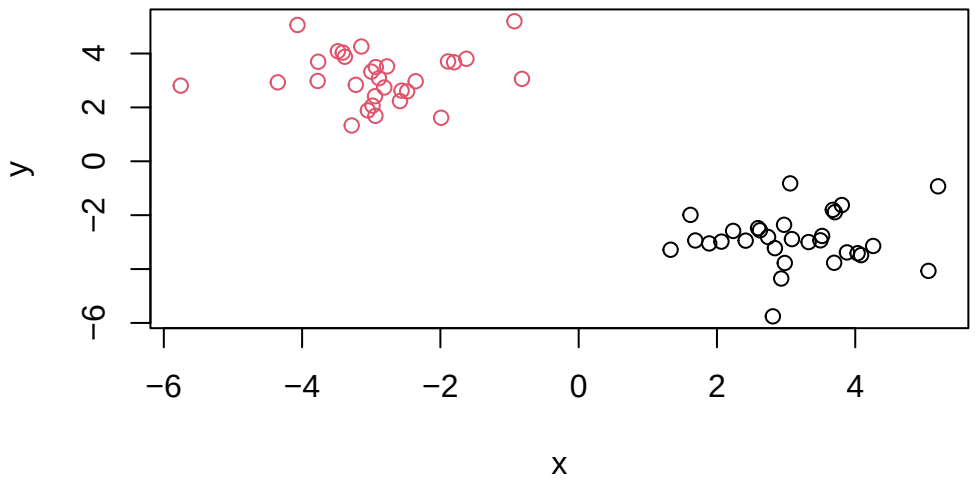


Function to get clusters/groups from a hclust object: `cutree()`

```
grps <- cutree(hc, h=8)
grps
```

```
 [1] 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2
[39] 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
```

```
plot(a, col=grps)
```



Principal Component Alalysis (PCA)

```
url <- "https://tinyurl.com/UK-foods"
x <- read.csv(url)
head(x)
```

```
              X England Wales Scotland N.Ireland
1        Cheese     105   103      103        66
2  Carcass_meat     245   227      242       267
3    Other_meat     685   803      750       586
4          Fish     147   160      122        93
5 Fats_and_oils     193   235      184       209
6        Sugars     156   175      147       139
```

```
dim(x)
```

```
[1] 17  5
```

Q1. 17 rows, 5 columns

```
rownames(x) <- x[,1]
x <- x[,-1]
head(x)
```

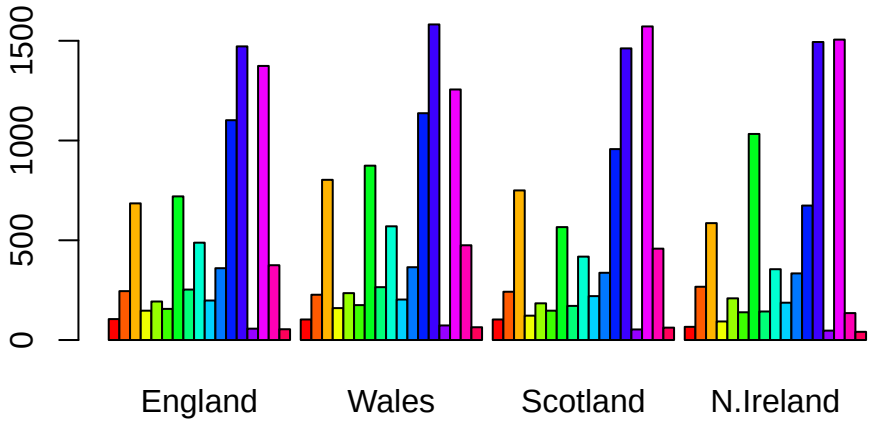
```
              England Wales Scotland N.Ireland
Cheese            105   103      103        66
Carcass_meat      245   227      242       267
Other_meat        685   803      750       586
Fish              147   160      122        93
Fats_and_oils     193   235      184       209
Sugars            156   175      147       139
```

```
x <- read.csv(url, row.names=1)
head(x)
```

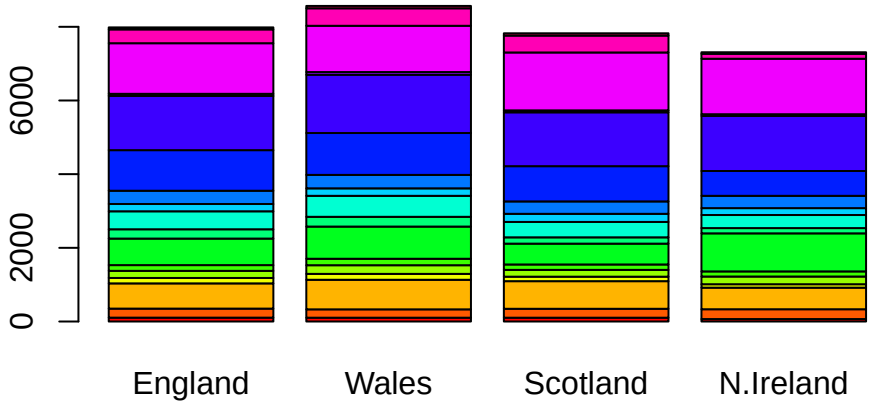
```
              England Wales Scotland N.Ireland
Cheese            105   103      103        66
Carcass_meat      245   227      242       267
Other_meat        685   803      750       586
Fish              147   160      122        93
Fats_and_oils     193   235      184       209
Sugars            156   175      147       139
```

Q2. `x <- read.csv(url, row.names=1)`. The dataframe can loose data if `x <- x[,-1]` is run multiple times.

```
barplot(as.matrix(x), beside=T, col=rainbow(nrow(x)))
```

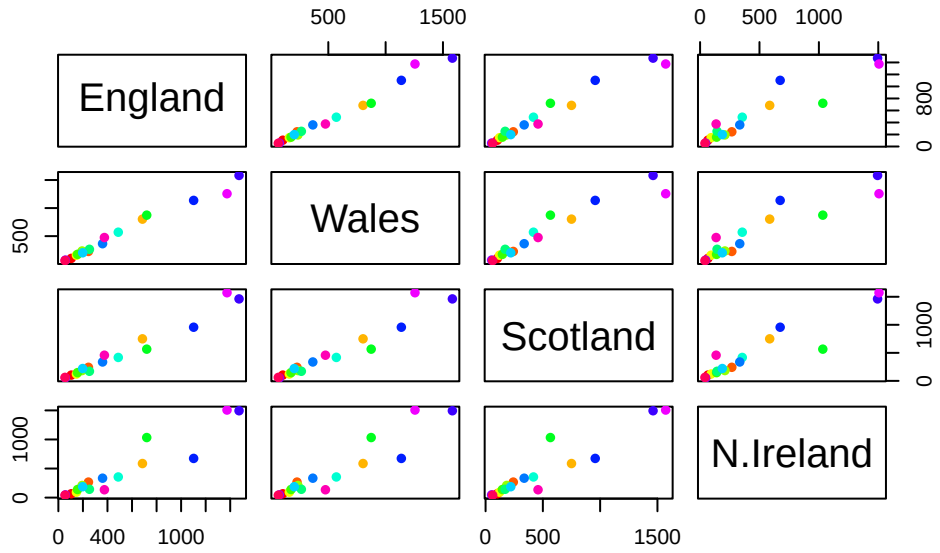



```
barplot(as.matrix(x), beside=F, col=rainbow(nrow(x)))
```



Q4. `beside=F` will create the stacked bar plot

```
pairs(x, col=rainbow(17), pch=16)
```



Q5. A given point lies on the diagonal for a given plot suggests that the two countries have the same values in the category that point belongs to.

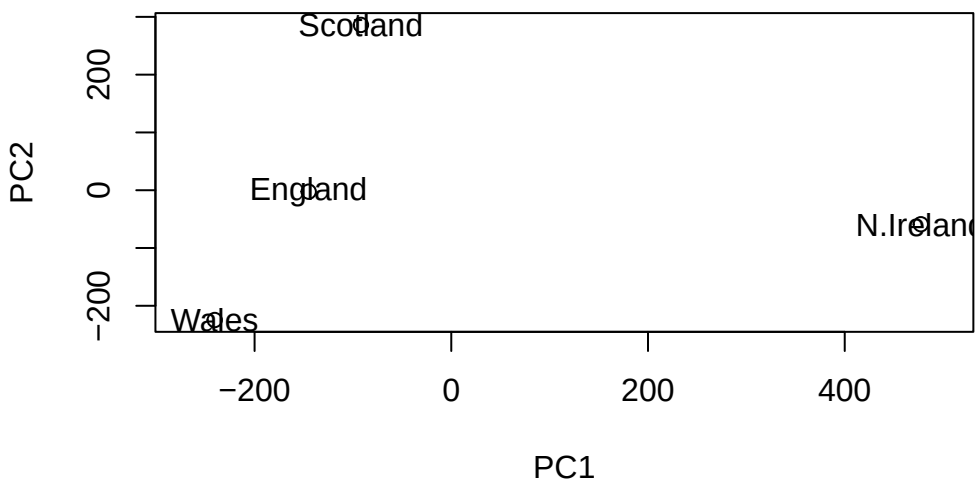
Q6. Points in plots comparing N.Ireland and other countries are further away from the diagonal of the plots.

Function (PCA in base R): `prcomp()`

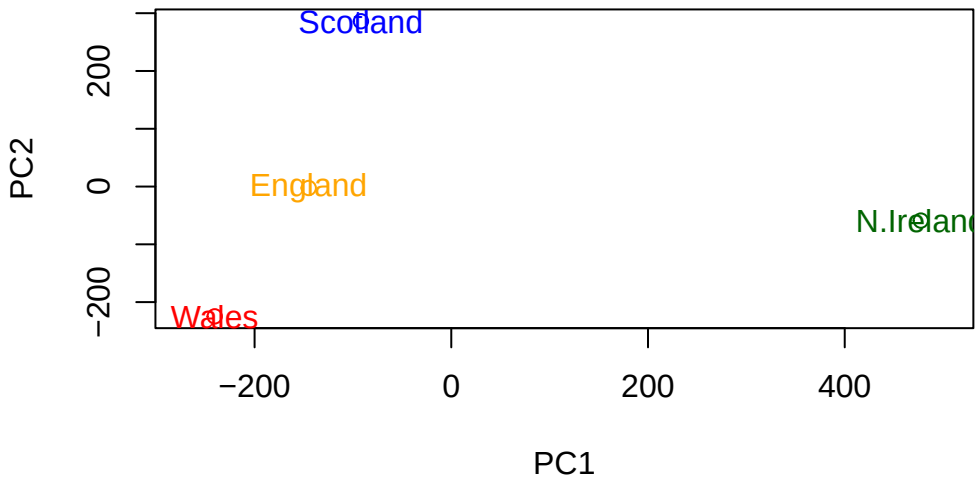
```
pca <- prcomp(t(x)) #t(): transpose df (row<->column)
summary(pca)
```

```
Importance of components:
                            PC1      PC2      PC3        PC4
Standard deviation     324.1502 212.7478 73.87622 3.176e-14
Proportion of Variance   0.6744   0.2905  0.03503 0.000e+00
Cumulative Proportion    0.6744   0.9650  1.00000 1.000e+00
```

```
plot(pca$x[,1], pca$x[,2], xlab="PC1", ylab="PC2", xlim=c(-270,500))
text(pca$x[,1], pca$x[,2], colnames(x))
```




```
plot(pca$x[,1], pca$x[,2], xlab="PC1", ylab="PC2", xlim=c(-270,500), col=c("orange","red",
text(pca$x[,1], pca$x[,2], colnames(x), col=c("orange","red","blue","darkgreen"))
```



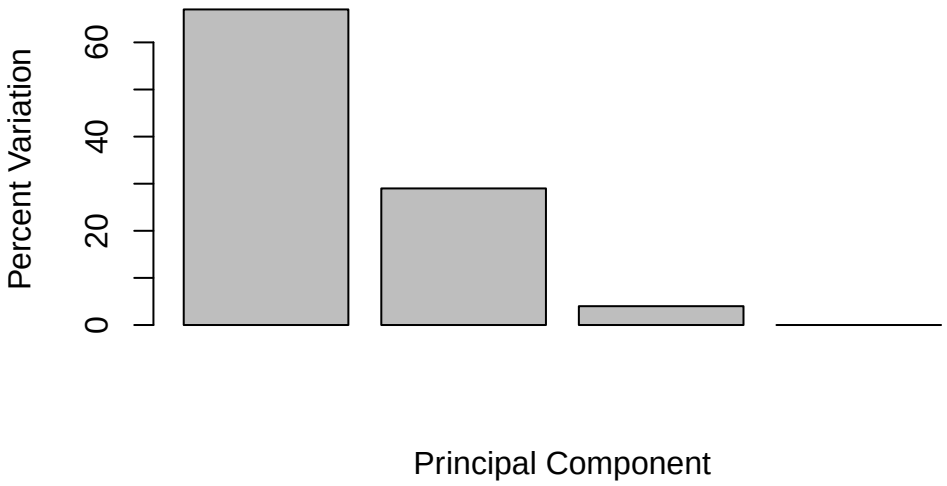

```
v <- round( pca$sdev^2/sum(pca$sdev^2) * 100 )
v
```

```
[1] 67 29  4  0
```

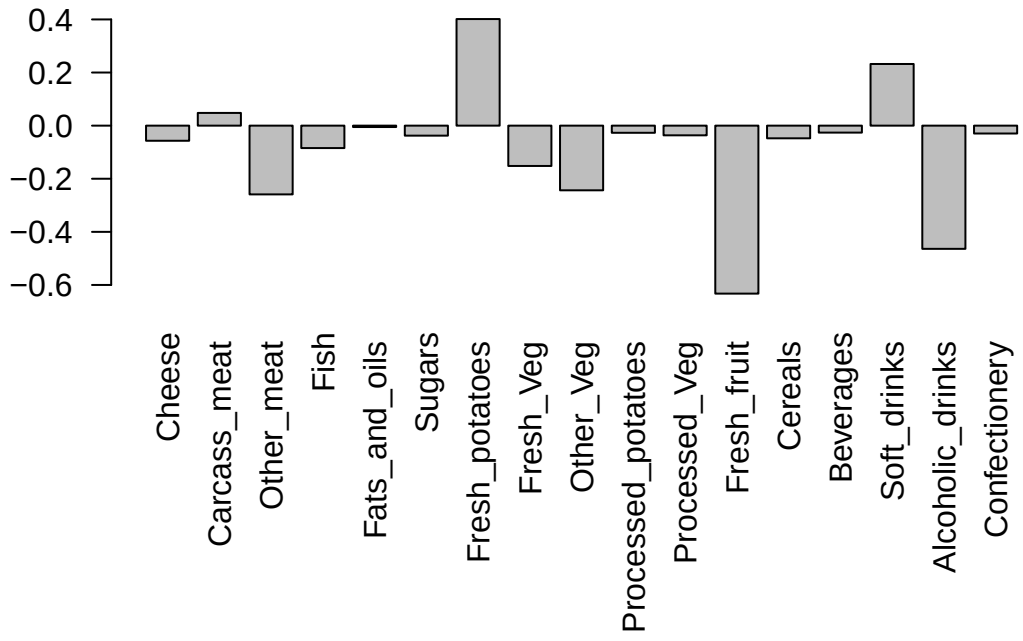
```
z <- summary(pca)
z$importance
```

```
                             PC1       PC2      PC3           PC4
Standard deviation     324.15019 212.74780 73.87622 3.175833e-14
Proportion of Variance   0.67444   0.29052  0.03503 0.000000e+00
Cumulative Proportion    0.67444   0.96497  1.00000 1.000000e+00
```

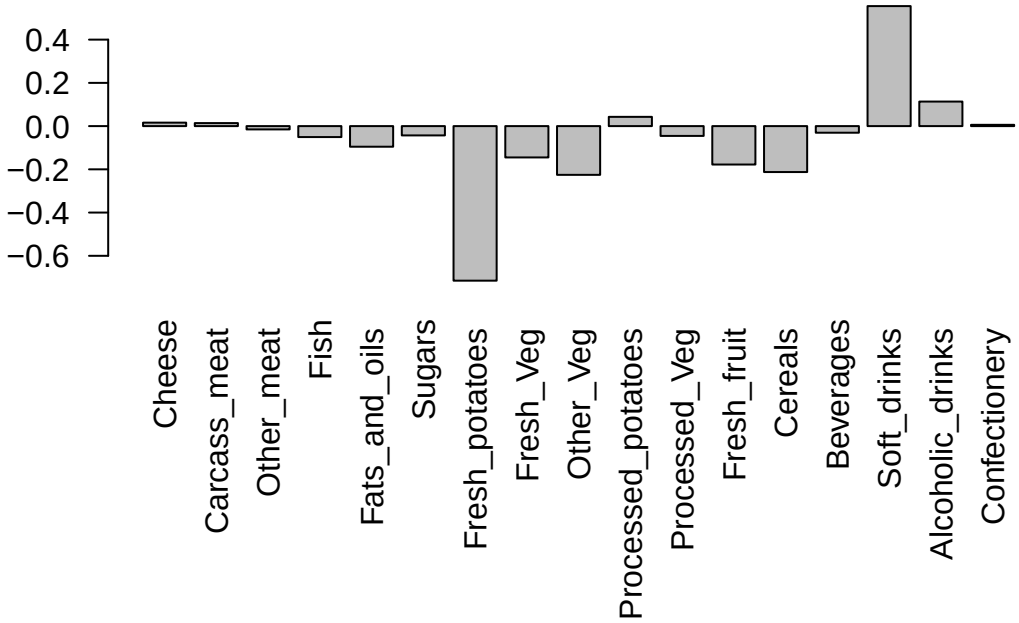
```
barplot(v, xlab="Principal Component", ylab="Percent Variation")
```




```
par(mar=c(10, 3, 0.35, 0))
barplot( pca$rotation[,1], las=2 )
```



```
par(mar=c(10, 3, 0.35, 0))
barplot( pca$rotation[,2], las=2 )
```

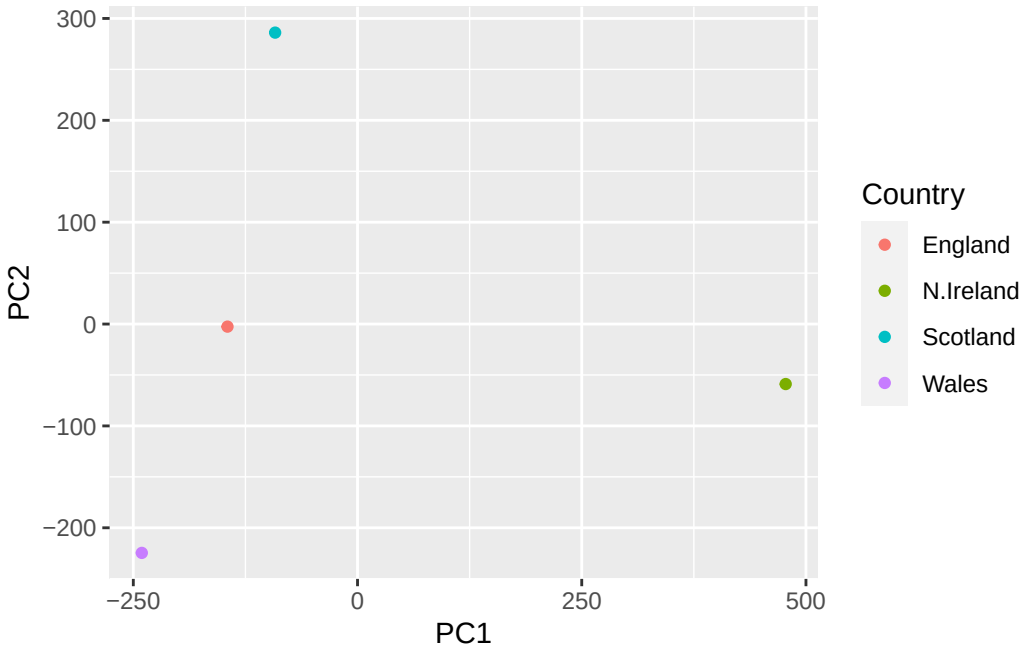


Q9. Fresh potatoes and soft drinks. PC2 tells the major difference between Whales and Scotland lies within these two categories.

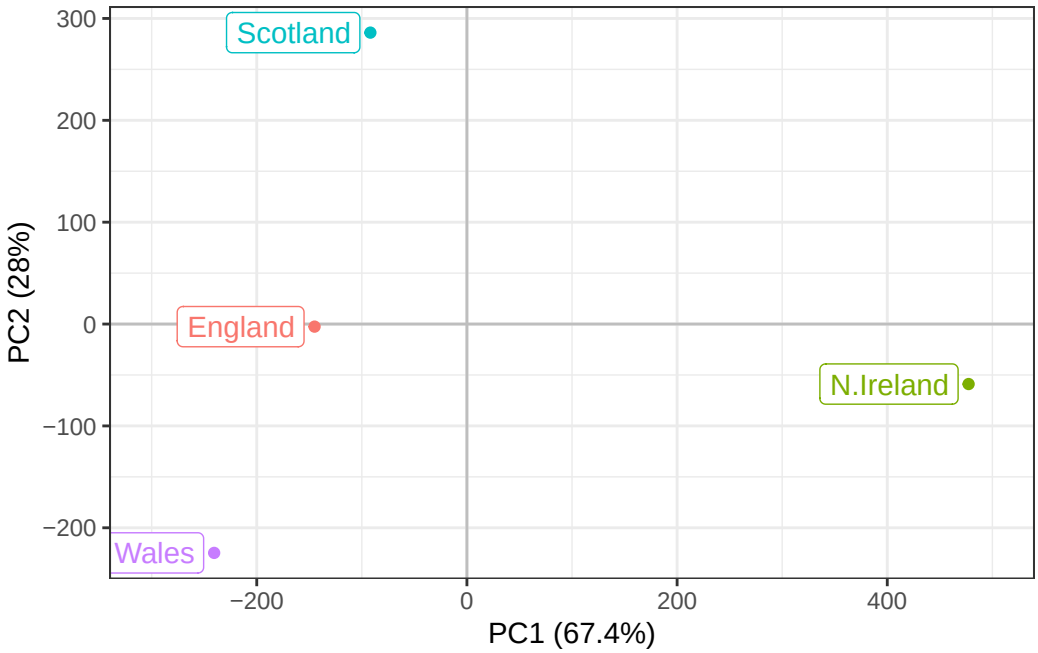
```
library(ggplot2)
```

```
Warning: package 'ggplot2' was built under R version 4.3.1
```

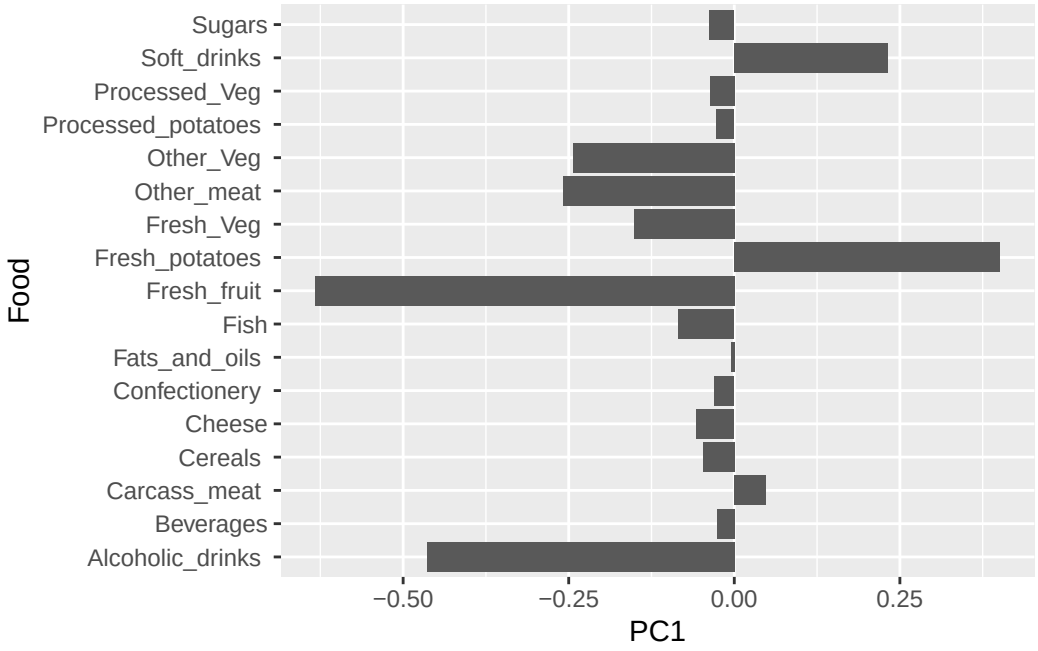
```
df <- as.data.frame(pca$x)
df_lab <- tibble::rownames_to_column(df, "Country")

ggplot(df_lab) +
  aes(PC1, PC2, col=Country) +
  geom_point()
```



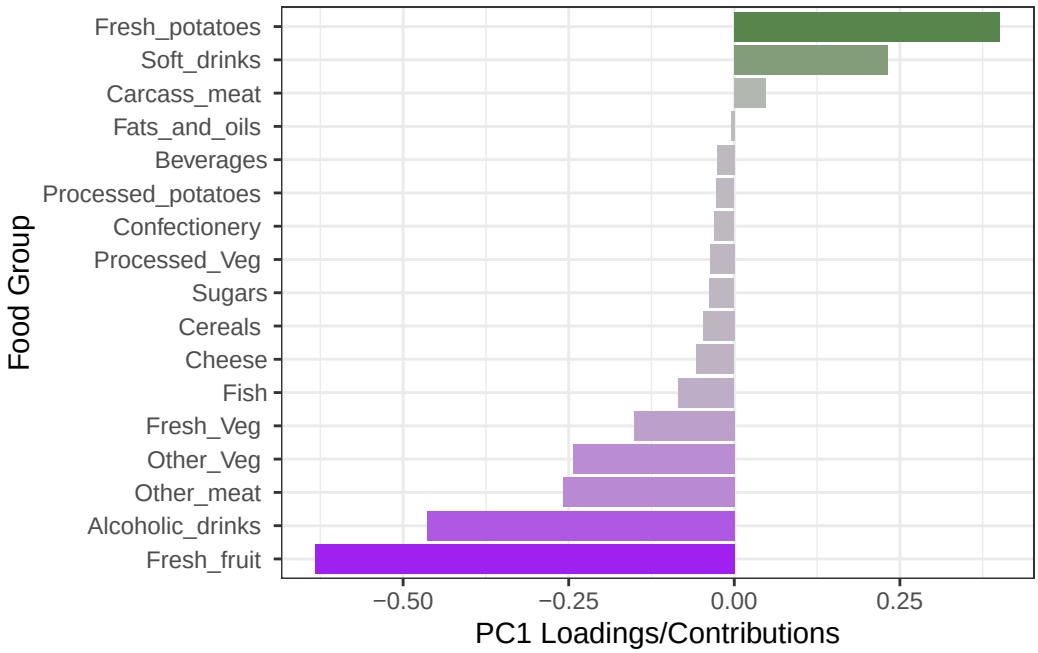

```
ggplot(df_lab) +
  aes(PC1, PC2, col=Country, label=Country) +
  geom_hline(yintercept = 0, col="gray") +
  geom_vline(xintercept = 0, col="gray") +
  geom_point(show.legend = FALSE) +
  geom_label(hjust=1, nudge_x = -10, show.legend = FALSE) +
  expand_limits(x = c(-300,500)) +
  xlab("PC1 (67.4%)") +
  ylab("PC2 (28%)") +
  theme_bw()
```



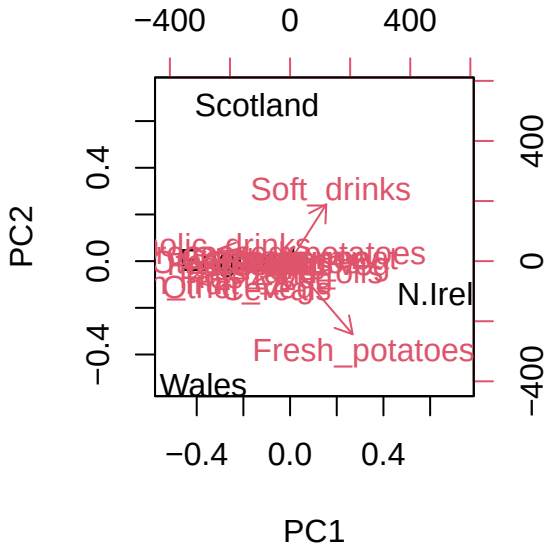

```
ld <- as.data.frame(pca$rotation)
ld_lab <- tibble::rownames_to_column(ld, "Food")

ggplot(ld_lab) +
  aes(PC1, Food) +
  geom_col()
```




```
ggplot(ld_lab) +
  aes(PC1, reorder(Food, PC1), bg=PC1) +
  geom_col() +
  xlab("PC1 Loadings/Contributions") +
  ylab("Food Group") +
  scale_fill_gradient2(low="purple", mid="gray", high="darkgreen", guide=NULL) +
  theme_bw()
```


Fresh_potatoes
Soft_drinks
Carcass_meat
Fats_and_oils
Beverages
Processed_potatoes
Confectionery
Processed_Veg
Sugars
Cereals
Cheese
Fish
Fresh_Veg
Other_Veg
Other_meat
Alcoholic_drinks
Fresh_fruit
Food Group
−0.50
−0.25
0.00
0.25
PC1 Loadings/Contributions

```
biplot(pca)
```


−400
0
400
Scotland
Soft_drinks
N.Irel
Fresh_potatoes
Wales
0.4
0.0
−0.4
PC2
400
0
−400
−0.4
0.0
0.4
PC1

```
url2 <- "https://tinyurl.com/expression-CSV"
rna.data <- read.csv(url2, row.names=1)
head(rna.data)
```

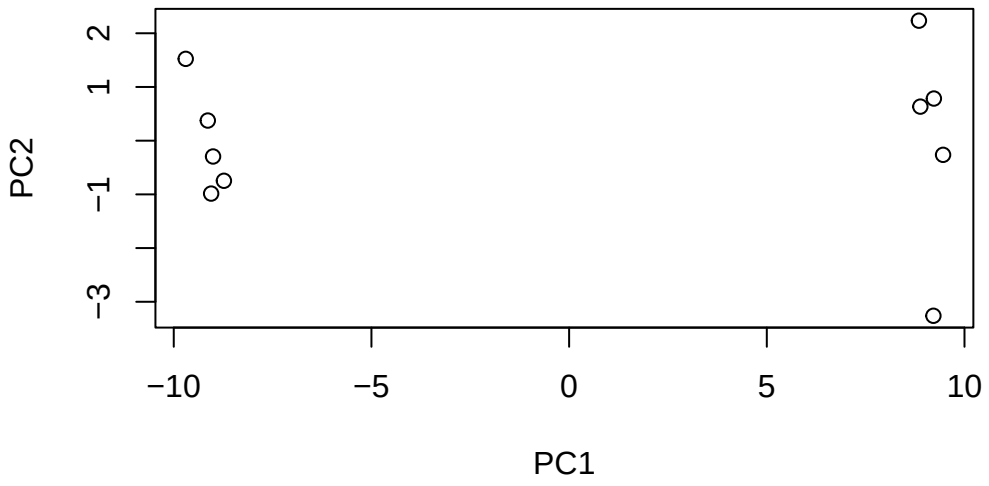
```
       wt1 wt2  wt3  wt4 wt5 ko1 ko2 ko3 ko4 ko5
gene1  439 458  408  429 420  90  88  86  90  93
gene2  219 200  204  210 187 427 423 434 433 426
gene3 1006 989 1030 1017 973 252 237 238 226 210
gene4  783 792  829  856 760 849 856 835 885 894
gene5  181 249  204  244 225 277 305 272 270 279
gene6  460 502  491  491 493 612 594 577 618 638
```

```
dim(rna.data)
```

```
[1] 100  10
```

Q10. 100 genes, 10 samples

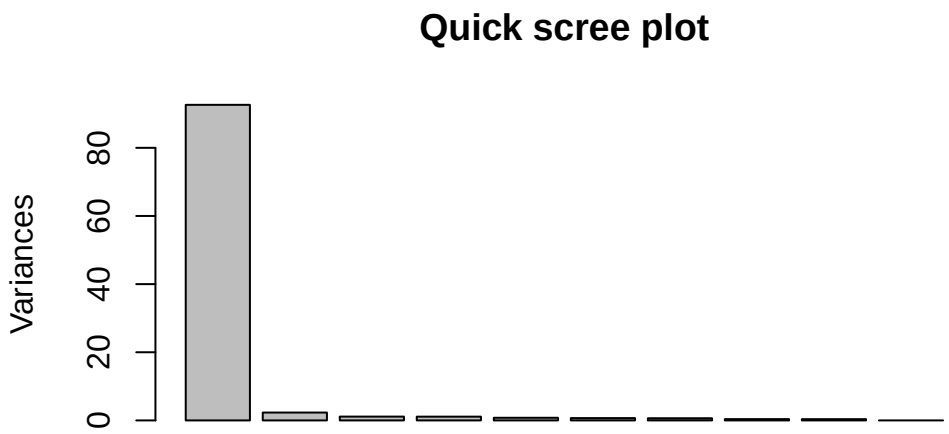
```
pca <- prcomp(t(rna.data), scale=TRUE)
plot(pca$x[,1], pca$x[,2], xlab="PC1", ylab="PC2")
```



```
summary(pca)
```

```
Importance of components:
                          PC1    PC2     PC3     PC4     PC5     PC6     PC7
Standard deviation     9.6237 1.5198 1.05787 1.05203 0.88062 0.82545 0.80111
Proportion of Variance 0.9262 0.0231 0.01119 0.01107 0.00775 0.00681 0.00642
Cumulative Proportion  0.9262 0.9493 0.96045 0.97152 0.97928 0.98609 0.99251
                           PC8     PC9      PC10
Standard deviation     0.62065 0.60342 3.457e-15
Proportion of Variance 0.00385 0.00364 0.000e+00
Cumulative Proportion  0.99636 1.00000 1.000e+00
```

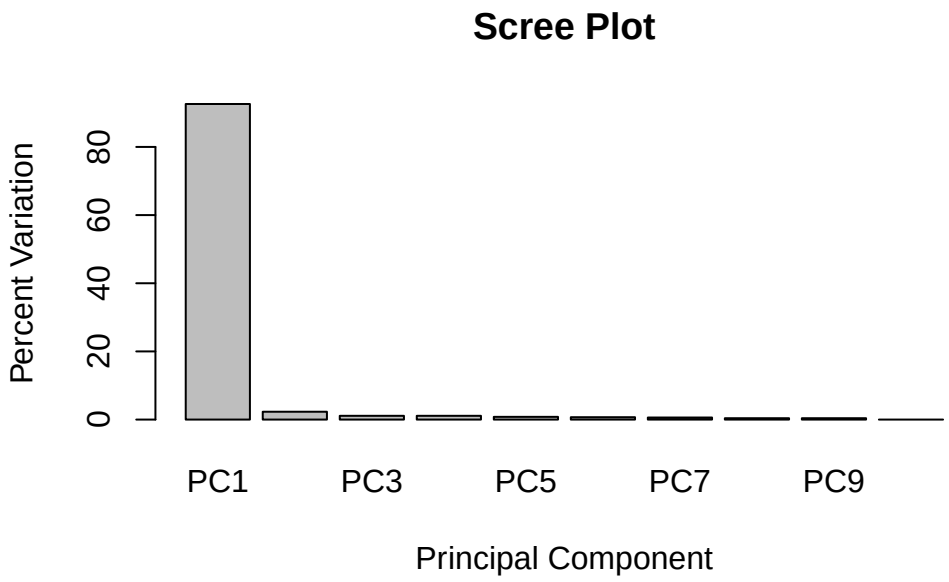
```
plot(pca, main="Quick scree plot")
```



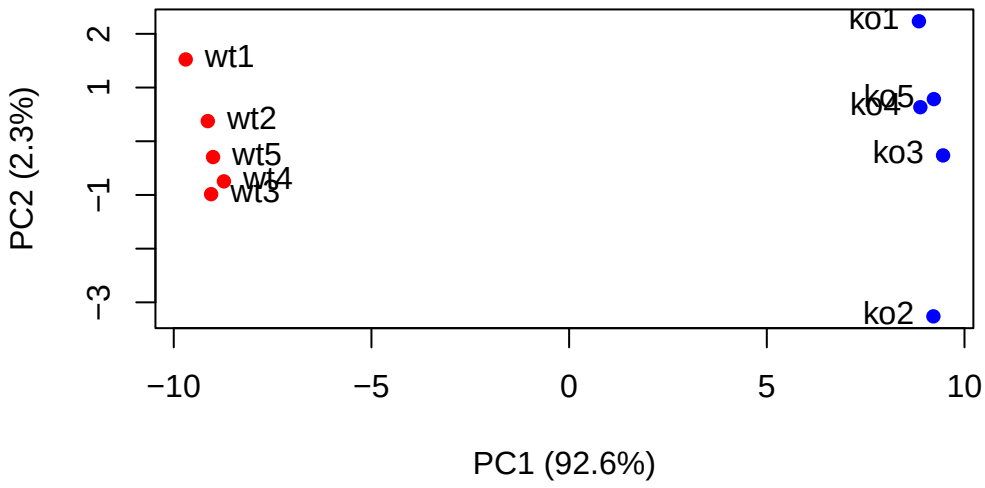

```
#Percent variance: percent of difference accounted for by each PC
pca.var <- pca$sdev^2

pca.var.per <- round(pca.var/sum(pca.var)*100, 1)
pca.var.per
```

```
[1] 92.6  2.3  1.1  1.1  0.8  0.7  0.6  0.4  0.4  0.0
```

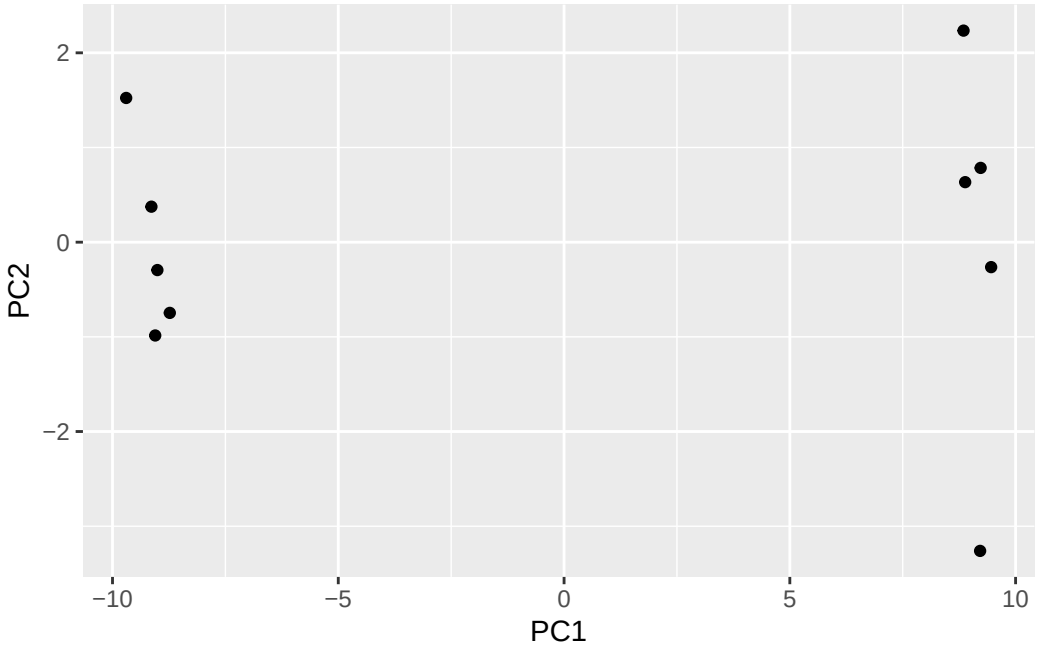
```
barplot(pca.var.per, main="Scree Plot",
        names.arg = paste0("PC", 1:10),
        xlab="Principal Component", ylab="Percent Variation")
```



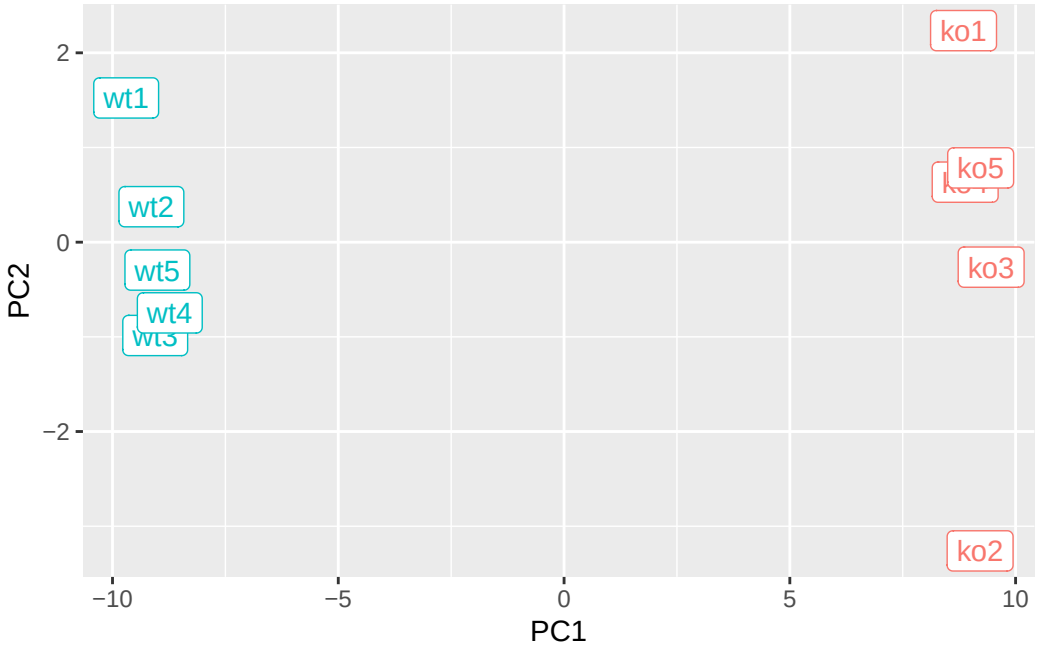

```
colvec <- colnames(rna.data)
colvec[grep("wt", colvec)] <- "red"
colvec[grep("ko", colvec)] <- "blue"

plot(pca$x[,1], pca$x[,2], col=colvec, pch=16,
     xlab=paste0("PC1 (", pca.var.per[1], "%)"),
     ylab=paste0("PC2 (", pca.var.per[2], "%)"))

text(pca$x[,1], pca$x[,2], labels = colnames(rna.data), pos=c(rep(4,5), rep(2,5)))
```



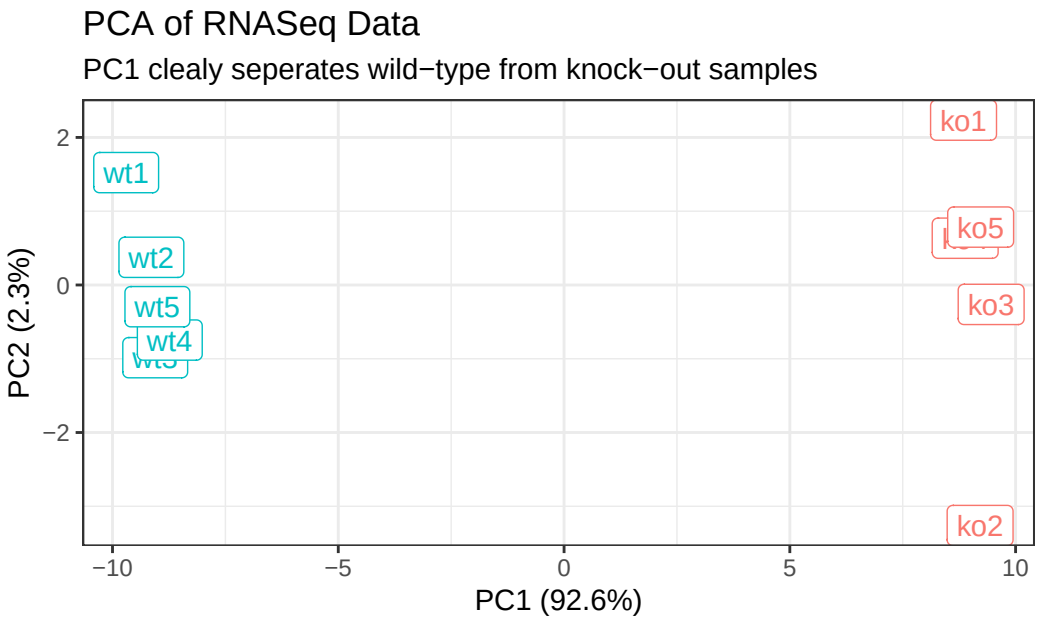

```
df <- as.data.frame(pca$x)

ggplot(df) +
  aes(PC1, PC2) +
  geom_point()
```




```
df$samples <- colnames(rna.data)
df$condition <- substr(colnames(rna.data),1,2)

p <- ggplot(df) +
        aes(PC1, PC2, label=samples, col=condition) +
        geom_label(show.legend = FALSE)
p
```




```
p + labs(title="PCA of RNASeq Data",
       subtitle = "PC1 clealy seperates wild-type from knock-out samples",
       x=paste0("PC1 (", pca.var.per[1], "%)"),
       y=paste0("PC2 (", pca.var.per[2], "%)"),
       caption="Class example data") +
     theme_bw()
```




```
#Gene load: find top 10 genes that contribute to PC1 in either direction
loading_scores <- pca$rotation[,1]
gene_scores <- abs(loading_scores)
gene_score_ranked <- sort(gene_scores, decreasing=TRUE)

## show the names of the top 10 genes
top_10_genes <- names(gene_score_ranked[1:10])
top_10_genes
```

```
[1] "gene100" "gene66"  "gene45"  "gene68"  "gene98"  "gene60"  "gene21"
[8] "gene56"  "gene10"  "gene90"
```