Class8: Breast Cancer Mini Project

Sawyer (PID: A16335277)

Before we get stuck into project work we will have a quick look at applying PCA to some example RNASeq data (tail en of lab 7).

Read the data (detailed in lab 7):

```
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
```

Q. How many genes are in this dataset?

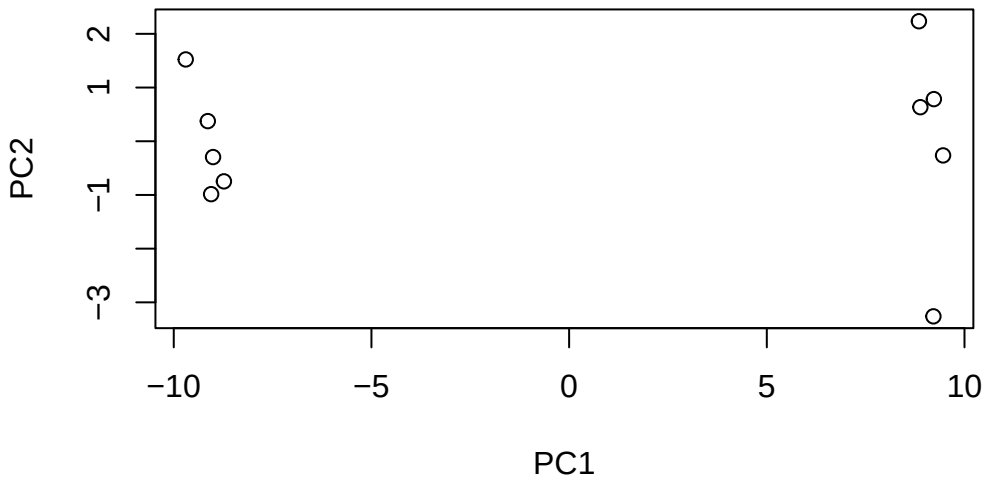
```
nrow(rna.data)
```

```
[1] 100
```

Run PCA

```
## 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")
```

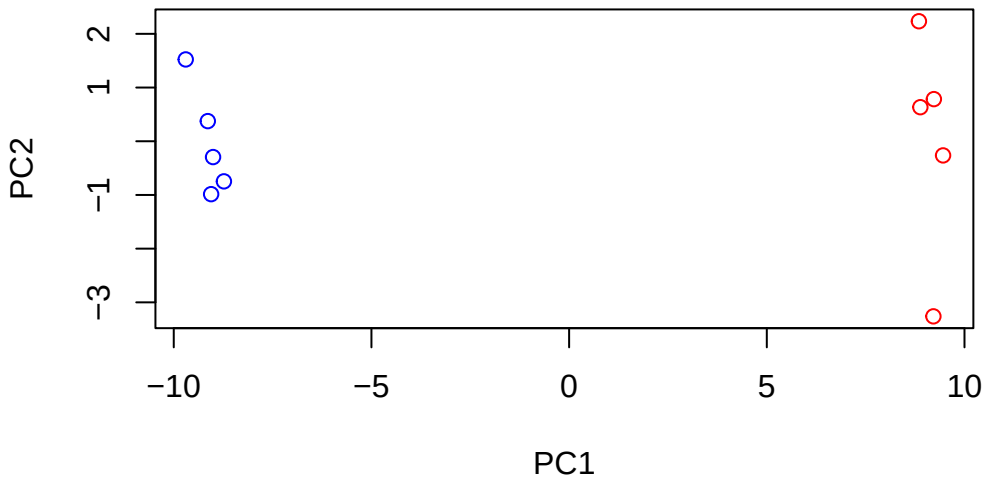



```
summary(pca)
```

```
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
```

```
# We have 5 wt and 5 ko samples
mycols <- c(rep("blue", 5), rep("red", 5))

plot(pca$x[,1], pca$x[,2], xlab="PC1", ylab="PC2", col=mycols)
```



I could examine which genes contribute most to this first PC

```
head(sort(abs(pca$rotation[,1]), decreasing = T))
```

```
  gene100     gene66     gene45     gene68     gene98     gene60
0.1038708 0.1038455 0.1038402 0.1038395 0.1038372 0.1038055
```

1. Analysis of Breast Cancer FNA data.

```
# Save your input data file into your Project directory
fna.data <- "WisconsinCancer.csv"

# Complete the following code to input the data and store as wisc.df
wisc.df <- read.csv(fna.data, row.names=1)
head(wisc.df)
```

```
        diagnosis radius_mean texture_mean perimeter_mean area_mean
842302          M       17.99        10.38         122.80    1001.0
842517          M       20.57        17.77         132.90    1326.0
```

```
84300903         M       19.69        21.25         130.00    1203.0
84348301         M       11.42        20.38          77.58     386.1
84358402         M       20.29        14.34         135.10    1297.0
843786           M       12.45        15.70          82.57     477.1
         smoothness_mean compactness_mean concavity_mean concave.points_mean
842302           0.11840          0.27760         0.3001             0.14710
842517           0.08474          0.07864         0.0869             0.07017
84300903         0.10960          0.15990         0.1974             0.12790
84348301         0.14250          0.28390         0.2414             0.10520
84358402         0.10030          0.13280         0.1980             0.10430
843786           0.12780          0.17000         0.1578             0.08089
         symmetry_mean fractal_dimension_mean radius_se texture_se perimeter_se
842302          0.2419                0.07871    1.0950     0.9053        8.589
842517          0.1812                0.05667    0.5435     0.7339        3.398
84300903        0.2069                0.05999    0.7456     0.7869        4.585
84348301        0.2597                0.09744    0.4956     1.1560        3.445
84358402        0.1809                0.05883    0.7572     0.7813        5.438
843786          0.2087                0.07613    0.3345     0.8902        2.217
         area_se smoothness_se compactness_se concavity_se concave.points_se
842302    153.40      0.006399        0.04904      0.05373           0.01587
842517     74.08      0.005225        0.01308      0.01860           0.01340
84300903   94.03      0.006150        0.04006      0.03832           0.02058
84348301   27.23      0.009110        0.07458      0.05661           0.01867
84358402   94.44      0.011490        0.02461      0.05688           0.01885
843786     27.19      0.007510        0.03345      0.03672           0.01137
         symmetry_se fractal_dimension_se radius_worst texture_worst
842302       0.03003             0.006193        25.38         17.33
842517       0.01389             0.003532        24.99         23.41
84300903     0.02250             0.004571        23.57         25.53
84348301     0.05963             0.009208        14.91         26.50
84358402     0.01756             0.005115        22.54         16.67
843786       0.02165             0.005082        15.47         23.75
         perimeter_worst area_worst smoothness_worst compactness_worst
842302            184.60     2019.0           0.1622            0.6656
842517            158.80     1956.0           0.1238            0.1866
84300903          152.50     1709.0           0.1444            0.4245
84348301           98.87      567.7           0.2098            0.8663
84358402          152.20     1575.0           0.1374            0.2050
843786            103.40      741.6           0.1791            0.5249
         concavity_worst concave.points_worst symmetry_worst
842302            0.7119               0.2654         0.4601
842517            0.2416               0.1860         0.2750
84300903          0.4504               0.2430         0.3613
```

```
84348301          0.6869                0.2575          0.6638
84358402          0.4000                0.1625          0.2364
843786            0.5355                0.1741          0.3985
         fractal_dimension_worst
842302                   0.11890
842517                   0.08902
84300903                 0.08758
84348301                 0.17300
84358402                 0.07678
843786                   0.12440
```

```
# Create diagnosis vector for later
diagnosis <- as.factor(wisc.df$diagnosis)

# We can use -1 here to remove the first column
wisc.data <- wisc.df[,-1]
```

- Q1. How many observations are in this dataset?

```
nrow(wisc.data)
```

```
[1] 569
```

- Q2. How many of the observations have a malignant diagnosis?

```
table(diagnosis)
```

```
diagnosis
  B   M
357 212
```

- Q3. How many variables/features in the data are suffixed with _mean?

```
# alternate method
sum(endsWith(names(wisc.data), '_mean'))
```

```
[1] 10
```

```
length(grep('_mean', names(wisc.data)))
```

```
[1] 10
```

2. PCA

Here we will use `prcomp()` on the `wisc.data` object - the one without the diagnosis column.

First, we have to decide whether to use the `scale=TRUE` argument when we run `prcomp()`.

we can look at the means and sd of each column. If they are similar then we are all good to go. If not we should use `scale=TRUE`

```
colMeans(wisc.data)
```

```
          radius_mean          texture_mean        perimeter_mean
         1.412729e+01          1.928965e+01          9.196903e+01
            area_mean       smoothness_mean      compactness_mean
         6.548891e+02          9.636028e-02          1.043410e-01
      concavity_mean   concave.points_mean         symmetry_mean
         8.879932e-02          4.891915e-02          1.811619e-01
fractal_dimension_mean             radius_se            texture_se
         6.279761e-02          4.051721e-01          1.216853e+00
       perimeter_se               area_se         smoothness_se
         2.866059e+00          4.033708e+01          7.040979e-03
      compactness_se         concavity_se    concave.points_se
         2.547814e-02          3.189372e-02          1.179614e-02
        symmetry_se    fractal_dimension_se          radius_worst
         2.054230e-02          3.794904e-03          1.626919e+01
      texture_worst       perimeter_worst            area_worst
         2.567722e+01          1.072612e+02          8.805831e+02
    smoothness_worst     compactness_worst       concavity_worst
         1.323686e-01          2.542650e-01          2.721885e-01
 concave.points_worst       symmetry_worst fractal_dimension_worst
         1.146062e-01          2.900756e-01          8.394582e-02
```

```
apply(wisc.data, 2, sd)
```

```
          radius_mean          texture_mean        perimeter_mean
         3.524049e+00          4.301036e+00          2.429898e+01
            area_mean       smoothness_mean      compactness_mean
         3.519141e+02          1.406413e-02          5.281276e-02
      concavity_mean   concave.points_mean         symmetry_mean
         7.971981e-02          3.880284e-02          2.741428e-02
fractal_dimension_mean             radius_se            texture_se
         7.060363e-03          2.773127e-01          5.516484e-01
```

```
     perimeter_se           area_se        smoothness_se
     2.021855e+00       4.549101e+01        3.002518e-03
   compactness_se        concavity_se   concave.points_se
     1.790818e-02       3.018606e-02        6.170285e-03
      symmetry_se  fractal_dimension_se        radius_worst
     8.266372e-03       2.646071e-03        4.833242e+00
    texture_worst     perimeter_worst          area_worst
     6.146258e+00       3.360254e+01        5.693570e+02
smoothness_worst   compactness_worst     concavity_worst
     2.283243e-02       1.573365e-01        2.086243e-01
concave.points_worst     symmetry_worst fractal_dimension_worst
     6.573234e-02       6.186747e-02        1.806127e-02
```

These are very different so we should set `scale=True`.

```
wisc.pr <- prcomp(wisc.data, scale=TRUE)
summary(wisc.pr)
```

```
Importance of components:
                          PC1    PC2     PC3     PC4     PC5     PC6     PC7
Standard deviation     3.6444 2.3857 1.67867 1.40735 1.28403 1.09880 0.82172
Proportion of Variance 0.4427 0.1897 0.09393 0.06602 0.05496 0.04025 0.02251
Cumulative Proportion  0.4427 0.6324 0.72636 0.79239 0.84734 0.88759 0.91010
                           PC8    PC9    PC10   PC11    PC12    PC13    PC14
Standard deviation     0.69037 0.6457 0.59219 0.5421 0.51104 0.49128 0.39624
Proportion of Variance 0.01589 0.0139 0.01169 0.0098 0.00871 0.00805 0.00523
Cumulative Proportion  0.92598 0.9399 0.95157 0.9614 0.97007 0.97812 0.98335
                          PC15    PC16    PC17    PC18    PC19    PC20   PC21
Standard deviation     0.30681 0.28260 0.24372 0.22939 0.22244 0.17652 0.1731
Proportion of Variance 0.00314 0.00266 0.00198 0.00175 0.00165 0.00104 0.0010
Cumulative Proportion  0.98649 0.98915 0.99113 0.99288 0.99453 0.99557 0.9966
                          PC22    PC23   PC24    PC25    PC26    PC27    PC28
Standard deviation     0.16565 0.15602 0.1344 0.12442 0.09043 0.08307 0.03987
Proportion of Variance 0.00091 0.00081 0.0006 0.00052 0.00027 0.00023 0.00005
Cumulative Proportion  0.99749 0.99830 0.9989 0.99942 0.99969 0.99992 0.99997
                          PC29    PC30
Standard deviation     0.02736 0.01153
Proportion of Variance 0.00002 0.00000
Cumulative Proportion  1.00000 1.00000
```

Q4. From your results, what proportion of the original variance is captured by the first principal components (PC1)?

0.4427 or 44.27%

> Q5. How many principal components (PCs) are required to describe at least 70% of the original variance in the data?

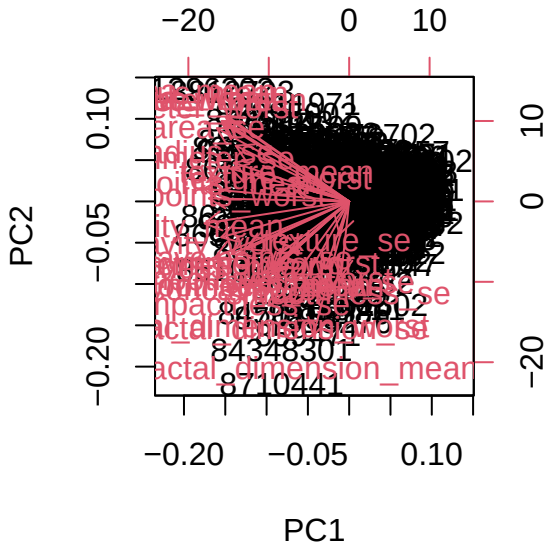
3 PCs capture 72.6% of the original variance.

> Q6. How many principal components (PCs) are required to describe at least 90% of the original variance in the data?

7 PCs capture 91% of the original variance.

Plotting the PCA results

```
biplot(wisc.pr)
```



> Q7. What stands out to you about this plot? Is it easy or difficult to understand? Why?

This plot is so cluttered and the individual data points are impossible to discern. ("Hot mess")

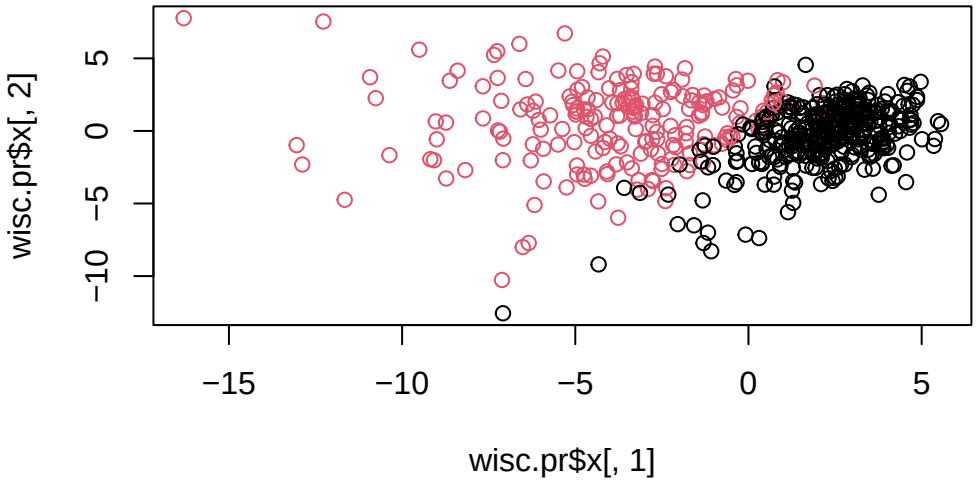
We need to make our own plot.

```
attributes(wisc.pr)
```

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

$class
[1] "prcomp"
```

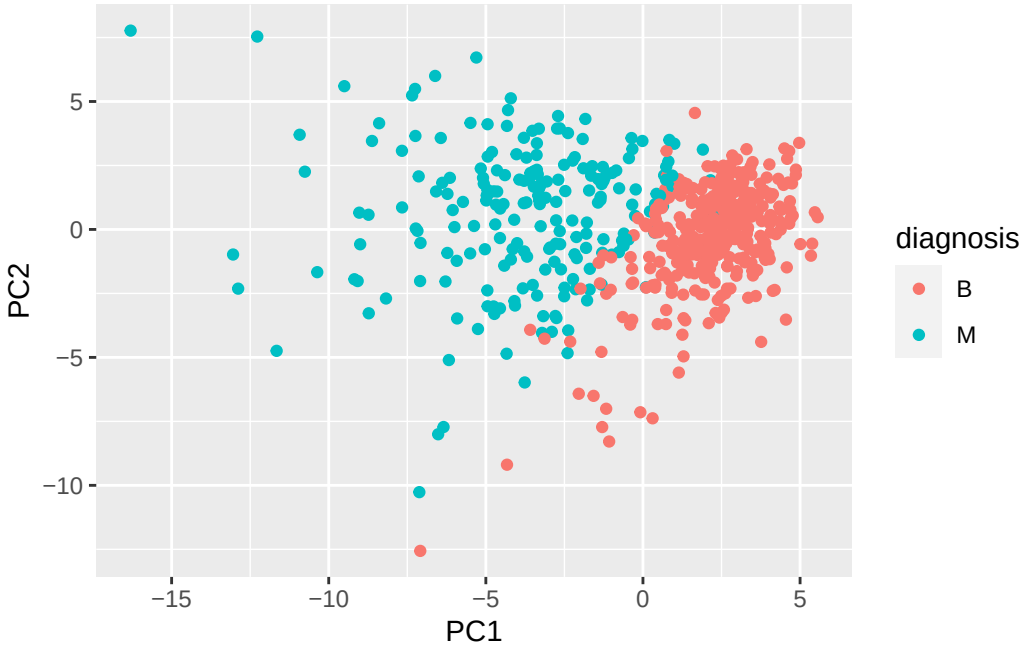
```
plot(wisc.pr$x[,1], wisc.pr$x[,2], col=diagnosis)
```




```
library(ggplot2)

pc <- as.data.frame(wisc.pr$x)

ggplot(pc) +
  aes(PC1, PC2, col=diagnosis) +
  geom_point()
```



Q9. For the first principal component, what is the component of the loading vector (i.e. wisc.pr$rotation[,1]) for the feature concave.points_mean?

```
wisc.pr$rotation["concave.points_mean",1]
```

```
[1] -0.2608538
```

Q10. What is the minimum number of principal components required to explain 80% of the variance of the data?

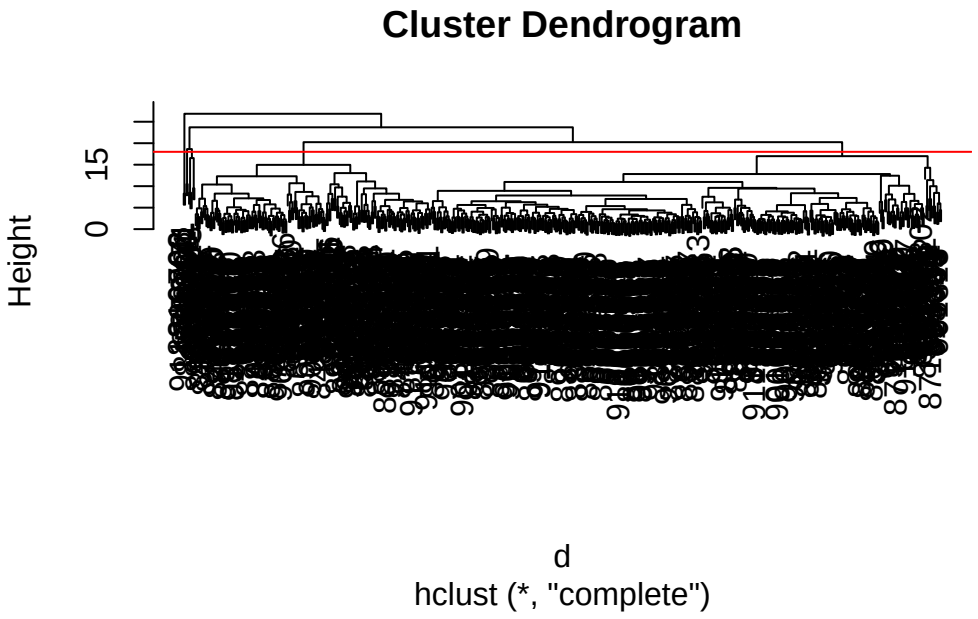
```
tbl <- summary(wisc.pr)
which(tbl$importance[3,] > 0.8)[1]
```

```
PC5
  5
```

3. Hierarchical clustering

The main function for Hierarchical clustering is called `hclust()` it takes a distance matrix as input.

```
d <- dist(scale(wisc.data))
wisc.hclust <- hclust(d)
plot(wisc.hclust)
abline(h=18, col="red")
```



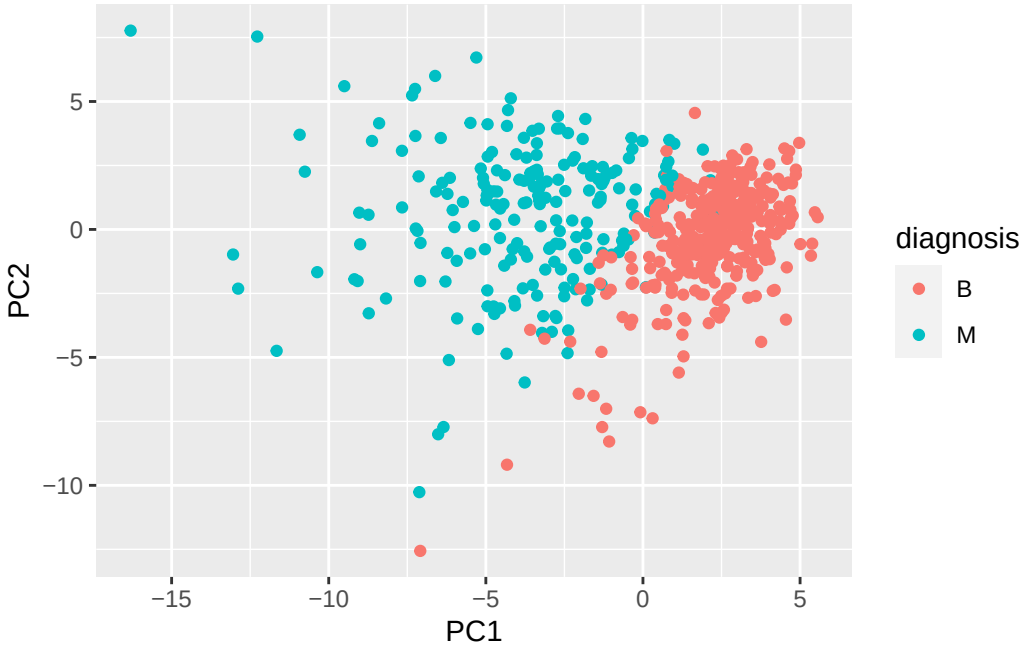

```
grps <- cutree(wisc.hclust, h=18)
table(grps)
```

```
grps
  1   2   3   4   5
177   5 383   2   2
```

Come back here later to see how our cluster grps correspond to M or B groups.

```
pc <- as.data.frame(wisc.pr$x)

ggplot(pc) +
  aes(PC1, PC2, col=diagnosis) +
  geom_point()
```

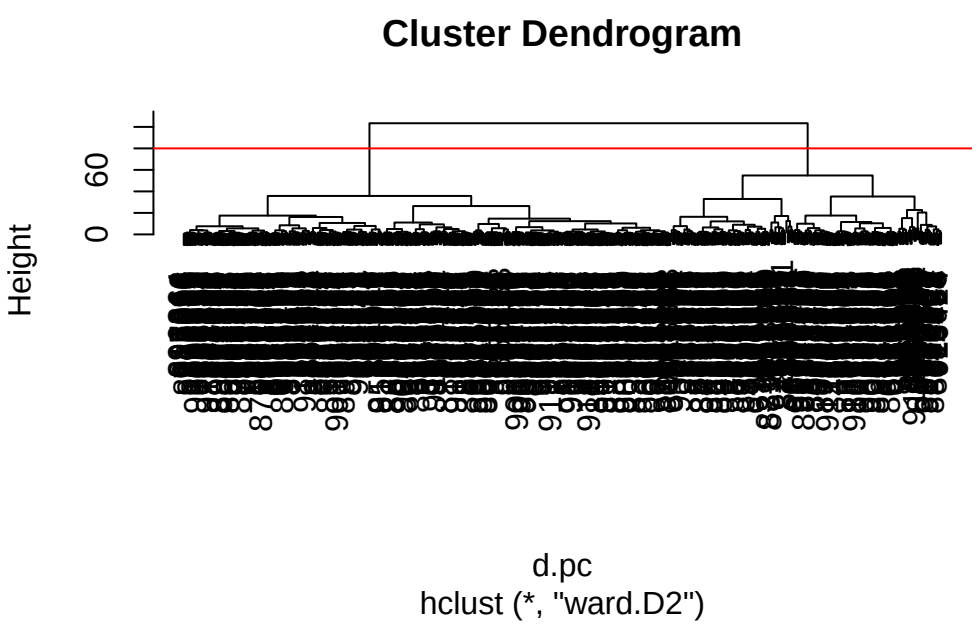


5. Combining Methods

Here we will perform clustering on PCA results rather than the original data.

In other words we will cluster using `wisc.pr$x` - our new better variables or PCs. We can chose as many or as few PCs to use as we like. It is your call!

```
d.pc <- dist(wisc.pr$x[,1:3])
wisc.pr.hclust <- hclust(d.pc, method="ward.D2")
plot(wisc.pr.hclust)
abline(h=80, col="red")
```




```
grps <- cutree(wisc.pr.hclust, h=80)
table(grps)
```

```
grps
  1   2
203 366
```

We can use `table()` function to make a cross-table as well as just a count table.

```
table(diagnosis)
```

```
diagnosis
  B   M
357 212
```

```
table(grps, diagnosis)
```

```
    diagnosis
grps   B   M
```

```
  1  24 179
  2 333  33
```

Write a note here about how to read this cross-table result:

This table shows the occurrence of both classes in the diagnosis vector and the clusters we've defined. We can see that cluster 1 corresponds to a Malignant diagnosis and cluster 2 corresponds to a Benign diagnosis. There are some false positives/negatives, but the overall result is pretty good.

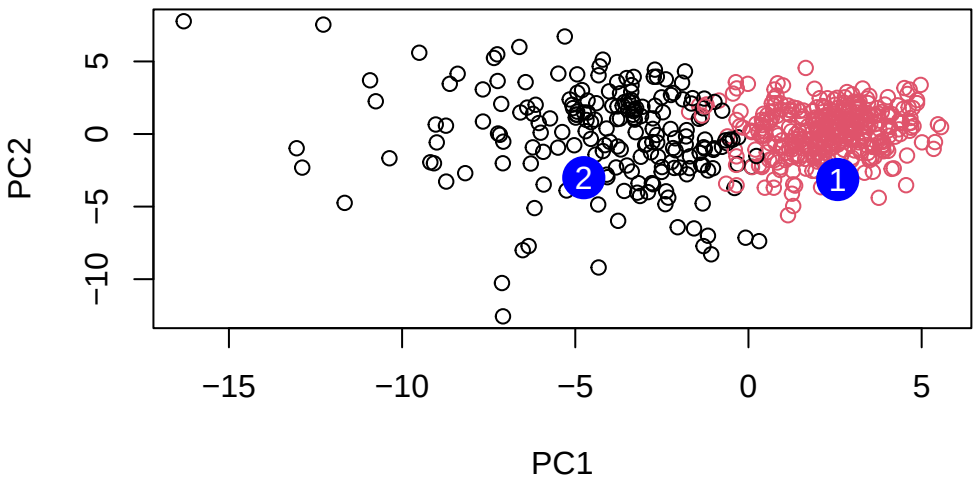
7. Prediction

```
#url <- "new_samples.csv"
url <- "https://tinyurl.com/new-samples-CSV"
new <- read.csv(url)
npc <- predict(wisc.pr, newdata=new)
npc
```

```
          PC1       PC2        PC3        PC4       PC5        PC6        PC7
[1,]  2.576616 -3.135913  1.3990492 -0.7631950  2.781648 -0.8150185 -0.3959098
[2,] -4.754928 -3.009033 -0.1660946 -0.6052952 -1.140698 -1.2189945  0.8193031
           PC8       PC9       PC10      PC11      PC12      PC13     PC14
[1,] -0.2307350 0.1029569 -0.9272861 0.3411457  0.375921 0.1610764 1.187882
[2,] -0.3307423 0.5281896 -0.4855301 0.7173233 -1.185917 0.5893856 0.303029
         PC15       PC16        PC17        PC18        PC19       PC20
[1,] 0.3216974 -0.1743616 -0.07875393 -0.11207028 -0.08802955 -0.2495216
[2,] 0.1299153  0.1448061 -0.40509706  0.06565549  0.25591230 -0.4289500
          PC21       PC22       PC23       PC24        PC25         PC26
[1,]  0.1228233 0.09358453 0.08347651  0.1223396  0.02124121  0.078884581
[2,] -0.1224776 0.01732146 0.06316631 -0.2338618 -0.20755948 -0.009833238
             PC27        PC28         PC29         PC30
[1,]  0.220199544 -0.02946023 -0.015620933  0.005269029
[2,] -0.001134152  0.09638361  0.002795349 -0.019015820
```

And plot this up

```
plot(wisc.pr$x[,1:2], col=grps)
points(npc[,1], npc[,2], col="blue", pch=16, cex=3)
text(npc[,1], npc[,2], c(1,2), col="white")
```



Q18. Which of these new patients should we prioritize for follow up based on your results?

We should prioritize group 2 (malignant) patients because their condition is likely more concerning.