Class 7

Jimmi Nguyen

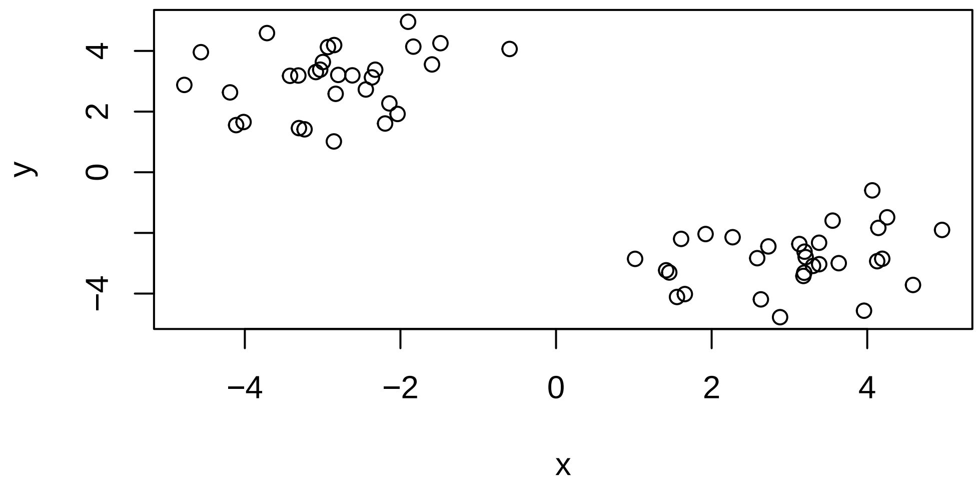
```
url <- "https://tinyurl.com/UK-foods"
x <- read.csv(url)
```

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

```
             x        y
[1,] -2.197415 1.607807
[2,] -2.037092 1.922347
[3,] -2.832612 2.585784
[4,] -2.618156 3.194086
[5,] -2.852086 4.192869
[6,] -2.997077 3.633939
```

Quick plot of x to see the two groups at -3,+3 and +3,-3

```
plot(x)
```



Use `kmeans()` function setting k to 2 and nstart = 20

```
km = kmeans(x, centers = 2, nstart = 20)
km
```

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

Cluster means:
          x         y
1 -2.852485  3.039061
2  3.039061 -2.852485

Clustering vector:
 [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 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

Within cluster sum of squares by cluster:
[1] 57.89978 57.89978
 (between_SS / total_SS =  90.0 %)

Available components:
```

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

Q. How many points are in each cluster?

```r
km$size
```

```
[1] 30 30
```

Q. What 'component' of your results object details - cluster assignment/membership - cluster center?

```r
km$cluster
```

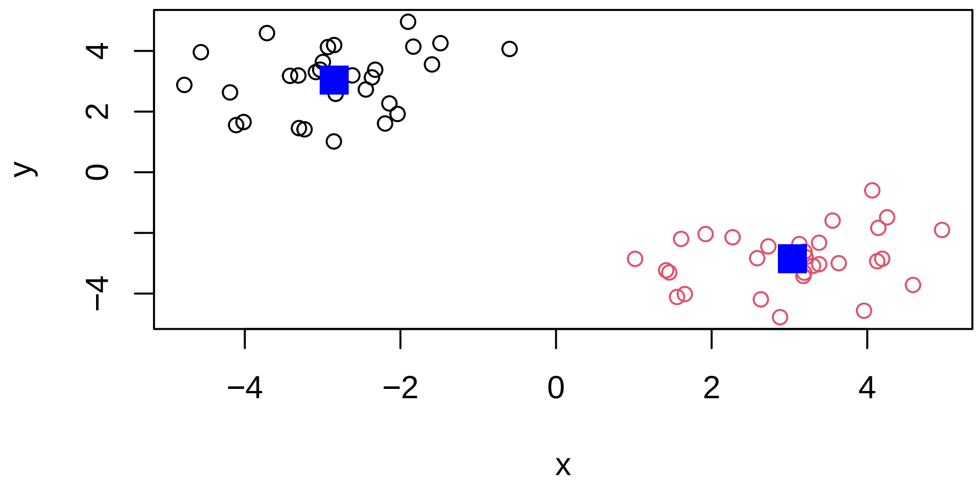
```
 [1] 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2
[39] 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
```

```r
km$centers
```

```
          x         y
1 -2.852485  3.039061
2  3.039061 -2.852485
```

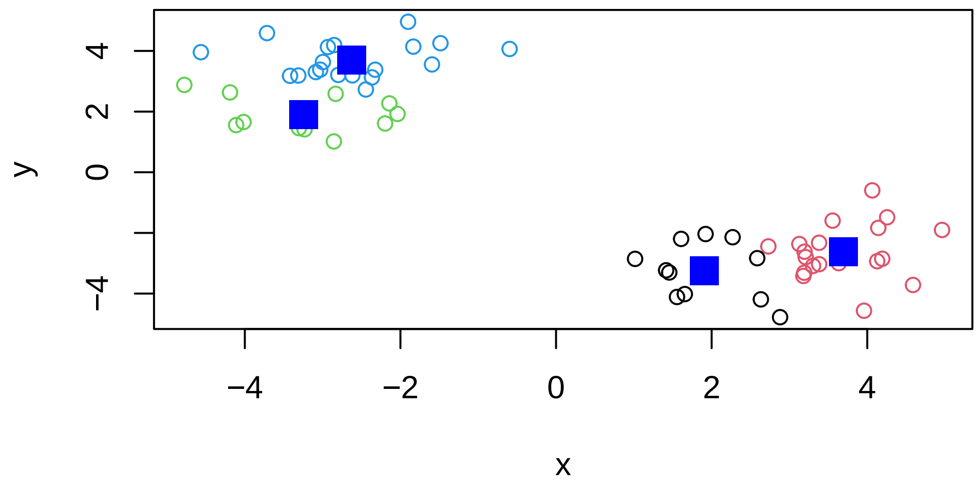
Q. Plot x colored by the kmeans cluster assignment and add cluster centers as blue points

```r
plot(x, col=km$cluster)
points(km$centers, col="blue", pch=15, cex=2)
```



Play with kmeans and ask for different number of clusters

```
km = kmeans(x, centers = 4, nstart = 20)
plot(x, col=km$cluster)
points(km$centers, col="blue", pch=15, cex=2)
```



Hierarchical Clustering

This is another very useful and widely emploued clusting method which has the advantage over kmeans in that it can help reveal the something of true grouping in your data.

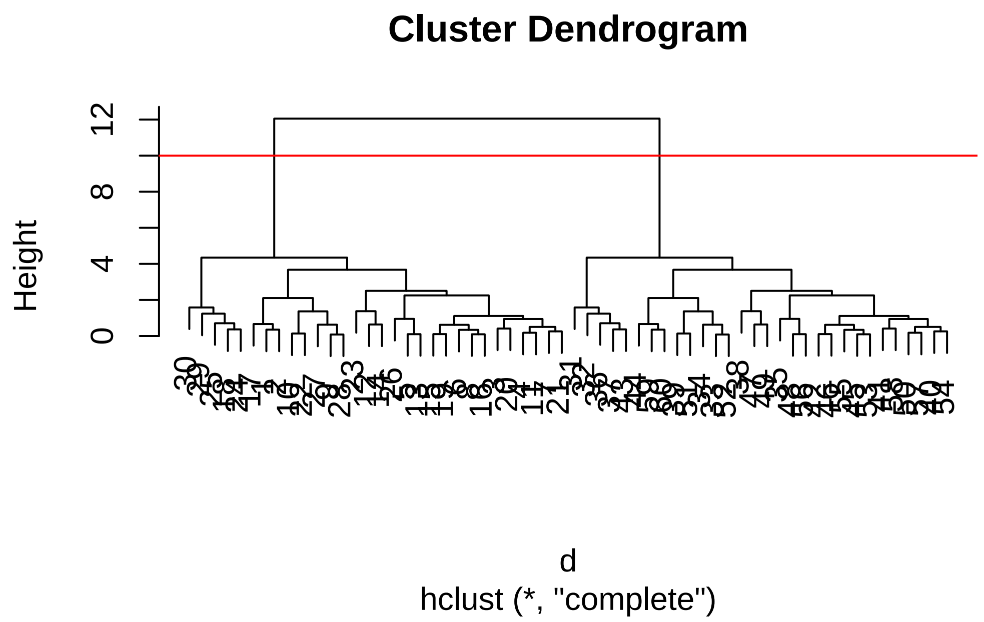
The `hclust()` function wants a distace matrix as input. We can get this from the `dist()` function.

```
d = dist(x)

hc = hclust(d)
hc
```

```
Call:
hclust(d = d)

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

There is a plot method for hclust results:

```
plot(hc)
abline(h=10, col="red")
```



To get my cluster membership vector I need to "cut" my tree to yield sub-trees or branches with all the members of a given cluster residing on the same cut branch. The function to do this is called `cutree()`

It is often helpful to use the `k=` argument to cutree rather than the `h=` height of cutting with `cutree()`. This will cut the tree to yield the number of clusters you want.

```
grps = cutree(hc, k=2)
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 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
```

Principle Component Analysis (PCA)

The base R function for PCA is called `prcomp()` Let's play with some 17D data (a very small dataset) and see how PCA can help.

PCA of UK food data

Import the data

```
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
```

> Q1. How many rows and columns are in your new data frame named x? What R functions could you use to answer this questions?

```
dim(x)
```

```
[1] 17  5
```

> Q2. Which approach to solving the 'row-names problem' mentioned above do you prefer and why? Is one approach more robust than another under certain circumstances?

```
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
```

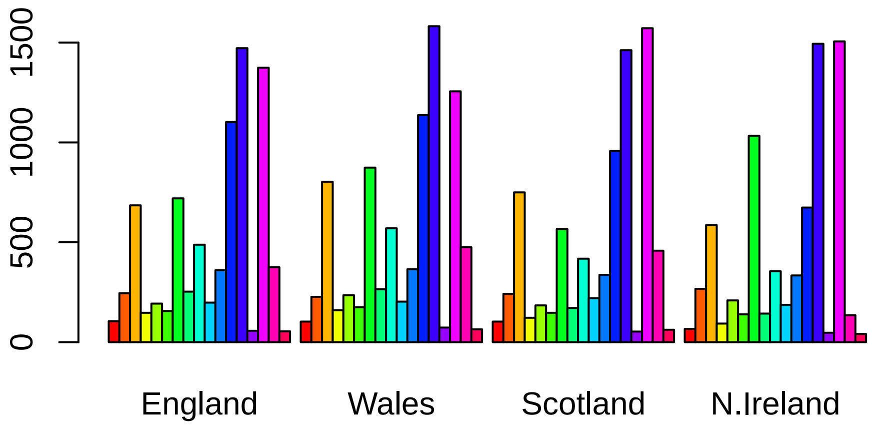
```
Fats_and_oils       193    235       184         209
Sugars              156    175       147         139
```

I prefer the `row.names=1` argument because it sets a specific column as the row names while the `x[,-1]` code will delete the first row after setting them to the row names. The `row.names` function is more robust because you can run as many times and it will produce the same output, while the `x[,-1]` code will continously delete rows as you run it repeatedly.

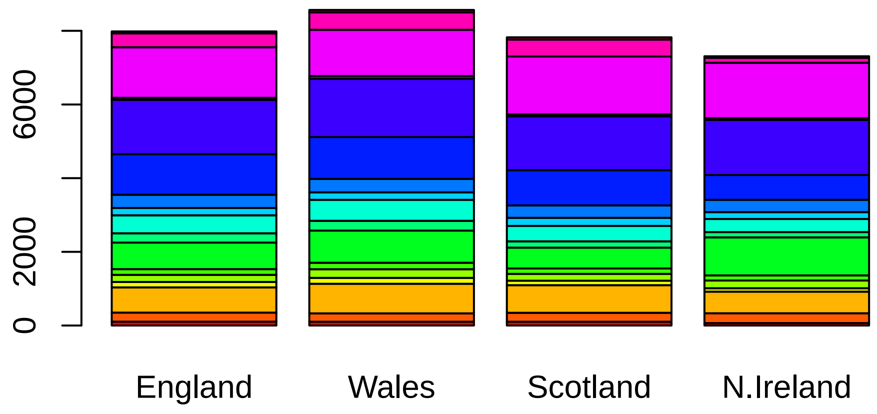
> Q3: Changing what optional argument in the above barplot() function results in the following plot?

Change the beside argument to false will result in the following plot.

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



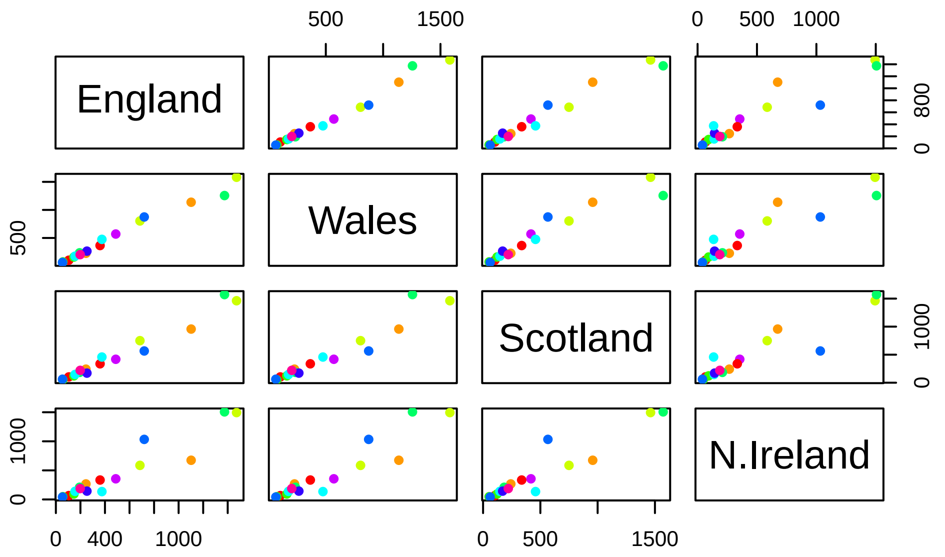
```r
barplot(as.matrix(x), beside=F, col=rainbow(nrow(x)))
```



> Q5: Generating all pairwise plots may help somewhat. Can you make sense of the following code and resulting figure? What does it mean if a given point lies on the diagonal for a given plot?

The code is cross comparing different countries by their food consumption. If points lie on the diagonal it means the both countries consume the same amount in that specific food.

```
pairs(x, col=rainbow(10), pch=16)
```



Q6. What is the main differences between N. Ireland and the other countries of the UK in terms of this data-set?

The main differences are their elevated consumption of fresh potatoes, lower alcohol consumption, and lower fresh fruit consumption.

```
pca = prcomp( t(x) )
summary(pca)
```

```
Importance of components:
                            PC1      PC2      PC3       PC4
Standard deviation     324.1502 212.7478 73.87622 4.189e-14
Proportion of Variance   0.6744   0.2905  0.03503 0.000e+00
Cumulative Proportion    0.6744   0.9650  1.00000 1.000e+00
```

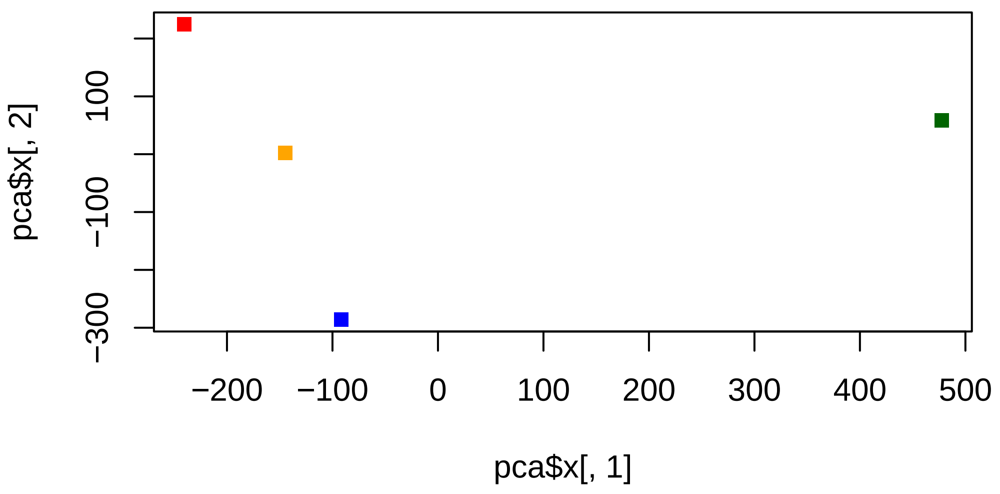
PCA plot

```
pca$x
```

```
                  PC1         PC2         PC3           PC4
England   -144.99315    2.532999 -105.768945   2.842865e-14
```

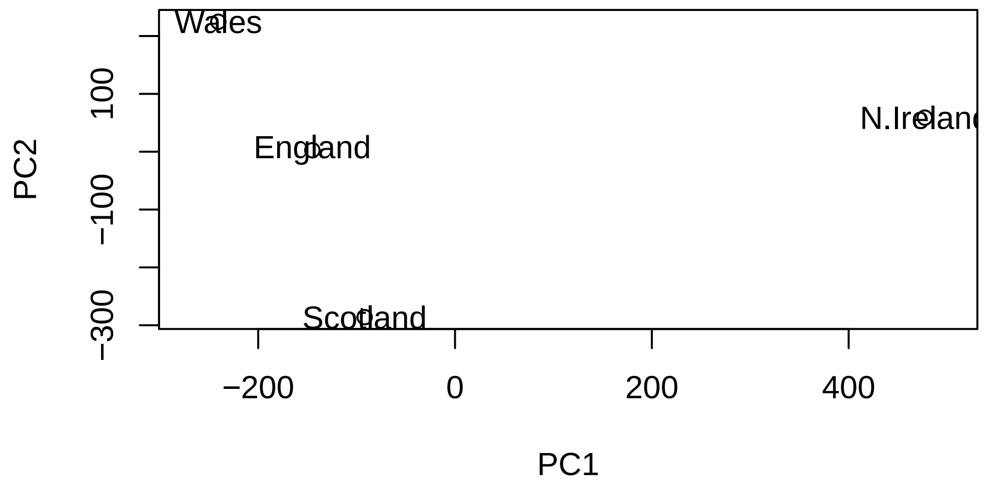
```
Wales      -240.52915  224.646925   56.475555  7.804382e-13
Scotland    -91.86934 -286.081786   44.415495 -9.614462e-13
N.Ireland   477.39164   58.901862    4.877895  1.448078e-13
```

```
plot(pca$x[,1],pca$x[,2], col=c("orange","red","blue","darkgreen"),pch=15)
```



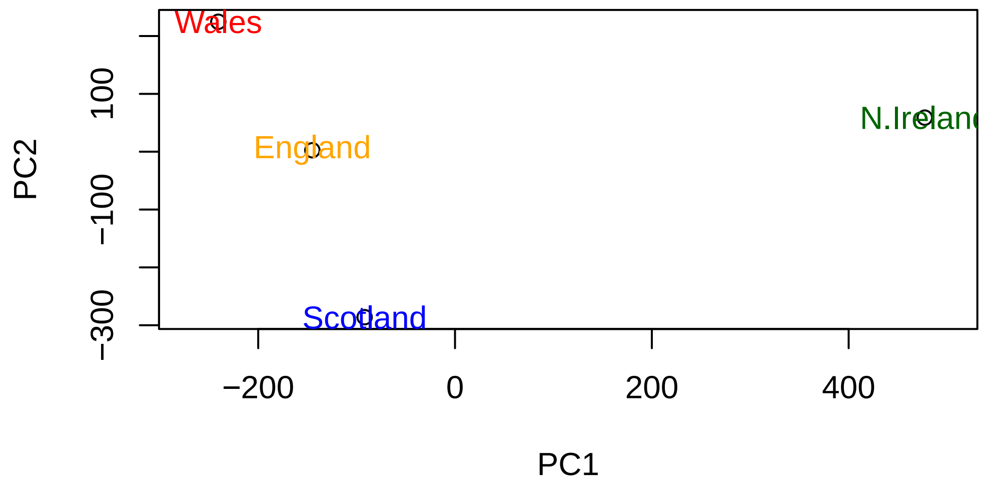
Q7. Complete the code below to generate a plot of PC1 vs PC2. The second line adds text labels over the data points.

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



Q8. Customize your plot so that the colors of the country names match the colors in our UK and Ireland map and table at start of this document.

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



Below we can use the square of `pca$sdev`, which stands for "standard deviation", to calculate how much variation in the original data each PC accounts for.

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

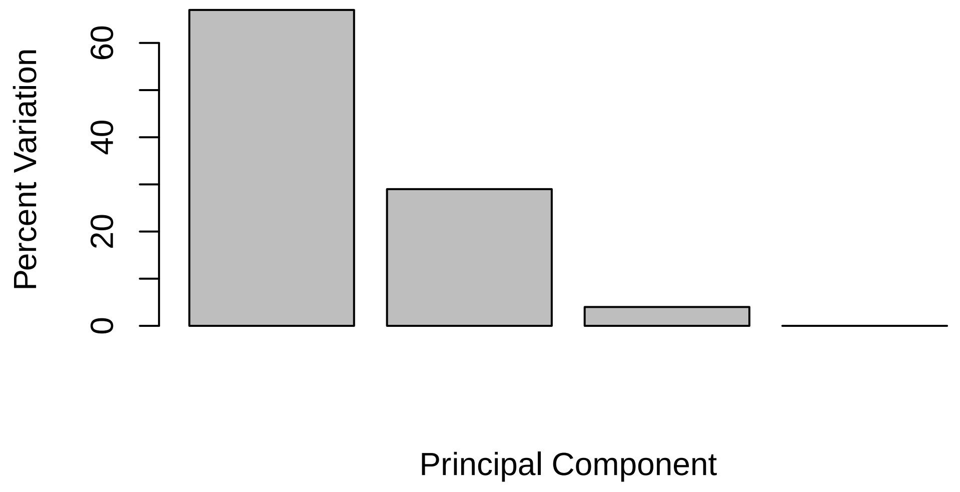
```
[1] 67 29  4  0
```

Summarized the variances with respect to the principal component using the `summary()` function and then using `barplot()`.

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

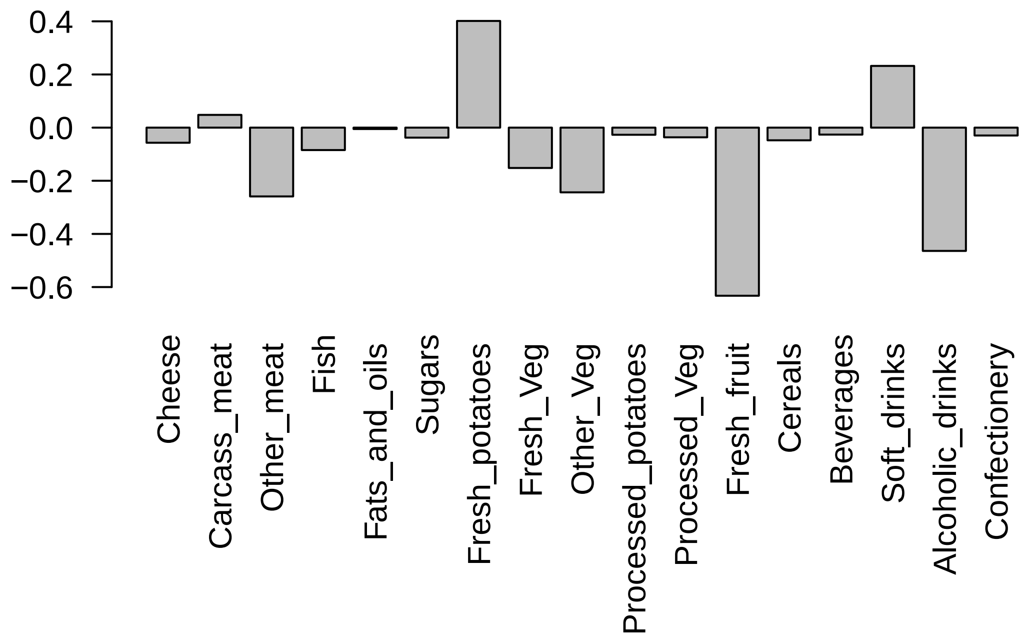
```
                             PC1       PC2      PC3          PC4
Standard deviation     324.15019 212.74780 73.87622 4.188568e-14
Proportion of Variance   0.67444   0.29052  0.03503 0.000000e+00
Cumulative Proportion    0.67444   0.96497  1.00000 1.000000e+00
```

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



Plotting the influences of original variables against the principal components using the `barplot()` function which gives us loading scores.

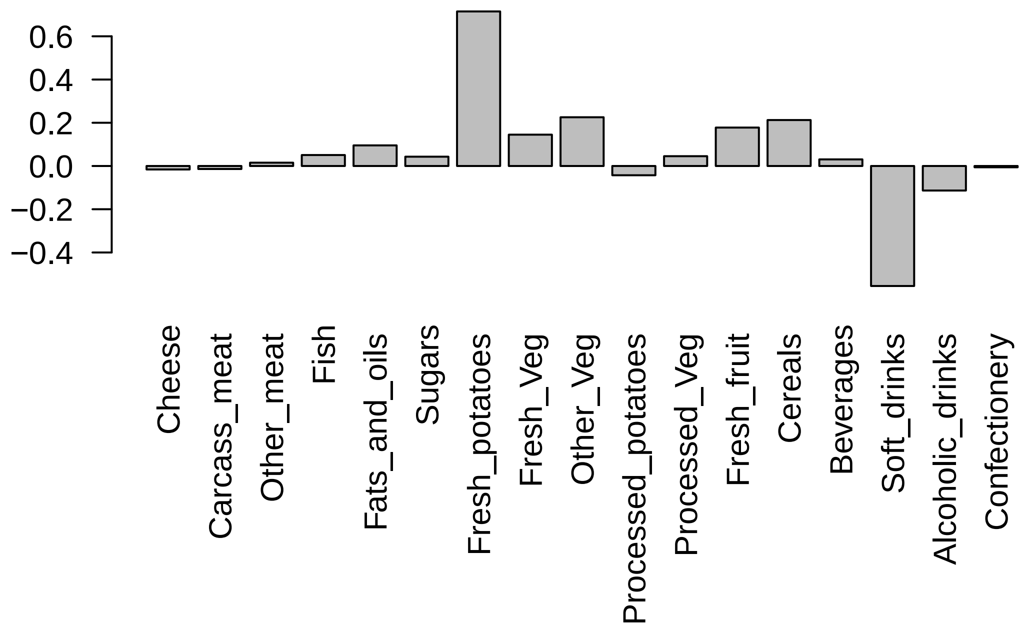
```r
par(mar=c(10, 3, 0.35, 0))
barplot( pca$rotation[,1], las=2 )
```



Q9: Generate a similar 'loadings plot' for PC2. What two food groups feature prominantely and what does PC2 mainly tell us about?

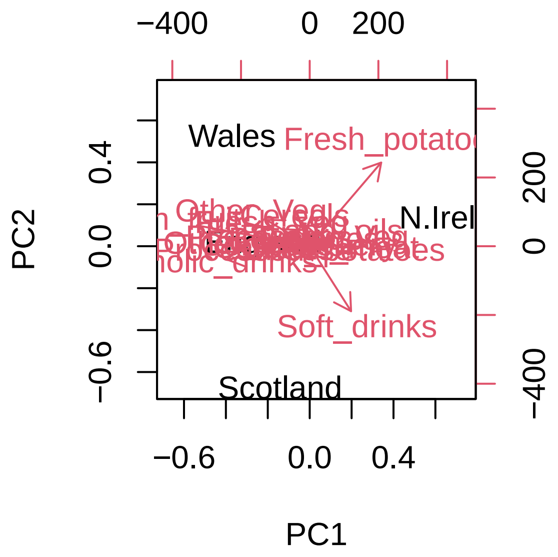
Fresh potatoes with the most positive loading score and soft drinks with the most negative loading score. PC2 tells us

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



information also can be summarized using the `biplot()` function.

```
biplot(pca)
```



New data of gene expression.

```
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
```

> Q10: How many genes and samples are in this data set?

There are 10 samples and 100 genes.

```
ncol(rna.data)
```

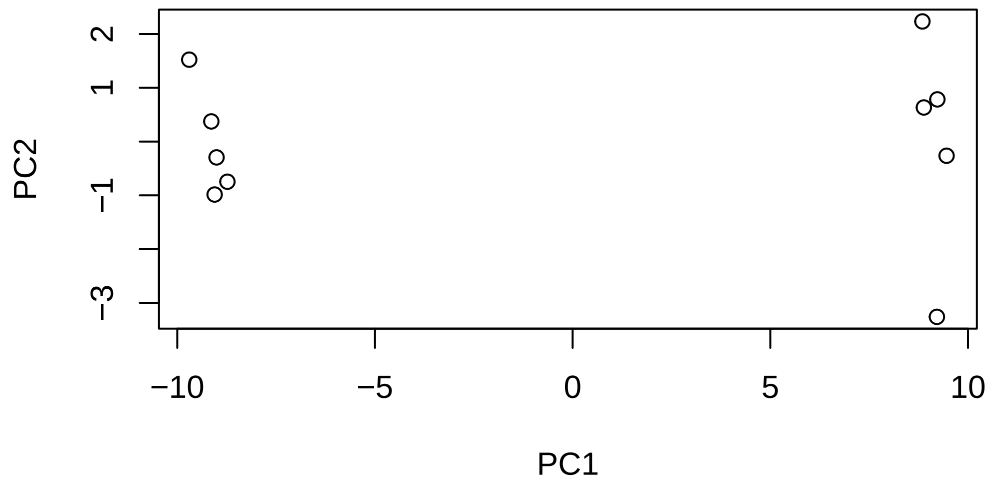
```
[1] 10
```

```
nrow(rna.data)
```

```
[1] 100
```

PCA and plot the results.

```
## Again we have to take the transpose of our data
pca <- prcomp(t(rna.data), scale=TRUE)

## Simple un polished plot of pc1 and pc2
plot(pca$x[,1], pca$x[,2], xlab="PC1", ylab="PC2")
```



summary of how much variation in the original data each PC account for.

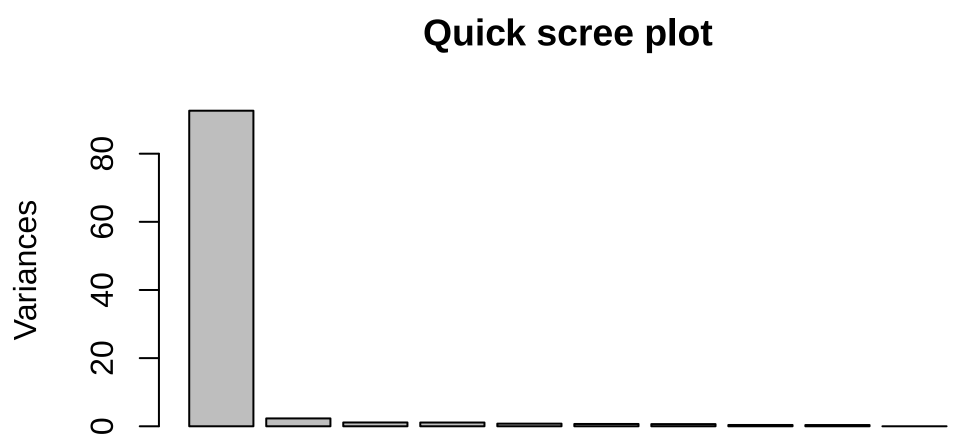
```
summary(pca)
```

```
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 3.348e-15
Proportion of Variance 0.00385 0.00364 0.000e+00
Cumulative Proportion  0.99636 1.00000 1.000e+00
```

barplot summary of this Proportion of Variance

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

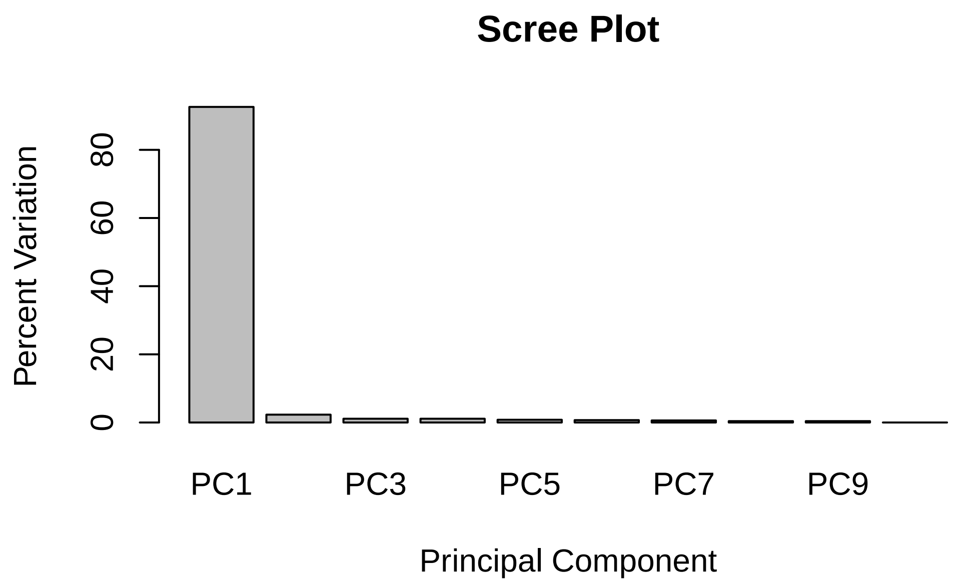


generate our own scree-plot.

```
## Variance captured per PC
pca.var <- pca$sdev^2

## Percent variance is often more informative to look at
pca.var.per <- round(pca.var/sum(pca.var)*100, 1)
pca.var.per
```

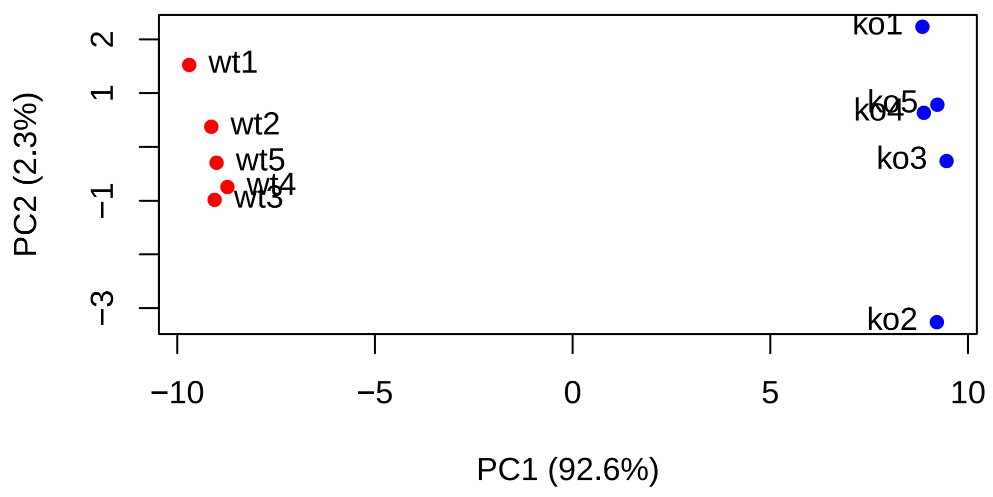
```
 [1] 92.6  2.3  1.1  1.1  0.8  0.7  0.6  0.4  0.4  0.0
```

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



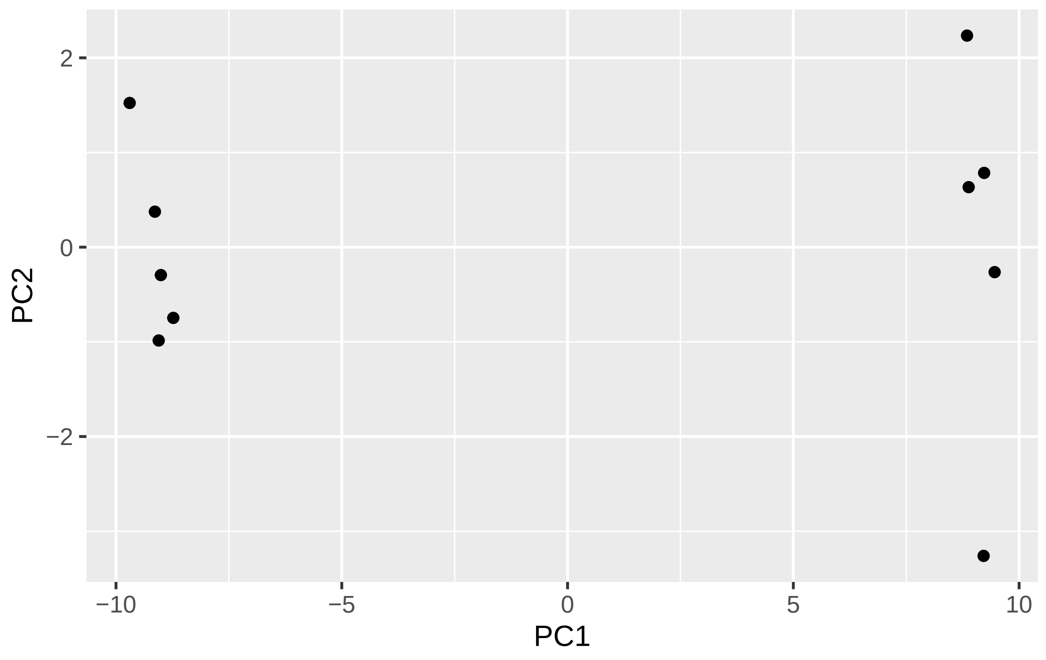
PCA plot a bit more attractive and useful...

```
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)))
```



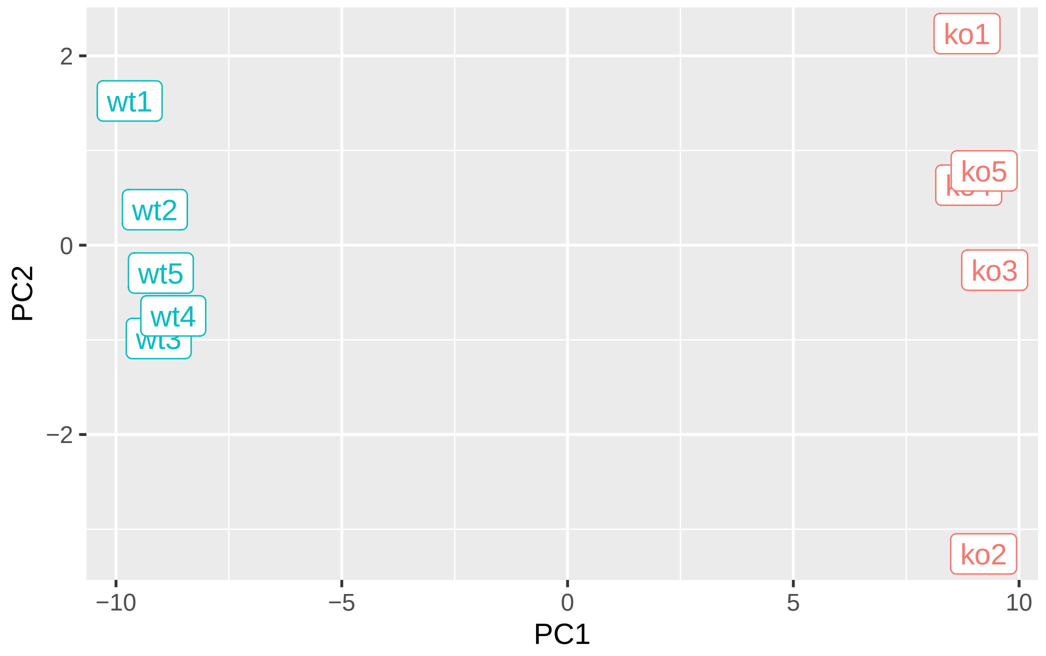
We could use the ggplot2 package here.

```
library(ggplot2)

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

# Our first basic plot
ggplot(df) +
  aes(PC1, PC2) +
  geom_point()
```



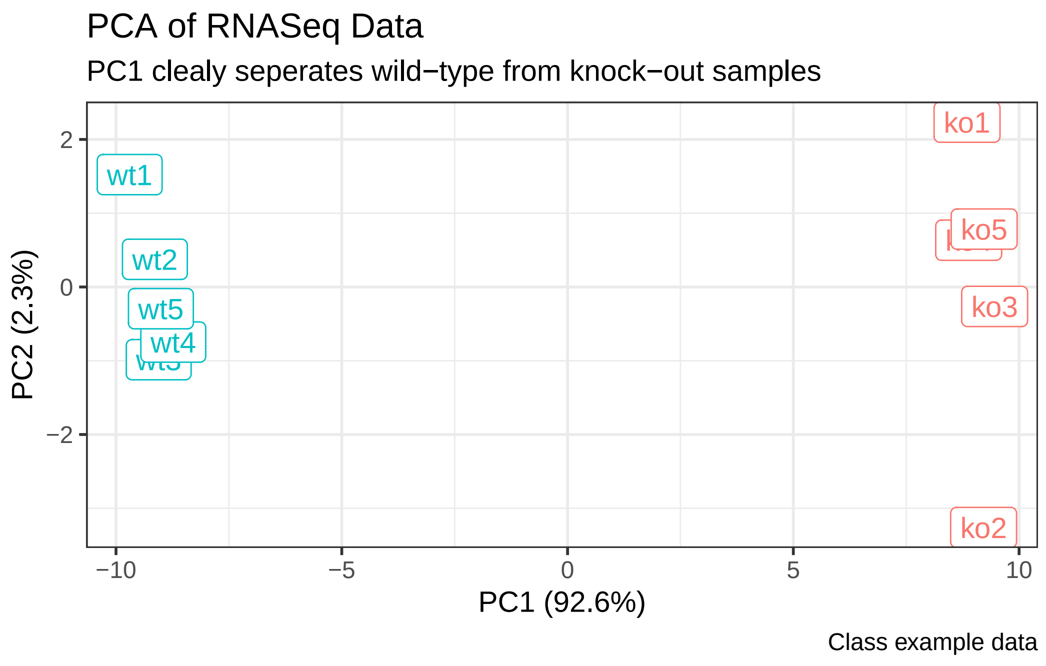
adding a condition specific color and sample label aesthetics.

```
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
```



some spit and polish

```
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()
```


PCA of RNASeq Data
PC1 clealy seperates wild−type from knock−out samples
ko1
wt1
ko5
wt2
ko3
wt5
wt4
ko2
2
0
−2
−10
−5
0
5
10
PC2 (2.3%)
PC1 (92.6%)
Class example data