class08

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

Read the data:

```
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?

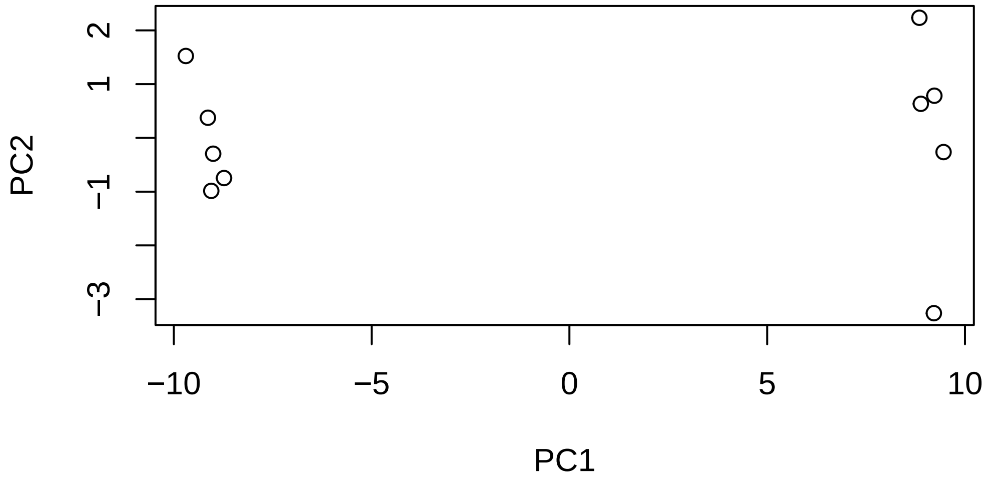
```
nrow(rna.data)
```

```
[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")
```



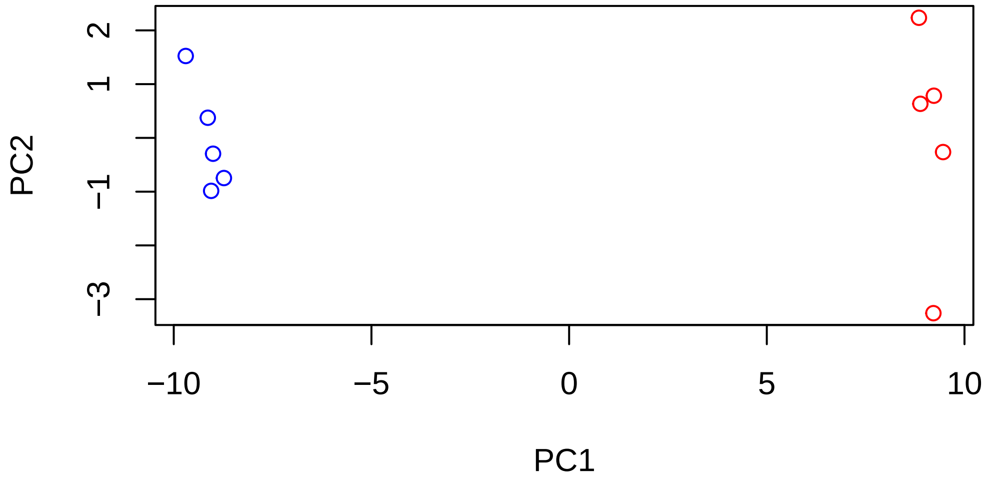
```
summary(pca)
```

```
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 3.345e-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))
mycols
```

```
 [1] "blue" "blue" "blue" "blue" "blue" "red"  "red"  "red"  "red"  "red"
```

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



I could examine which genes contribute most to this first PC

```
pca$rotation[,1]
```

```
       gene1        gene2        gene3        gene4        gene5        gene6 
-0.103666005  0.103514749 -0.103761385  0.075320862  0.087428334  0.099670829 
       gene7        gene8        gene9       gene10       gene11       gene12 
 0.103609009  0.100759370 -0.085460936  0.103783379 -0.103719665 -0.102001924 
      gene13       gene14       gene15       gene16       gene17       gene18 
-0.103399591  0.102478762  0.099993193  0.103598474 -0.103013773 -0.103774699 
      gene19       gene20       gene21       gene22       gene23       gene24 
-0.103390599  0.103121803  0.103787935  0.102725125  0.103681565 -0.098284250 
      gene25       gene26       gene27       gene28       gene29       gene30 
-0.103302326 -0.085745836 -0.103374849  0.103638752 -0.102739689  0.103044435 
      gene31       gene32       gene33       gene34       gene35       gene36 
-0.101768804 -0.100677376  0.103592988  0.103525731  0.102382706  0.103412422 
      gene37       gene38       gene39       gene40       gene41       gene42 
 0.100467583  0.102080752 -0.103744482 -0.102003831  0.103716818 -0.098746675 
      gene43       gene44       gene45       gene46       gene47       gene48 
-0.092001819 -0.103504699  0.103840183 -0.096571619  0.103502386  0.103682769 
      gene49       gene50       gene51       gene52       gene53       gene54 
-0.103188532 -0.103743341 -0.103265591  0.102519795 -0.103245619 -0.103584153 
```

```
      gene55       gene56       gene57       gene58       gene59       gene60 
 0.103695870 -0.103783479 -0.103703675 -0.103503980 -0.103607438  0.103805515 
      gene61       gene62       gene63       gene64       gene65       gene66 
-0.103308945  0.103713893  0.101468649 -0.078643996 -0.094219475  0.103845454 
      gene67       gene68       gene69       gene70       gene71       gene72 
 0.103453646  0.103839510  0.048197107 -0.101365212  0.095664760  0.102347342 
      gene73       gene74       gene75       gene76       gene77       gene78 
 0.102001050  0.101747637 -0.103592371  0.103514464 -0.097473626 -0.100499426 
      gene79       gene80       gene81       gene82       gene83       gene84 
-0.103639415 -0.093476477 -0.100659777 -0.103481127  0.066065263 -0.009263882 
      gene85       gene86       gene87       gene88       gene89       gene90 
 0.103698370 -0.102122719 -0.103448562  0.098226585  0.100038548  0.103777744 
      gene91       gene92       gene93       gene94       gene95       gene96 
-0.103698408  0.092534408  0.102950950 -0.102692869  0.102142492 -0.096658194 
      gene97       gene98       gene99      gene100 
 0.100787961  0.103837190  0.103649598  0.103870820 
```

Q. take absolute value then sort it; top 6 genes that contribute the most?

```
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 
```

Analysis of Breast Cancer RNA data

Read the 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)
```

Note that the first column here wisc.df$diagnosis is a pthologist provided expert diagnosis.

```
diagnosis <- as.factor(wisc.df$diagnosis)
```

Now I want to make sure I remove that column from my dataset for analysis

```r
wisc.data <- wisc.df[,-1]
head(wisc.data)
```

```
         radius_mean texture_mean perimeter_mean area_mean smoothness_mean
842302         17.99        10.38         122.80    1001.0         0.11840
842517         20.57        17.77         132.90    1326.0         0.08474
84300903       19.69        21.25         130.00    1203.0         0.10960
84348301       11.42        20.38          77.58     386.1         0.14250
84358402       20.29        14.34         135.10    1297.0         0.10030
843786         12.45        15.70          82.57     477.1         0.12780
         compactness_mean concavity_mean concave.points_mean symmetry_mean
842302            0.27760         0.3001             0.14710        0.2419
842517            0.07864         0.0869             0.07017        0.1812
84300903          0.15990         0.1974             0.12790        0.2069
84348301          0.28390         0.2414             0.10520        0.2597
84358402          0.13280         0.1980             0.10430        0.1809
843786            0.17000         0.1578             0.08089        0.2087
         fractal_dimension_mean radius_se texture_se perimeter_se area_se
842302                  0.07871    1.0950     0.9053        8.589  153.40
842517                  0.05667    0.5435     0.7339        3.398   74.08
84300903                0.05999    0.7456     0.7869        4.585   94.03
84348301                0.09744    0.4956     1.1560        3.445   27.23
84358402                0.05883    0.7572     0.7813        5.438   94.44
843786                  0.07613    0.3345     0.8902        2.217   27.19
         smoothness_se compactness_se concavity_se concave.points_se
842302        0.006399        0.04904      0.05373           0.01587
842517        0.005225        0.01308      0.01860           0.01340
84300903      0.006150        0.04006      0.03832           0.02058
84348301      0.009110        0.07458      0.05661           0.01867
84358402      0.011490        0.02461      0.05688           0.01885
843786        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
```

> Q1. How many observations are in this dataset?

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

```
[1] 569
```

569 rows

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

```
table(wisc.df$diagnosis)
```

```

  B   M 
357 212 
```

212 observations have a malignant diagnosis

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

```
length(grep("_mean", colnames(wisc.data)))
```

```
[1] 10
```

10 variables

adding a `value = TRUE` argument will print the names of the coluumns that have the match

Principal Component Analysis

Here we will use `prcomp()` on the `wisc.data` obkect - 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 the 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 
```

Means are pretty different.

```
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 also pretty different. As a result, we should `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
```

Capturing 44% in the first PC is pretty good; that combined with PC2 and PC3 (through a 3D plot) covers 73% of the variance, which is pretty good!

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

44.27%

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

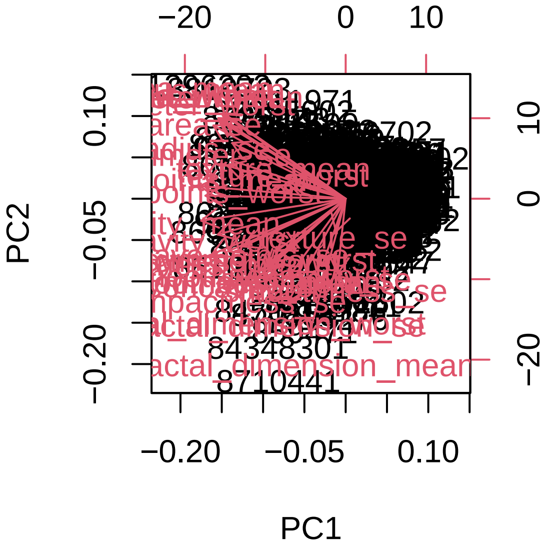
3 PCs captured 72.64%.

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

7 PCs captured 91.01%.

Plotting the PCA results

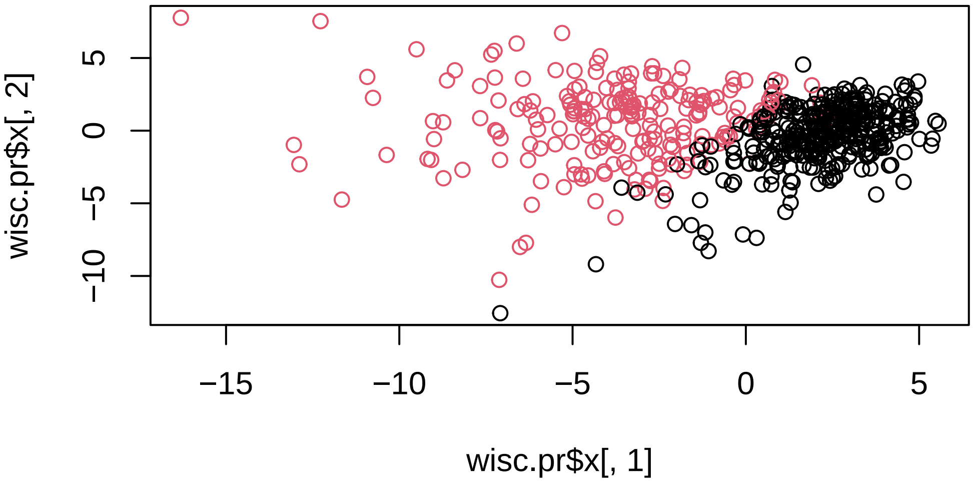
```
biplot(wisc.pr)
```



Usually made for smaller datasets, this is a big mess!

We need to make our own plot.

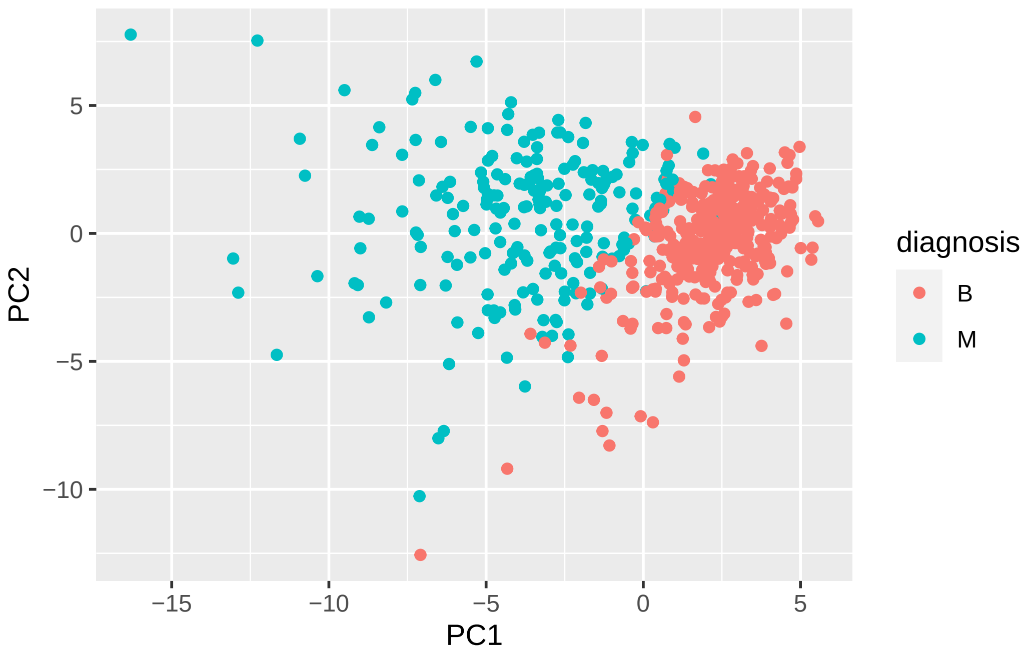
```r
plot(wisc.pr$x[,1], wisc.pr$x[,2], col = diagnosis)
```



```
library(ggplot2)

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

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



Communicating PCA results

> 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
```

-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)
tbl$importance[3,] > 0.8
```

```
  PC1   PC2   PC3   PC4   PC5   PC6   PC7   PC8   PC9  PC10  PC11  PC12  PC13 
FALSE FALSE FALSE FALSE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE 
 PC14  PC15  PC16  PC17  PC18  PC19  PC20  PC21  PC22  PC23  PC24  PC25  PC26 
 TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE  TRUE
 PC27  PC28  PC29  PC30
 TRUE  TRUE  TRUE  TRUE
```

```r
which(tbl$importance[3,] > 0.8)[1]
```

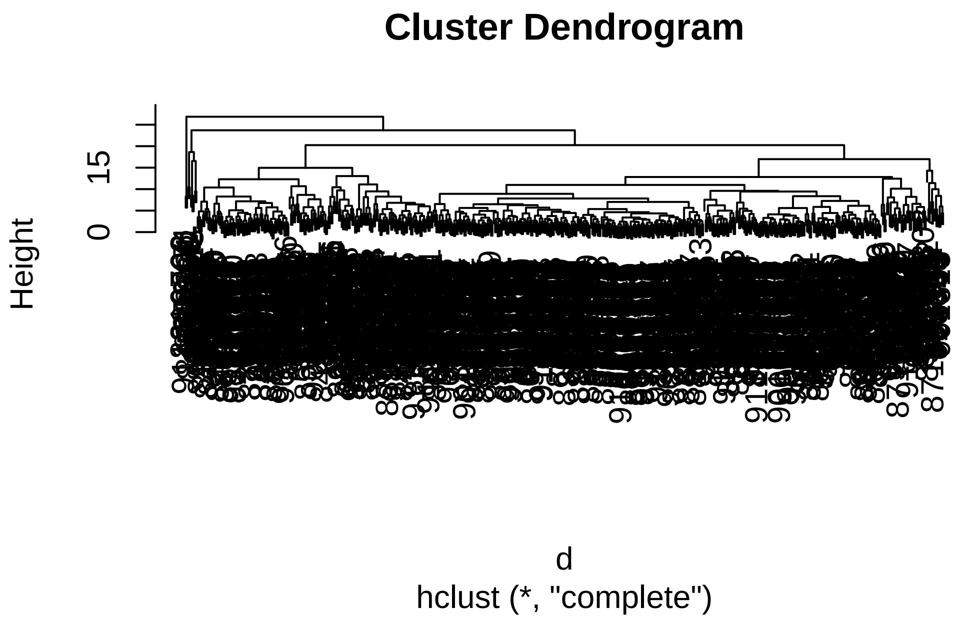
```
PC5
  5
```

Takes 5 PCs to explain 84.73% of the variance of the data.

##Hierarchial clustering

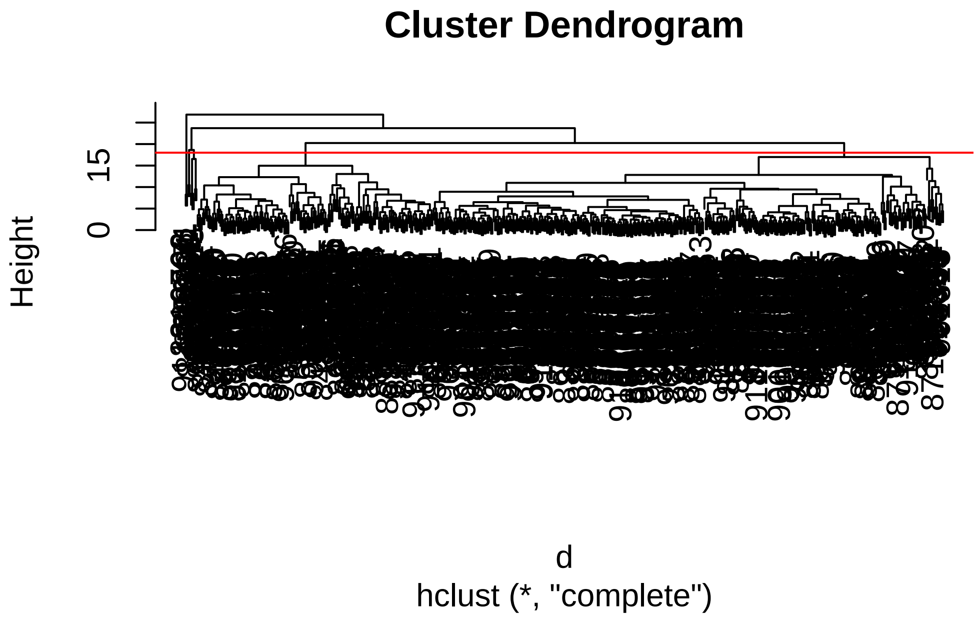
The main function for Hierarchial clustering is called `hclust()`; it takes a distance matrix as input.

```r
d <- dist(scale( wisc.data))
wisc.hclust <- hclust(d)
plot(wisc.hclust)
```



That result does not look good!!

```
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 
```

Unlikely that these will produce good results.

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

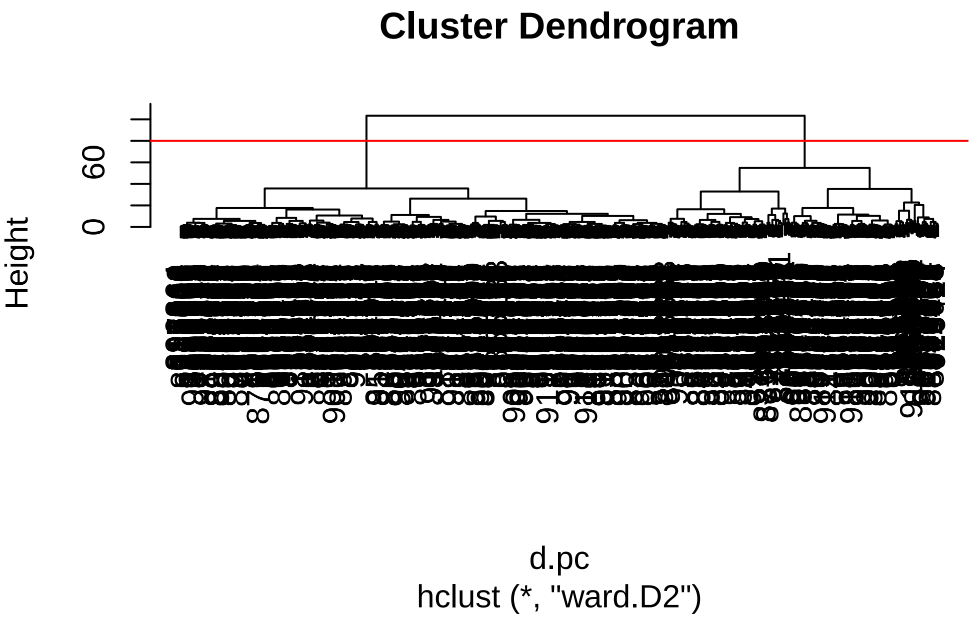
5. Combining methods

Here we will perform clustering on our 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 choose as many or as few PCs to use as we like. It is your call!

```r
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")
```



```r
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.

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

```
    diagnosis
grps   B   M
   1  24 179
```

```
  2 333  33
```

Showing you the clustering based on the diagnosis variable (out of the 357 benign, 333 belong in group 2, etc.).

The results indicate that our cluster 1 mostly captures cancer (M) and our cluster 2 mainly captures healthy (B) samples/individuals.

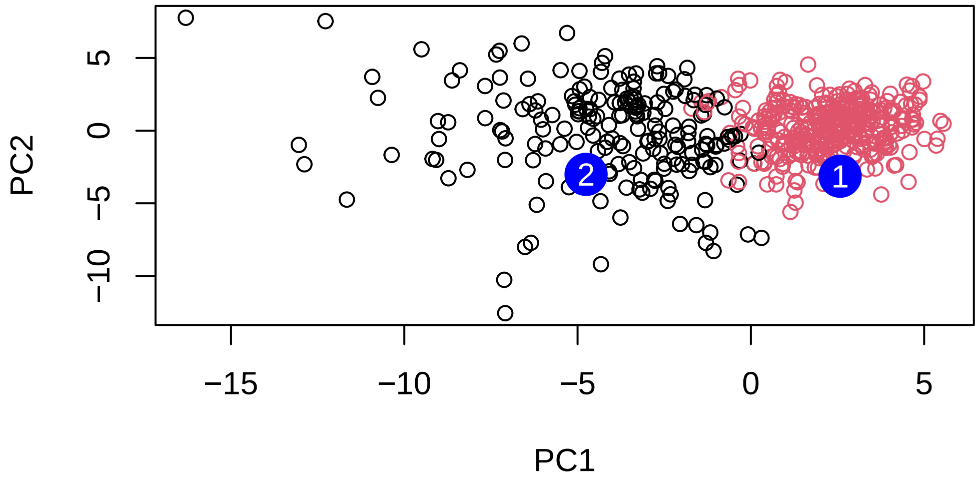
7. Prediction

```
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
```

Make predictions from what we found from Wisconson to see where these new people lie.

```
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?

Should be worried about patient 2 since they have characteristics of cells that look like other malignent cells.