Class 7: Hands on with Principal Component Analysis (PCA)

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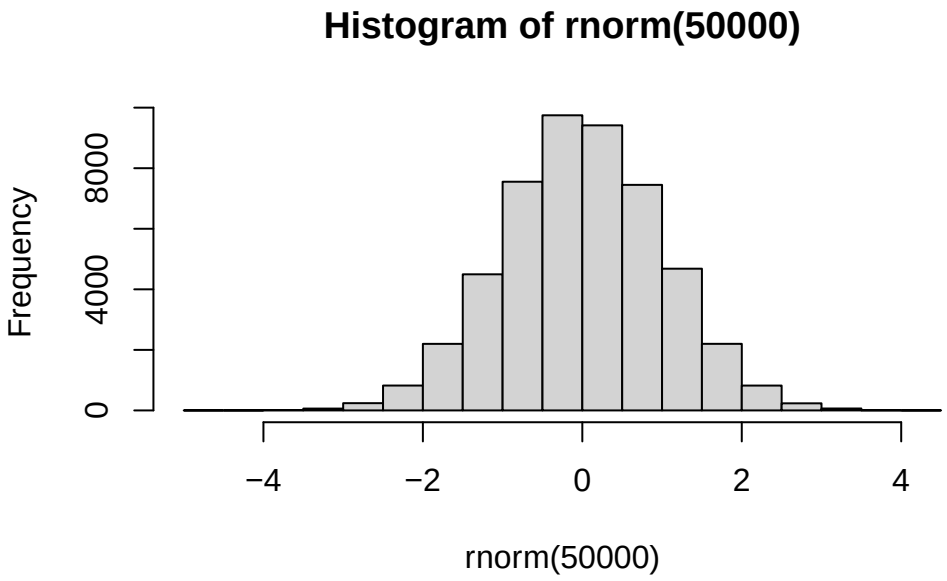
Today we are going to explore some core machine learning methods. Namely clustering and dimensionality

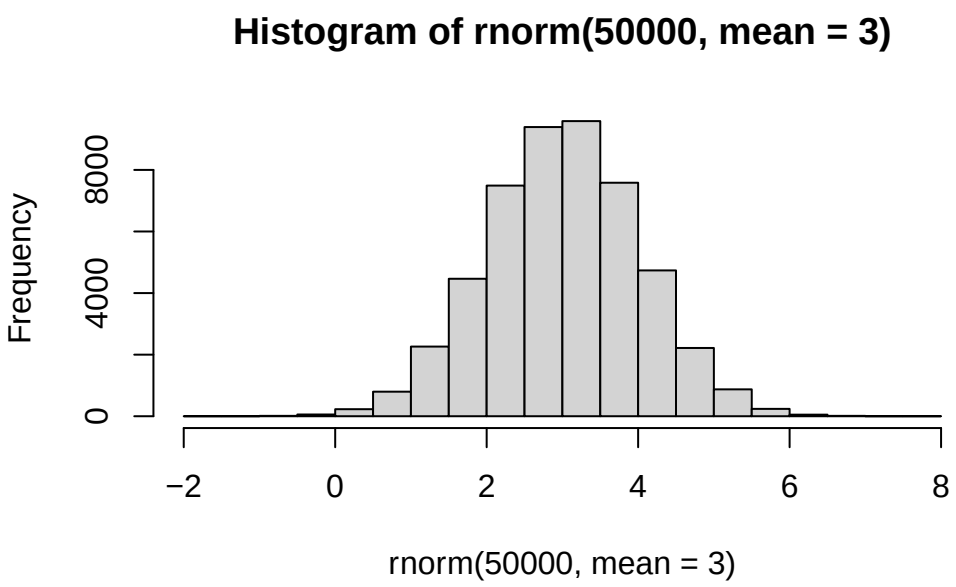
#Kmeans clustering

The main function for k-means in "base" R is called `kmeans()`. Let's first make up some data to see how kmeans works and to get at the results.

```
hist(rnorm(50000))
```



```r
hist(rnorm(50000,mean=3))
```



Make a vector with 60 total points, half centered at +3 and half at -3

```r
a<-rnorm(30,mean=3)
b<-rnorm(30,mean=-3)
c<-c(a,b)
c
```

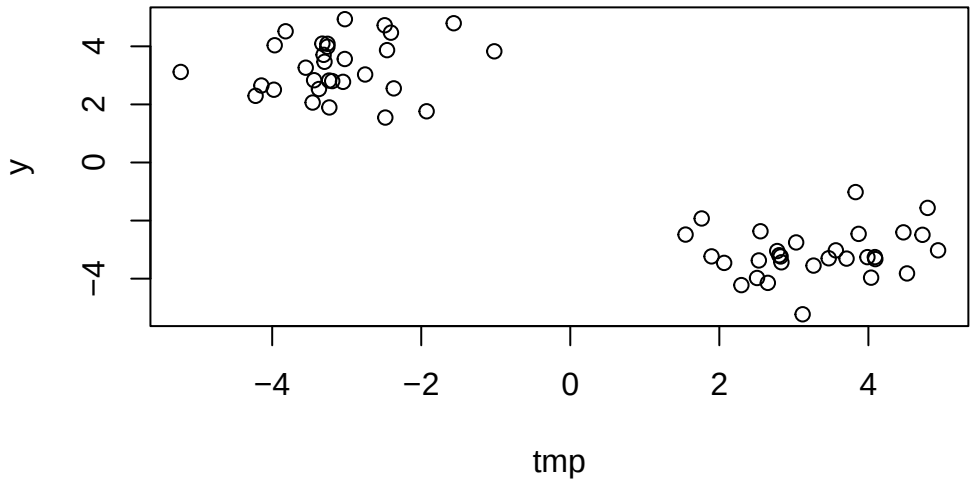
```
 [1]  2.3528800  2.3825327  4.6336049  2.6435367  2.6449683  3.2906067
 [7]  5.0471201  1.7351646  3.1181898  2.8886063  4.8068260  3.0740585
[13]  3.0969820  2.1784827  3.7244528  2.0831049  3.4514274  3.1020192
[19]  2.1059275  3.6496811  0.6097571  2.1145827  4.2485247  2.6803676
[25]  3.6405011  2.9664145  3.7688834  1.4294385  3.4595809  1.8815709
[31] -3.2269207 -2.8563175 -2.5139383 -3.4173538 -4.3044345 -4.0237469
[37] -2.7289281 -4.4263353 -2.1847563 -2.7628133 -2.8915667 -4.4026909
[43] -3.0411664 -3.7071289 -2.3841524 -3.3608755 -3.1740999 -1.3834901
[49] -2.2489636 -4.6417180 -2.0262573 -3.2649721 -2.8492250 -2.0302466
[55] -2.6110372 -3.6971119 -3.8816839 -1.9484898 -3.6812059 -2.7466132
```

Can shorten code ading this way.

```
tmp<-c(rnorm(30,3),rnorm(30,-3))
```

Get the reverse to make another vector

```
x<-cbind(tmp,y=rev(tmp))
plot(x)
```



Now run the kmeans function to see how they cluster

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

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

Cluster means:
        tmp         y
1 -3.128774  3.284547
2  3.284547 -3.128774

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] 46.15512 46.15512
 (between_SS / total_SS =  93.0 %)

Available components:

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

Whats in this results?

```
attributes(k)
```

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

$class
[1] "kmeans"
```

What are the cluster centers?

```
k$centers
```

```
        tmp         y
1 -3.128774  3.284547
2  3.284547 -3.128774
```

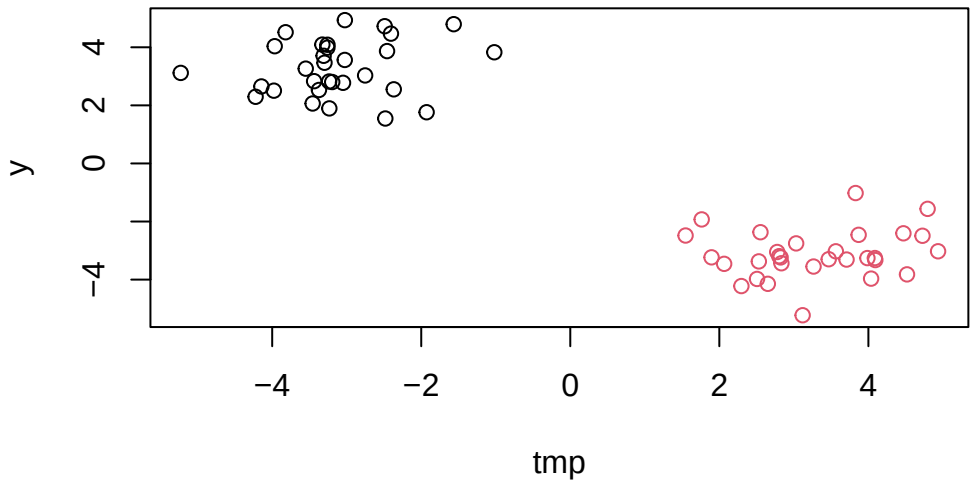
What are the clustering results?

```
k$cluster
```

```
 [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 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
```

Q. Plot your data “x” showing your clustering results and the center point for each cluster?

```r
plot(x,col=k$cluster)
```



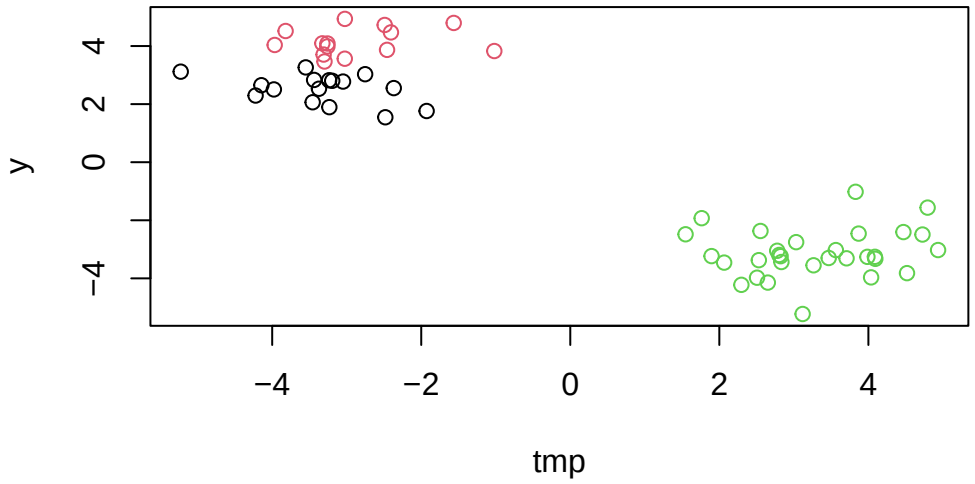
Q. Run kmeans and cluster into 3 groups and plot thre results

```r
k2<-kmeans(x,centers=3,nstart=20)
k2
```

```
K-means clustering with 3 clusters of sizes 16, 14, 30

Cluster means:
        tmp         y
1 -3.352120  2.528271
2 -2.873520  4.148863
3  3.284547 -3.128774

Clustering vector:
 [1] 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 1 1 1 2 2 2 1 1
[39] 1 1 1 2 1 2 1 2 1 2 2 1 2 2 2 1 1 1 2 2 2 1

Within cluster sum of squares by cluster:
[1] 13.29902 11.53594 46.15512
 (between_SS / total_SS =  94.6 %)
```

```
Available components:

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

```
plot(x,col=k2$cluster)
```



The big limitations of kmeans is that it imposes a structure on your data (it will force your data to fit your told it to, aka, not real). Process requires arbitrarily but sistematcally (manually) applying until you find the best one.

Hierarchical Clustering

The main function in "base" R for this is called `hlcust()`. It wants a distance matrix as input not the data itself.

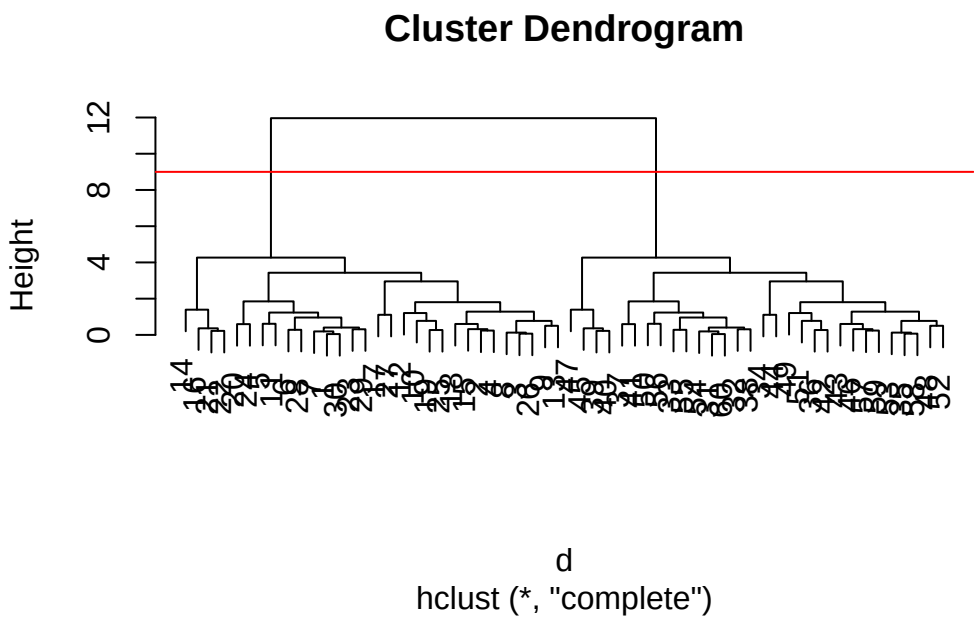
We can calculate a distance matrix in lots of different ways but here we will use the `dist()` function.

```
d<-dist(x)
hc<-hclust(d)
hc
```

```
Call:
hclust(d = d)

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

There is a specific plot

```
plot(hc)
abline(h=9,col="red")
```



To get the cluster membership vector we nee to "cut" the tree at a given height that we pick. The function to do this is called `cutree()`

```
cutree(hc, h=9)
```

```
 [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
```

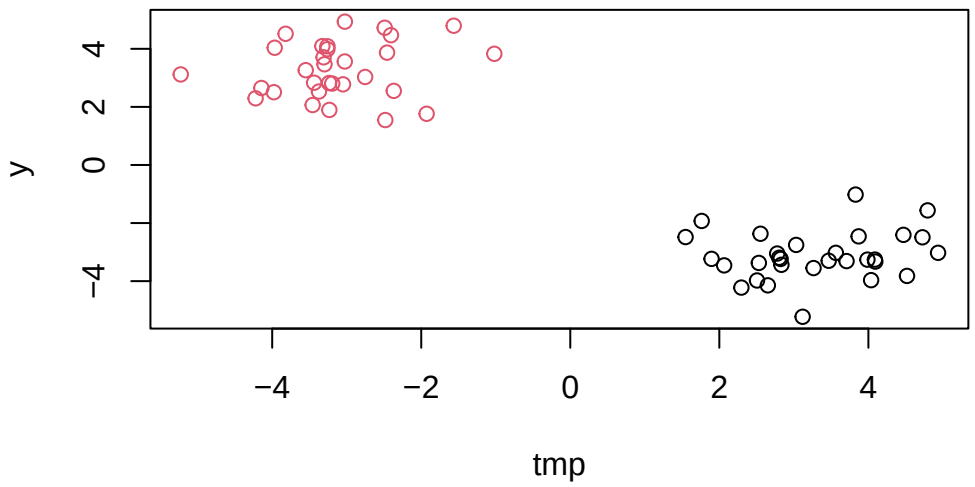
```
cutree(hc, k=4)
```

```
 [1] 1 1 1 1 1 1 1 1 1 1 1 1 2 1 2 1 1 1 1 2 2 1 1 1 1 1 1 1 3 3 3 3 3 3 3 3
[39] 4 4 3 3 3 3 4 3 4 3 3 3 3 3 3 3 3 3 3 3 3 3
```

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

Q. Plot our data(x) colored by our hclust result.

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



Data import

Principal Component Analysis (PCA)

We will start with PCA of a tiny tiny datase and make fun of stuff barry eats

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

One useful plot in this case (because we only have 4 countries to look across) is a so called pairse plot

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

ANS: x has 17 rows and 5 columns

```
dim(x)
```

```
[1] 17  5
```

```
nrow(x)
```

```
[1] 17
```

```
ncol(x)
```

```
[1] 5
```

Checking your data

```
## Preview the first 6 rows
head(x,6)
```

```
              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
```

Hmm, it looks like the row-names here were not set properly as we were expecting 4 columns (one for each of the 4 countries of the UK - not 5 as reported from the dim() function).

Here it appears that the row-names are incorrectly set as the first column of our x data frame (rather than set as proper row-names). This is very common and sometimes what we want - but not in this case. Lets try to fix this up with the following code, which sets the rownames() to the first column and then removes the troublesome first column (with the -1 column index):

```
# Note how the minus indexing works
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
```

lets check the dimensions again:

```
dim(x)
```

```
[1] 17  4
```

An alternative approach to setting the correct row-names in this case would be to read the data filie again and this time set the row.names argument of read.csv() to be the first column (i.e. use argument setting row.names=1), see below:

```
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
```

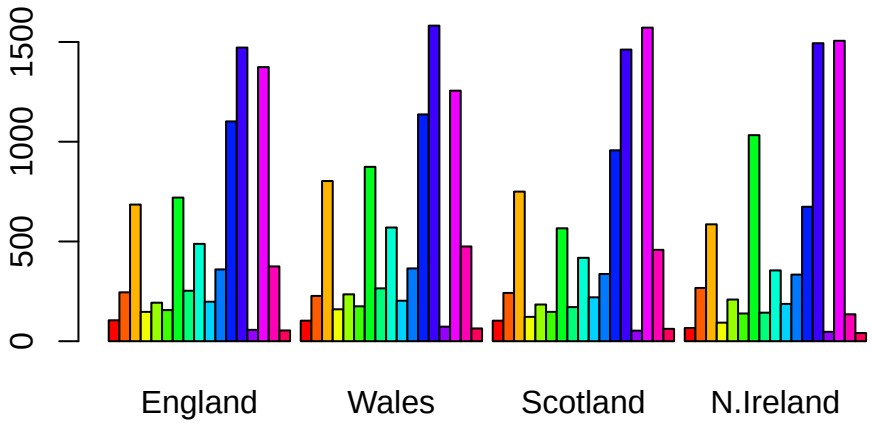
Q2. Which approach to solving the 'row-names problem' mentioned above do you prefer and why? Is one approach more robust than another under certain circumstances?

ANS: I prefer to use the row.names argument of read.csv() since it requires less coding lines

Spotting major differences and trends

A cursory glance over the numbers in this table does not reveal much of anything. Indeed in general it is difficult to extract meaning in regard to major differences and trends from any given array of numbers. Generating regular bar-plots and various pairwise plots does not help too much either:

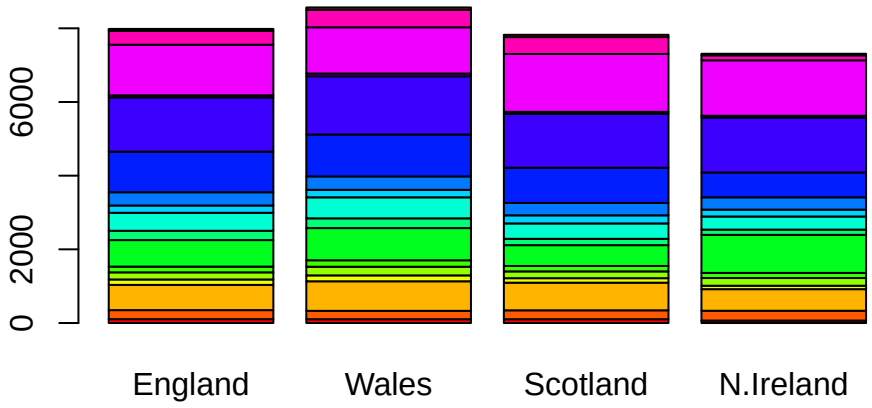
```
barplot(as.matrix(x), beside=T, col=rainbow(nrow(x)))
```



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

ANS: Changing the "beside" argument from TRUE to FALSE

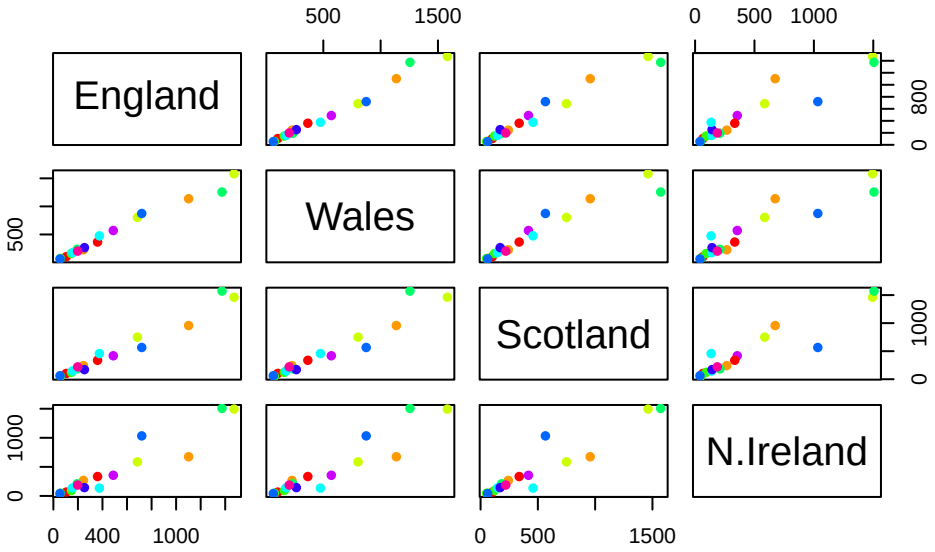
```
barplot(as.matrix(x), beside=F, col=rainbow(nrow(x)))
```



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?

ANS:Each scatterplot below or above the diagonal represents the relationship between two regions. For example, the scatterplot in the first column and second row compares the values for England (x-axis) and Wales (y-axis). The argument color set as "rainbow" assigns a color to a type of food (for example, yellow=cheese, green=Carcass_meat, etc.). The pch parameter stands for "plotting character" or "point character". Finally, each point in a plot represents the comparison of the consumption of different types of food between two regions. If a point lies on the diagonal of a given plot it means that both regions consume the same amount of a specific type of 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?

ANS: N. Ireland consumes more fresh potatoes and soft drinks than other countries, which it seems to correspond to the dark blue and orange points in the scatterplots aligned to the N.Ireland column. These point are located very far from the diagonal.

Enter PCA

The main function to do PCA in "base" R is called `prcomp()`.

It wants our food as the columns and the countries as the rows. It basically wants the tanspose of the data we have

```
# Use the prcomp() PCA function
pca<-prcomp(t(x))
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
```

```
attributes(pca)
```

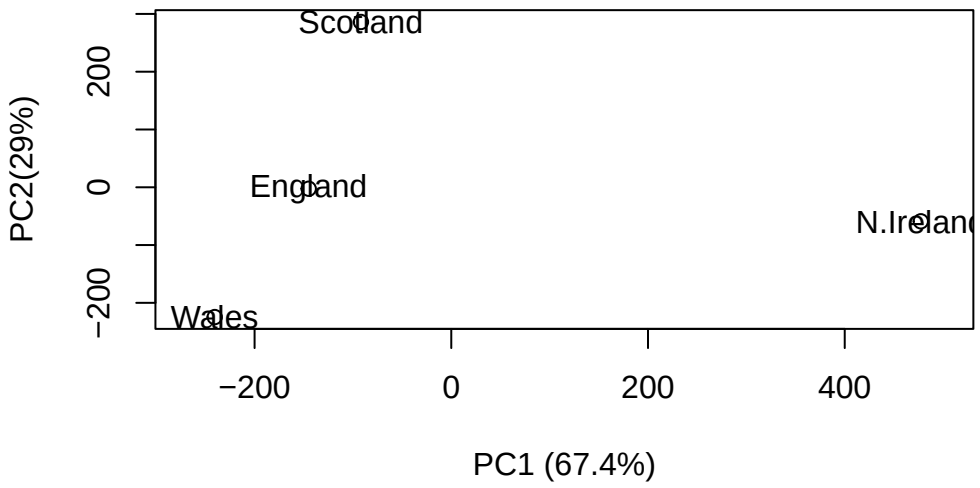
```
$names
[1] "sdev"     "rotation" "center"   "scale"    "x"

$class
[1] "prcomp"
```

```
pca$x
```

```
                PC1         PC2        PC3           PC4
England   -144.99315   -2.532999 105.768945 -4.894696e-14
Wales     -240.52915 -224.646925 -56.475555  5.700024e-13
Scotland   -91.86934  286.081786 -44.415495 -7.460785e-13
N.Ireland  477.39164  -58.901862  -4.877895  2.321303e-13
```

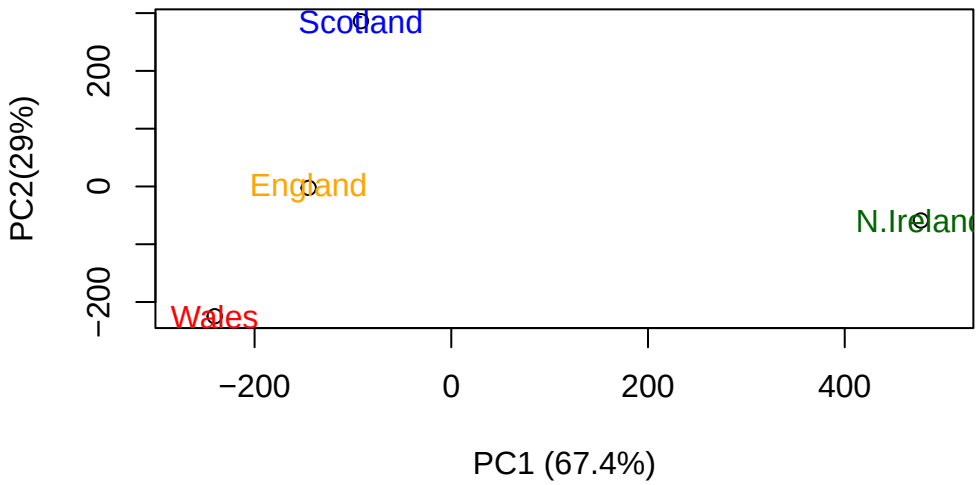
Q7. Complete the code below to generate a plot of PC1 vs PC2. The second line adds text labels over the data points.

```
plot(pca$x[,1],pca$x[,2],xlab="PC1 (67.4%)",ylab="PC2(29%)", 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.

```
plot(pca$x[,1],pca$x[,2],xlab="PC1 (67.4%)",ylab="PC2(29%)", xlim=c(-270,500))
text(pca$x[,1], pca$x[,2], colnames(x),col=c("orange","red","blue","darkgreen"))
```



Once the principal components have been obtained, we can use them to map the relationship between variables (i.e. countries) in therms of these major PCs (i.e. new axis that maximally describe the original data variance).

As part of the PCA method, we automatically obtain information about the contributions of each principal component to the total variance of the coordinates. This is typically contained in the Eigenvectors returned from such calculations. In the prcomp() function we can use the summary() command above or examine the returned pca$sdev

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

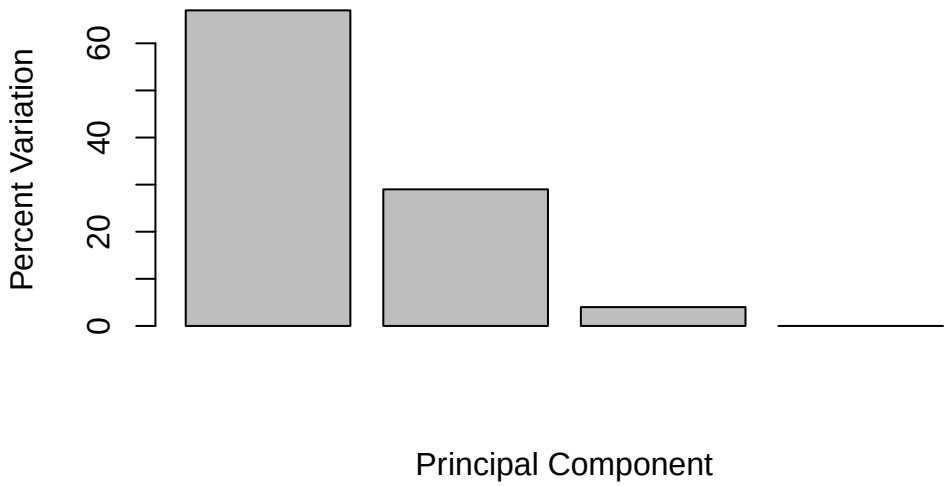
```
[1] 67 29  4  0
```

```
## or the second row here...
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
```

This information can be summarized in a plot of the variances (eigenvalues) with respect to the principal component number (eigenvector number), which is given below.

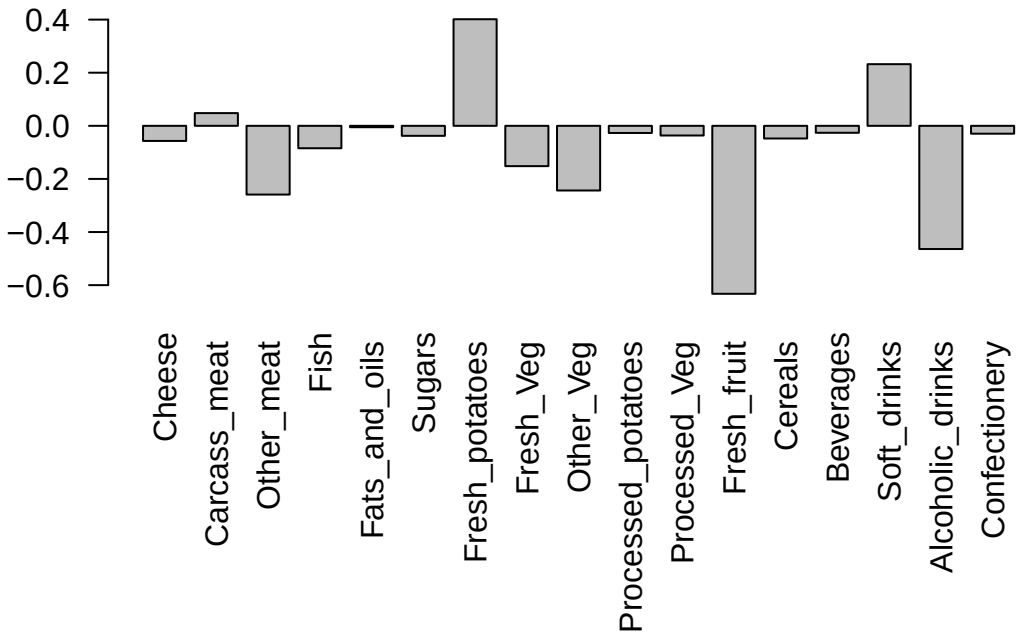
```
barplot(v, xlab="Principal Component", ylab="Percent Variation")
```



Digging deeper (variable loadings)

We can also consider the influence of each of the original variables upon the principal components (typically known as loading scores). This information can be obtained from the prcomp() returned $rotation component. It can also be summarized with a call to biplot(), see below:

```
## Lets focus on PC1 as it accounts for > 90% of variance
par(mar=c(10, 3, 0.35, 0))
barplot( pca$rotation[,1], las=2 )
```



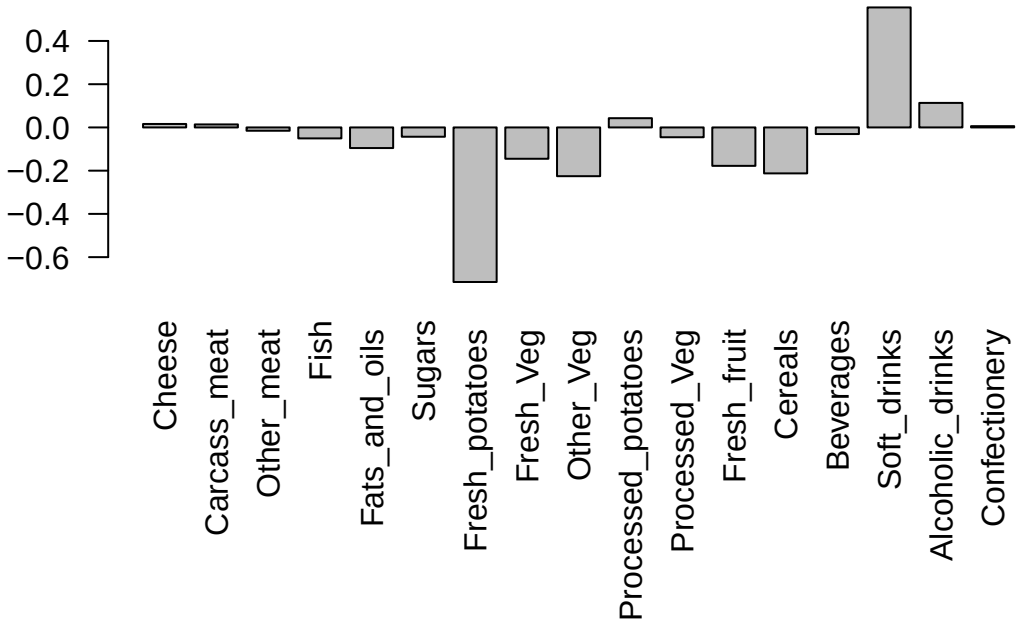
Here we see observations (foods) with the largest positive loading scores that effectively "push" N. Ireland to right positive side of the plot (including Fresh_potatoes and Soft_drinks).

We can also see the observations/foods with high negative scores that push the other countries to the left side of the plot (including Fresh_fruit and Alcoholic_drinks).

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

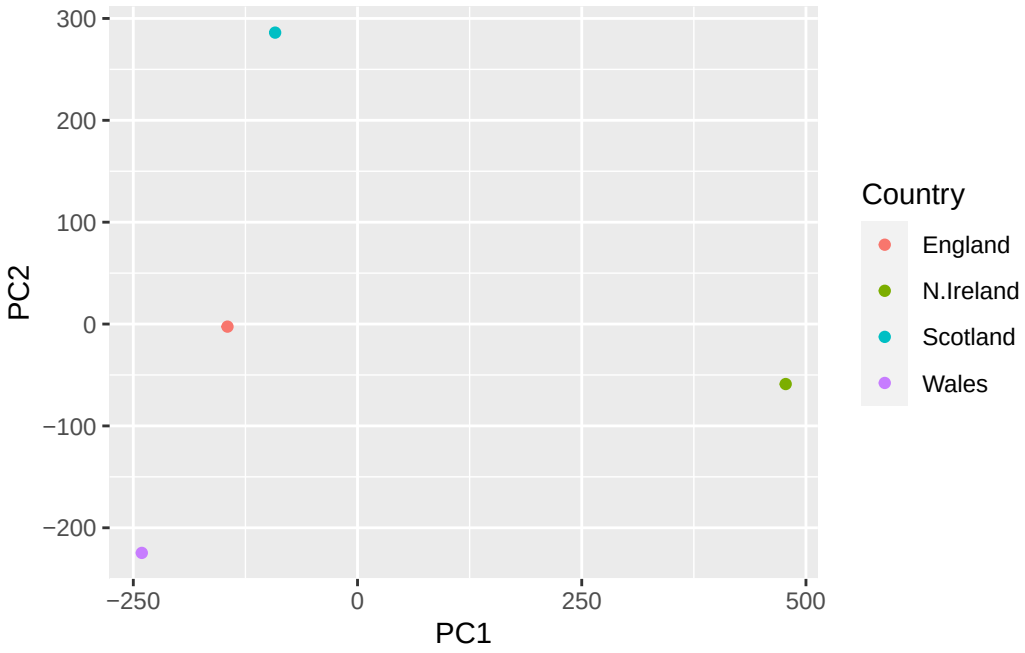
ANS: While Fresh-potatoes are in the negative side of the plot, soft drinks are in the positive side. The PC2 shows that there are more variance between these two groups. However, it does not show alcoholic drinks and fresh fruit as predominant food like PC1 does.

```
## Lets focus on PC2
par(mar=c(10, 3, 0.35, 0))
barplot( pca$rotation[,2], las=2 )
```



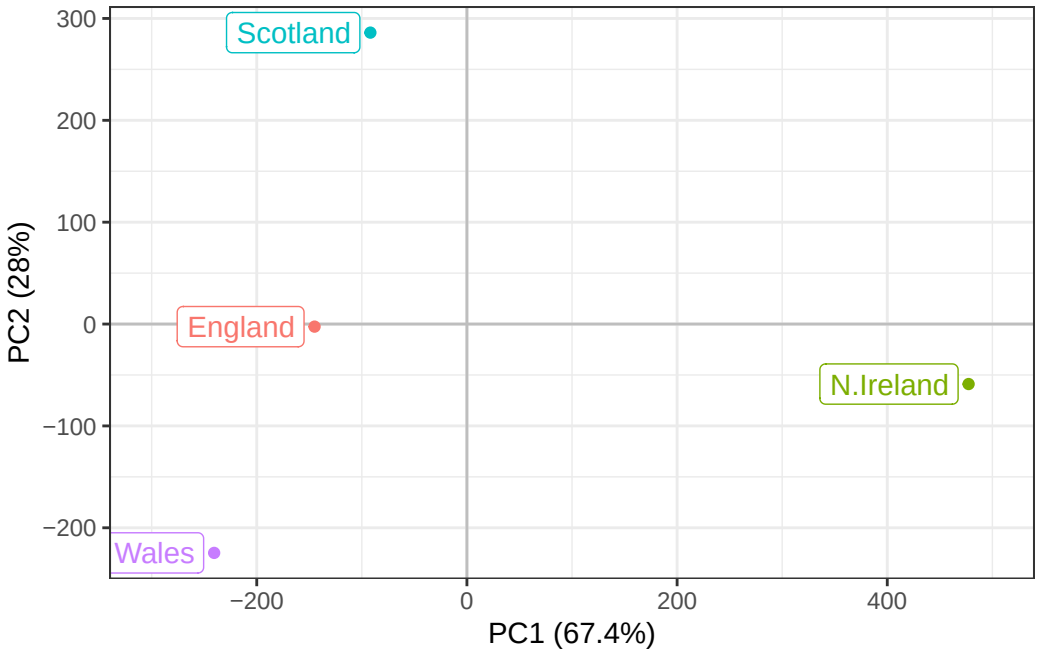
Using ggplot for these figures

```
library(ggplot2)

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

# Our first basic plot
ggplot(df_lab) +
  aes(PC1, PC2, col=Country) +
  geom_point()
```



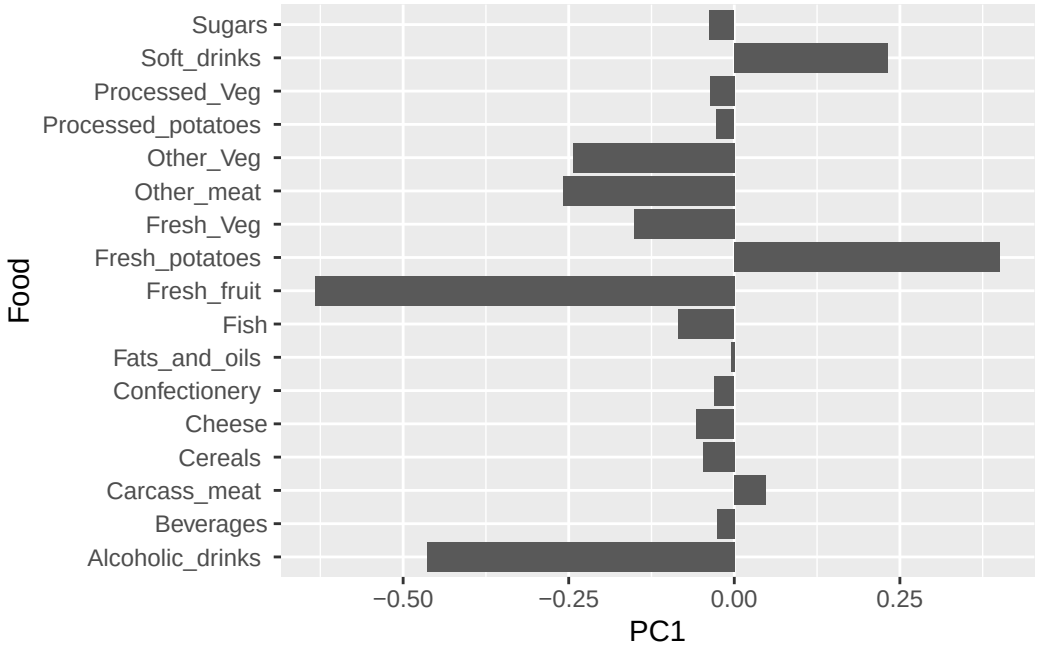
And then we can get carried away and make this look much nicer:

```
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()
```



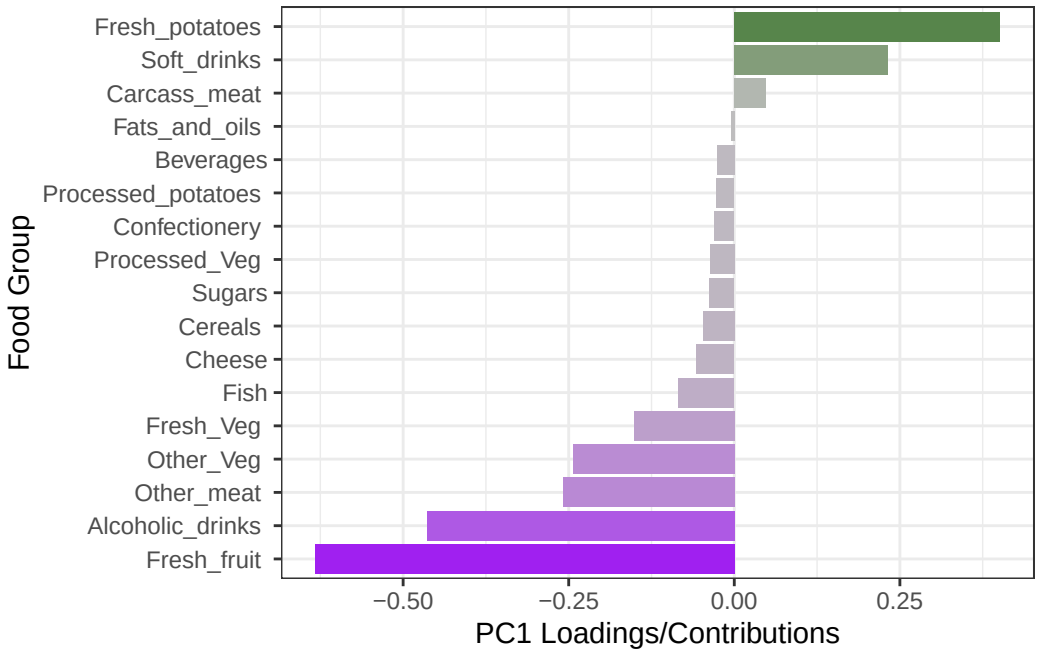
Let's do the same for our loadings/PC contributions figures. This data is stored in the pca$rotation object that we convert to a data frame, add the useful row names as a new column and then plot and customize with additional ggplot layers. Which do you prefer, base graphics or ggplot?

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

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



We can now add some additional features to the plot, such as reordering the y axis by the PC1 loadings and selecting a rather ugly color scale (to match our country colors) and our prefered theme layer.

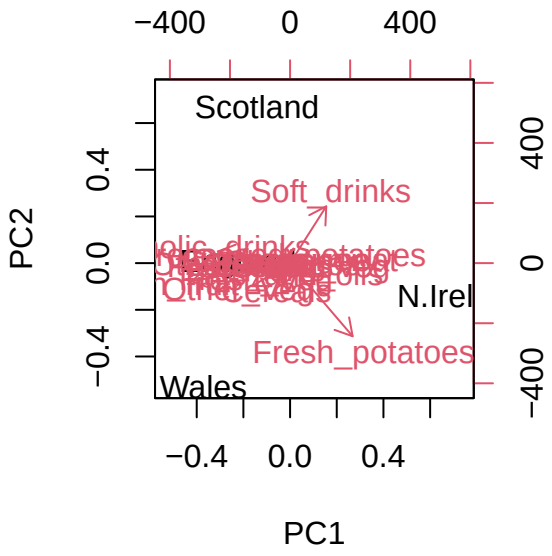
```
ggplot(ld_lab) +
  aes(PC1, reorder(Food, PC1), bg=PC1) +
  geom_col() +
  xlab("PC1 Loadings/Contributions") +
  ylab("Food Group") +
  scale_fill_gradient2(low="purple", mid="gray", high="darkgreen", guide=NULL) +
  theme_bw()
```



Biplots

Another way to see this information together with the main PCA plot is in a so-called biplot:

```
## The inbuilt biplot() can be useful for small datasets
biplot(pca)
```



2. PCA of RNA-seq data

In this example, a small RNA-seq count data set (available from the class website (expression.csv and the tinyurl short link: "https://tinyurl.com/expression-CSV") is read into a data frame called rna.data where the columns are individual samples (i.e. cells) and rows are measurements taken for all the samples (i.e. genes).

```
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?

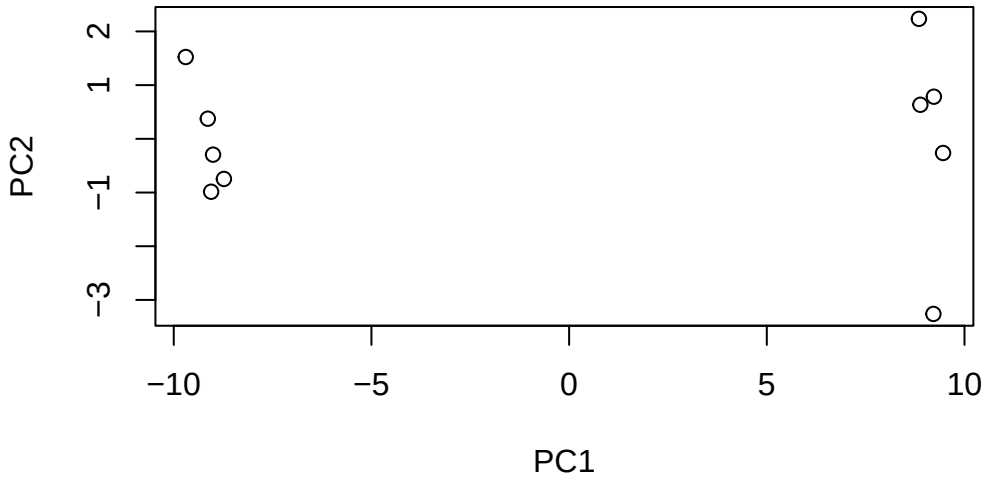
ANS: 100 genes and 10 samples

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

```
[1] 100  10
```

Generating barplots etc. to make sense of this data is really not an exciting or worthwhile option to consider. So lets do 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")
```



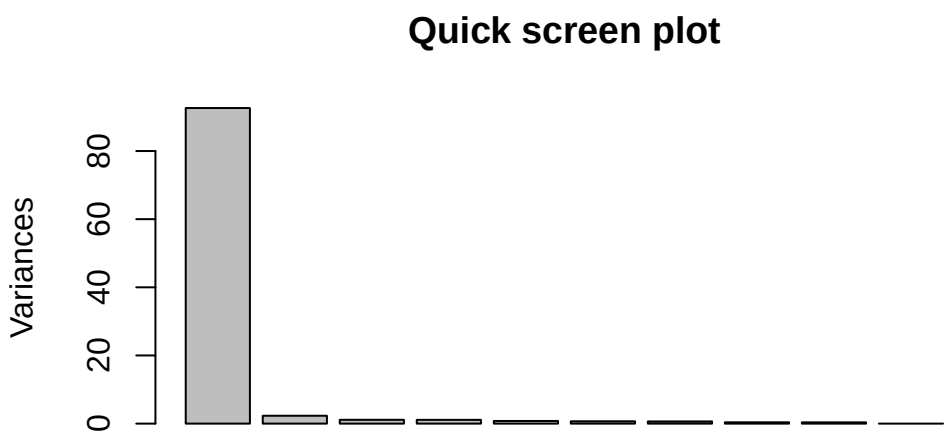
This quick plot looks interesting with a nice separation of samples into two groups of 5 samples each. Before delving into the details of this grouping let's first examine a summary of how much variation in the original data each PC accounts for:

```
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 3.457e-15
Proportion of Variance 0.00385 0.00364 0.000e+00
Cumulative Proportion  0.99636 1.00000 1.000e+00
```

A quick barplot summary of this Proportion of Variance for each PC can be obtained by calling the plot() function directly on our prcomp result object.

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



Let's make the above scree plot ourselves and in so doing explore the object returned from prcomp() a little further. 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:

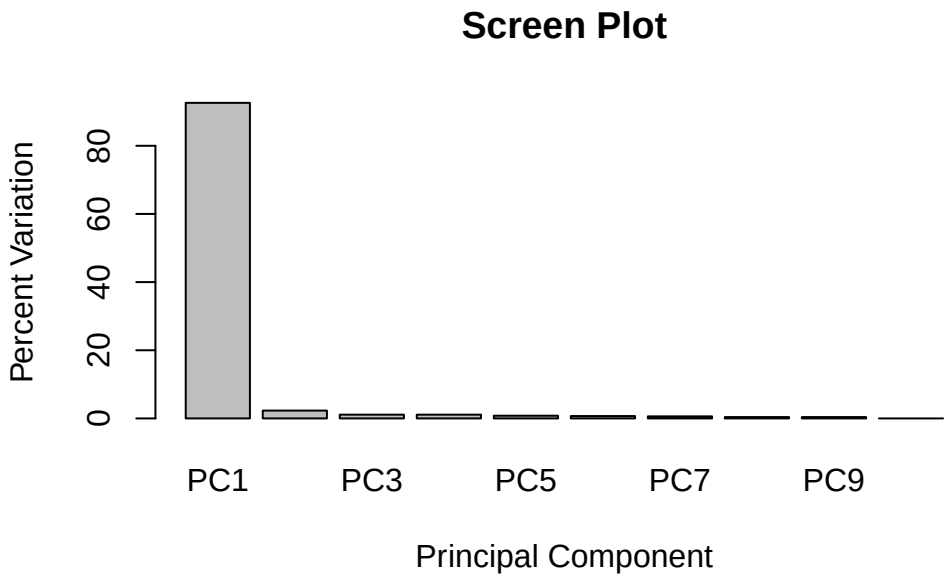
```
## Variance captured per PC
pca.var <- pca$sdev^2
```

```
## 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
```

We can use this to generate our own screen-plot like this:

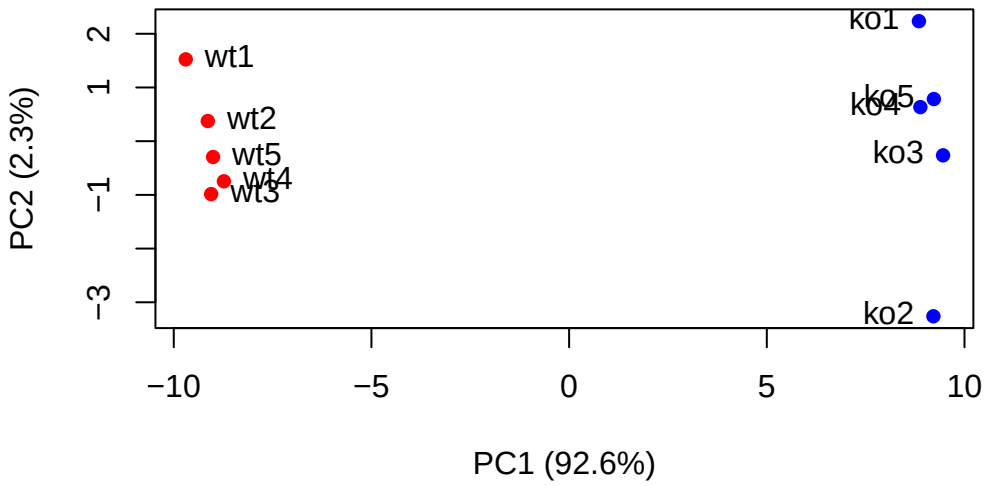
```
barplot(pca.var.per, main="Screen Plot",
        names.arg = paste0("PC", 1:10),
        xlab="Principal Component", ylab="Percent Variation")
```



Now lets make our main PCA plot a bit more attractive and useful...

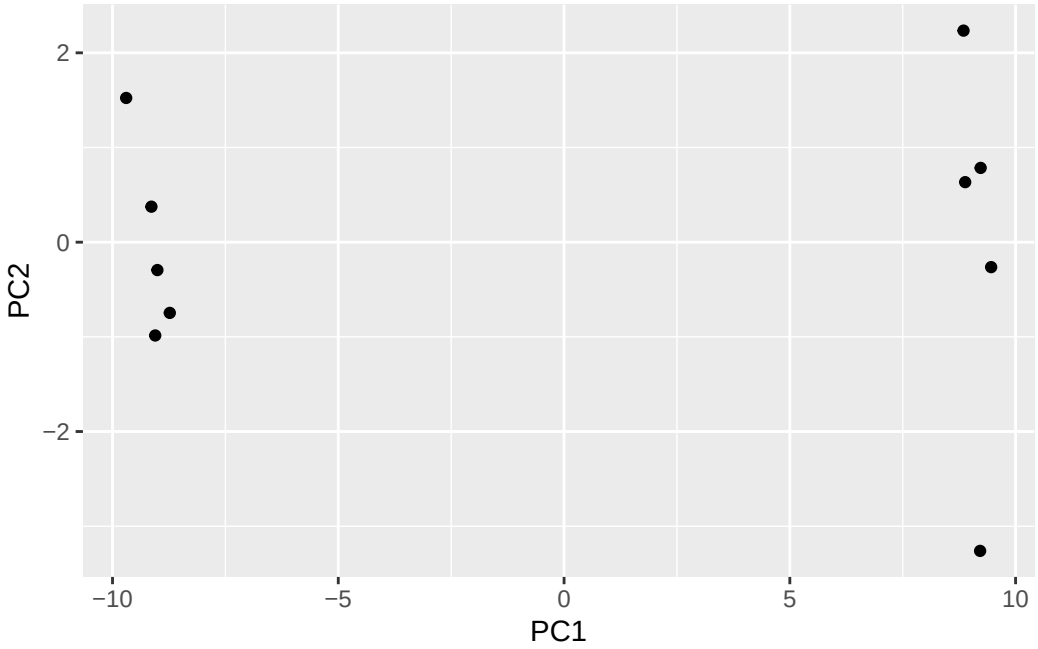
```
## A vector of colors for wt and ko samples
colvec <- colnames(rna.data)
colvec[grep("wt", colvec)] <- "red"
colvec[grep("ko", colvec)] <- "blue"

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)))
```



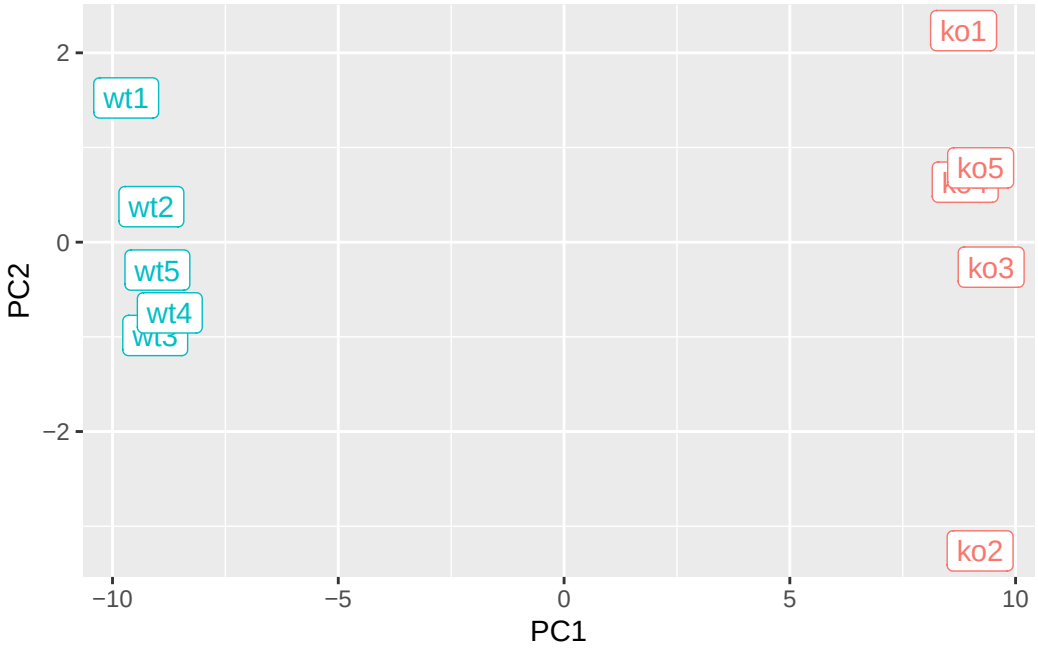
Using ggplot

```
library(ggplot2)

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

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



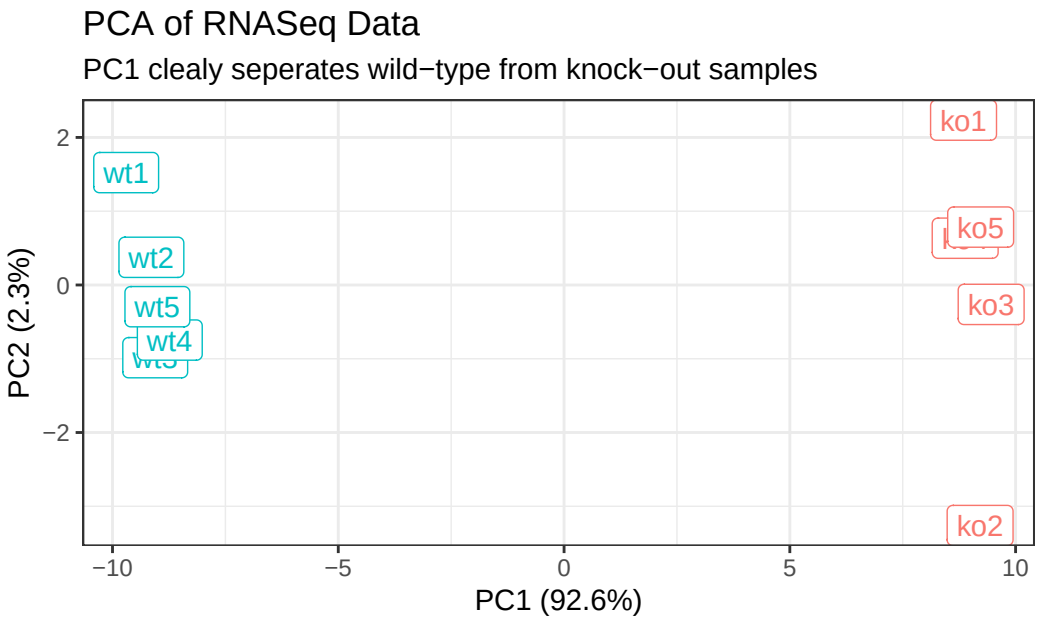
If we want to add a condition specific color and perhaps sample label aesthetics for wild-type and knock-out samples we will need to have this information added to our data.frame:

```
# Add a 'wt' and 'ko' "condition" column
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
```



And finally add 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()
```



Optional: Gene loadings

```
loading_scores <- pca$rotation[,1]

## Find the top 10 measurements (genes) that contribute
## most to PC1 in either direction (+ or -)
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"
```