class08

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Before we get stuck into project work we will have a quick look at applying PCA to some example RNASeq data (tail end of 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
```

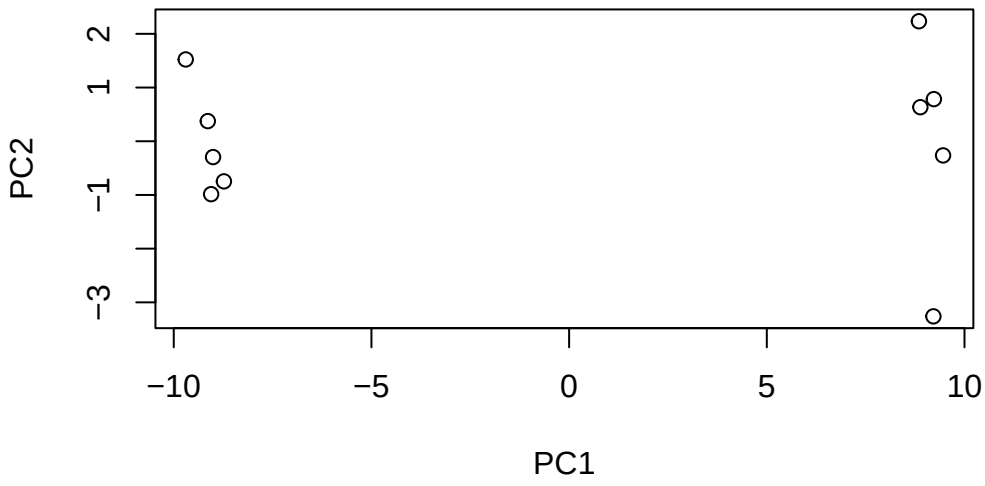
Q10: How many genes and samples are in this data set?

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

```
[1] 100
```

##Run PCA

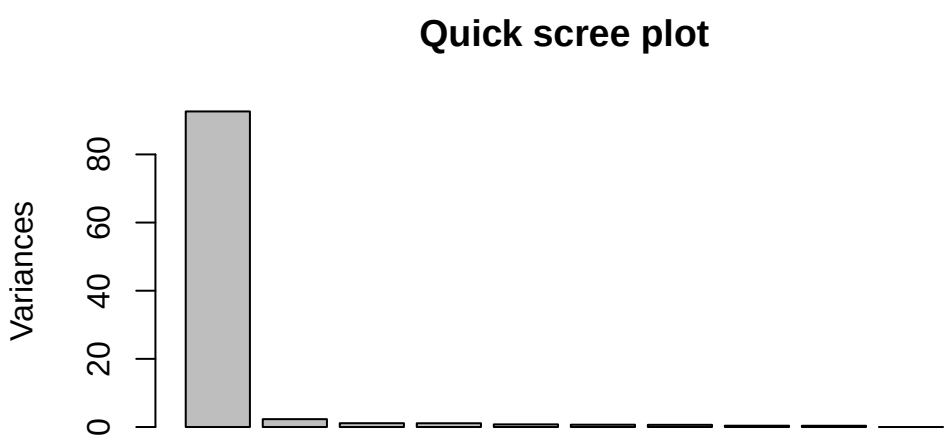
```
## Again we have to take the transpose of our data
pca <- prcomp(t(rna.data), scale=TRUE)

## 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
```

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




```
pca$x
```

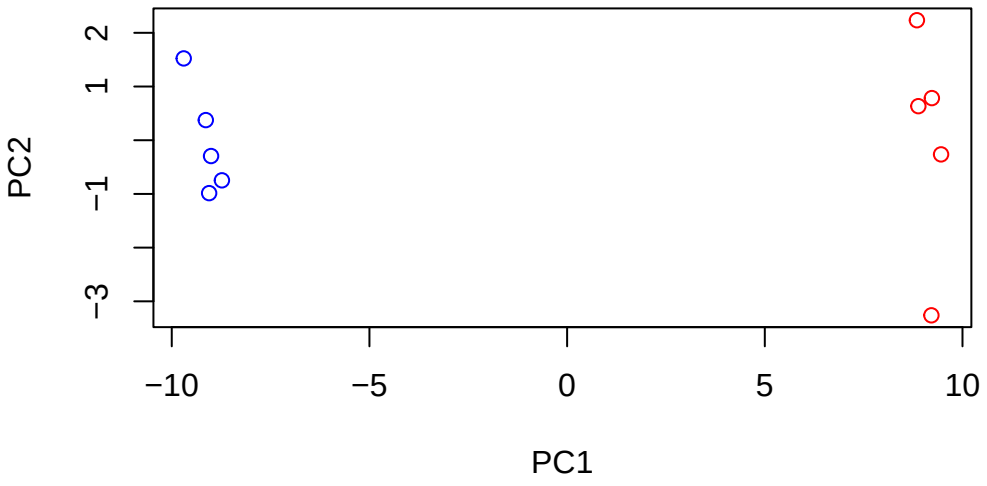
```
          PC1        PC2        PC3        PC4        PC5        PC6
wt1 -9.697374  1.5233313 -0.2753567  0.7322391 -0.6749398  1.1823860
wt2 -9.138950  0.3748504  1.0867958 -1.9461655  0.7571209 -0.4369228
wt3 -9.054263 -0.9855163  0.4152966  1.4166028  0.5835918  0.6937236
wt4 -8.731483 -0.7468371  0.5875748  0.2268129 -1.5404775 -1.2723618
wt5 -9.006312 -0.2945307 -1.8498101 -0.4303812  0.8666124 -0.2496025
ko1  8.846999  2.2345475 -0.1462750 -1.1544333 -0.6947862  0.7128021
ko2  9.213885 -3.2607503  0.2287292 -0.7658122 -0.4922849  0.9170241
ko3  9.458412 -0.2636283 -1.5778183  0.2433549  0.3654124 -0.5837724
ko4  8.883412  0.6339701  1.5205064  0.7760158  1.2158376 -0.1446094
ko5  9.225673  0.7845635  0.0103574  0.9017667 -0.3860869 -0.8186668
           PC7         PC8         PC9         PC10
wt1 -0.24446614  1.03519396  0.07010231 3.073930e-15
wt2 -0.03275370  0.26622249  0.72780448 1.963707e-15
wt3 -0.03578383 -1.05851494  0.52979799 2.893519e-15
wt4 -0.52795595 -0.20995085 -0.50325679 2.872702e-15
wt5  0.83227047 -0.05891489 -0.81258430 1.693090e-15
ko1 -0.07864392 -0.94652648 -0.24613776 4.052314e-15
ko2  0.30945771  0.33231138 -0.08786782 3.268219e-15
ko3 -1.43723425  0.14495188  0.56617746 2.636780e-15
```

```
ko4 -0.35073859  0.30381920 -0.87353886 3.615164e-15
ko5  1.56584821  0.19140827  0.62950330 3.379241e-15
```

```
#We have 5wt and 5 ko samples, so add color
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

```
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 Breat Cancer FNA data

First, load csv file

```r
# Assign name for csv file
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
```

Note that the first column here wisc.df$diagnosis is a pathologist provided expert diagnosis. Now I want to make sure I remove that column from my dataset for analysis

```
wisc.data <- wisc.df[,-1] #Creating dataset without diagnosis
diagnosis <- as.factor(wisc.df$diagnosis) #Creating vector with just diagnosis
```

Q1. How many observations are in this dataset?

```
nrow(wisc.df)
```

```
[1] 569
```

There are 569 observation in this dataset.

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

```
table(diagnosis)
```

```
diagnosis
  B   M
357 212
```

There are 212 maglignant diagnosis observed.

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

```
grp <- grep("_mean", colnames(wisc.df), ignore.case = T, value = T)
grp
```

```
 [1] "radius_mean"            "texture_mean"           "perimeter_mean"
 [4] "area_mean"              "smoothness_mean"        "compactness_mean"
 [7] "concavity_mean"         "concave.points_mean"    "symmetry_mean"
[10] "fractal_dimension_mean"
```

```
length(grp)
```

```
[1] 10
```

There are 10 features in the data suffixed with "_mean."

##Principal Component Analysis

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

First, we have 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 scale=TRUE.

```
wisc.pr <- prcomp(wisc.data, scale = T)
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)?

44.27% of original variance is captured by PC1.

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

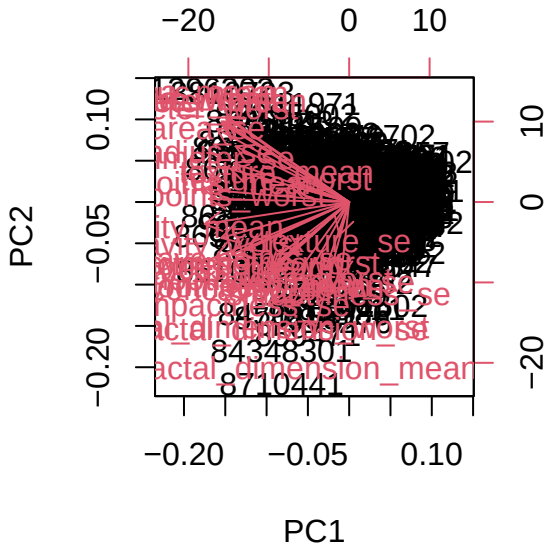
Cumulative proportion (C.P) exceeds 70% from third principal component, where C.P at PC3 is 72.239%. Therefore, three PCs are required to capture at least 70% of the original variance.

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

C.P exceeds 90% from seventh principal component, where C.P at PC7 is 91.010%. Therefore, seven PCs are required to capture at least 90% 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?

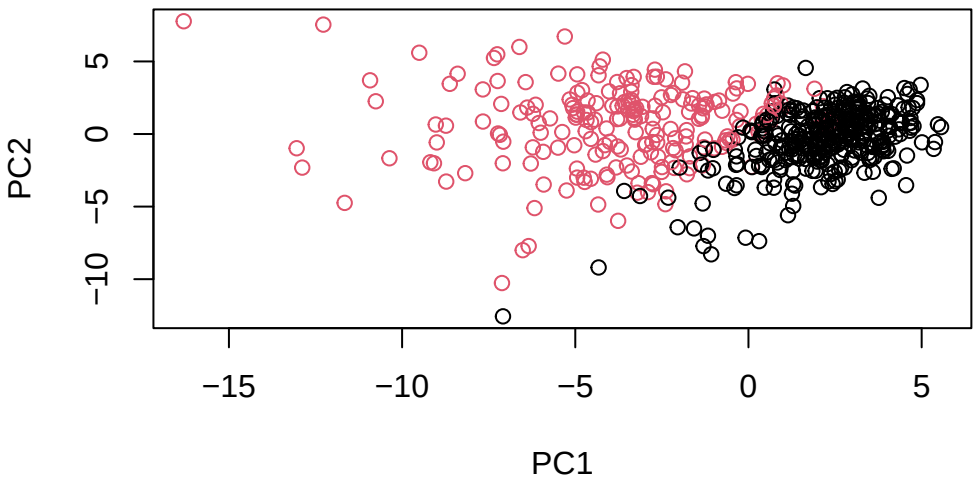
A lot of benign data points are clusted in same area with patient numbers. It's impossible to see actual data points (because of size of patient id) that understanding plot is challenging.

We need to make our own plot

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

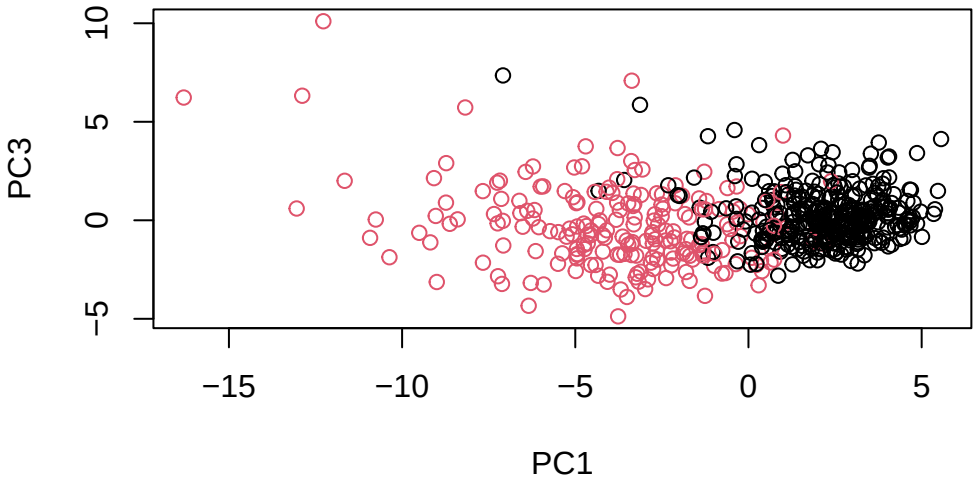
```
$names
[1] "sdev"     "rotation" "center"   "scale"    "x"

$class
[1] "prcomp"
```

```
plot(wisc.pr$x[ ,1], wisc.pr$x[ ,2], col= diagnosis, xlab = "PC1", ylab = "PC2")
```



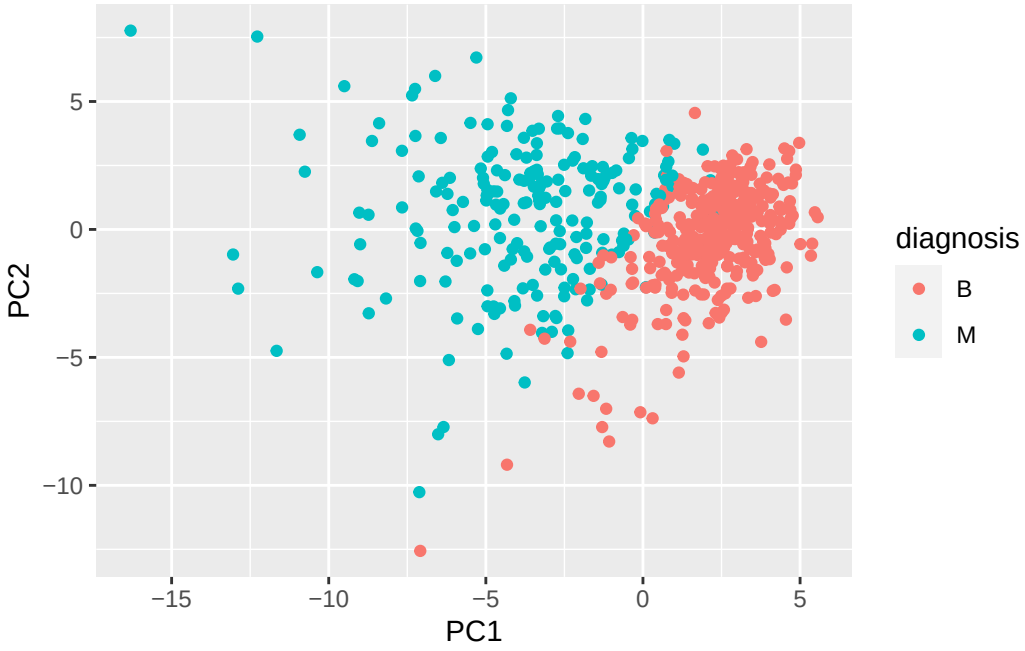
Q8. Generate a similar plot for principal components 1 and 3. What do you notice about these plots?

```
plot(wisc.pr$x[ ,1], wisc.pr$x[ ,3], col= diagnosis, xlab = "PC1", ylab = "PC3")
```



PC3 vs PC1 (graph 2) looks similar to PC2 vs PC1 (graph 1), but inverted by x-axis. While spread of dots on PC1 stays the same, their vertical spread changes as PC changed from PC2 to PC3. Also, graph 2 seems benign and malignant groups are closer/more overlapping than graph 1. This makes sense since graph 1 captures more variance (cumulative proportion >60%) than graph 2 (cumulative proportion <60%) that seperation of dots are more clear in graph 1.

```
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
```

Loading vector for concanve.points_mean is -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]
```

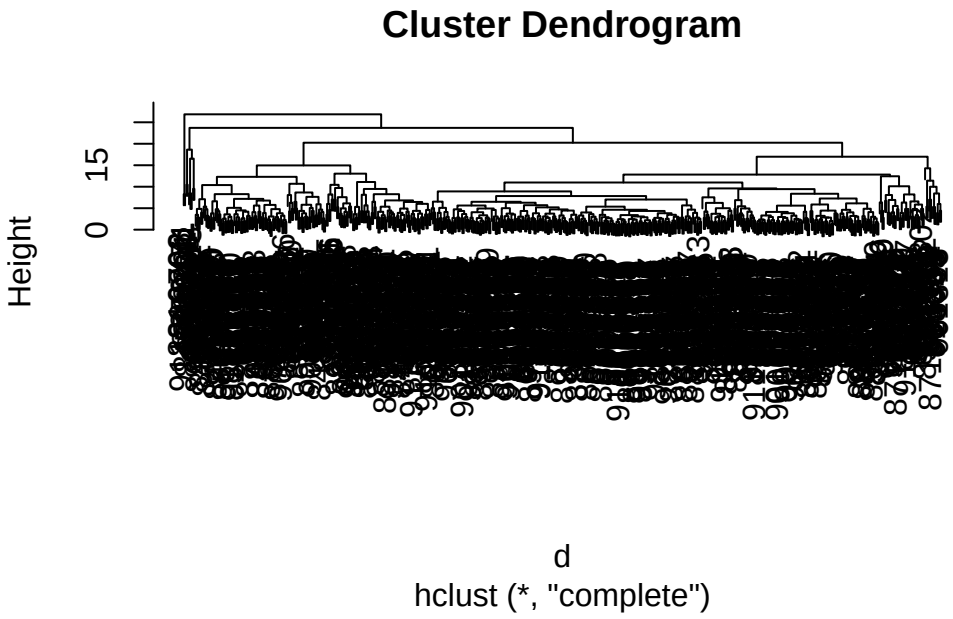
```
PC5
  5
```

At least 5 PCs are required to capture 80% of the variance.

Hierarchical clustering

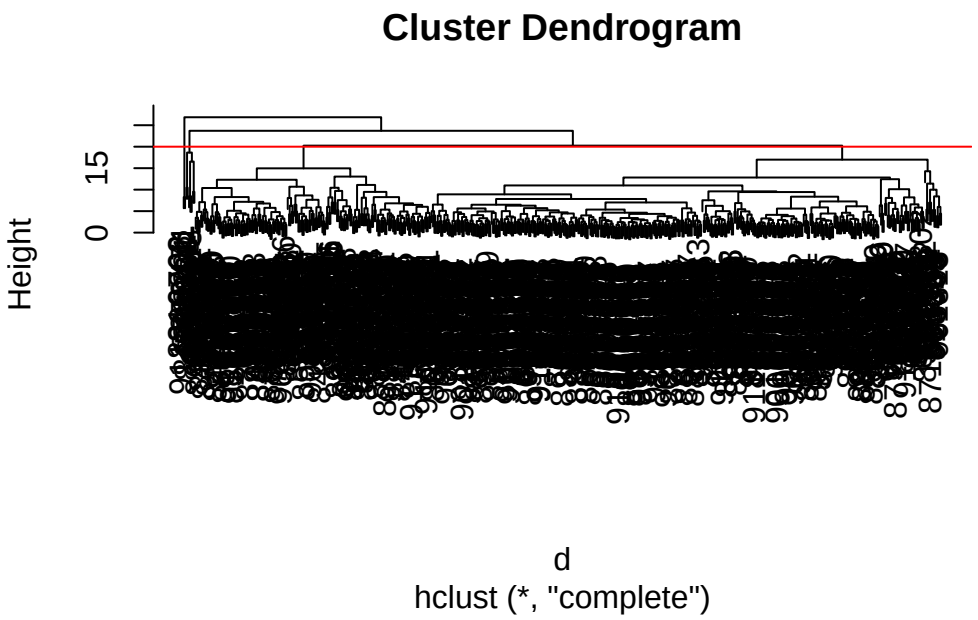
The main function for Hierarchical clustering is called 'hclust()' it takes a distance matrix as input.

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



Q11. Using the plot() and abline() functions, what is the height at which the clustering model has 4 clusters?

```
plot(wisc.hclust)
abline(h = 20, col = "red")
```




```
grps <- cutree(wisc.hclust, h = 20)
table(grps)
```

```
grps
  1   2   3   4
177   7 383   2
```

height between 19 and 20 shows 4 clusters. Example plot with height of 20 is drawn above.

```
wisc.hclust.clusters <- cutree(wisc.hclust, h = 20)
table(wisc.hclust.clusters, diagnosis)
```

```
                    diagnosis
wisc.hclust.clusters   B   M
                   1  12 165
                   2   2   5
                   3 343  40
                   4   0   2
```

Q12. Can you find a better cluster vs diagnoses match by cutting into a different number of clusters between 2 and 10?

```
grps <- cutree(wisc.hclust, h = 20)
table(grps, diagnosis)
```

```
    diagnosis
grps   B   M
   1  12 165
   2   2   5
   3 343  40
   4   0   2
```

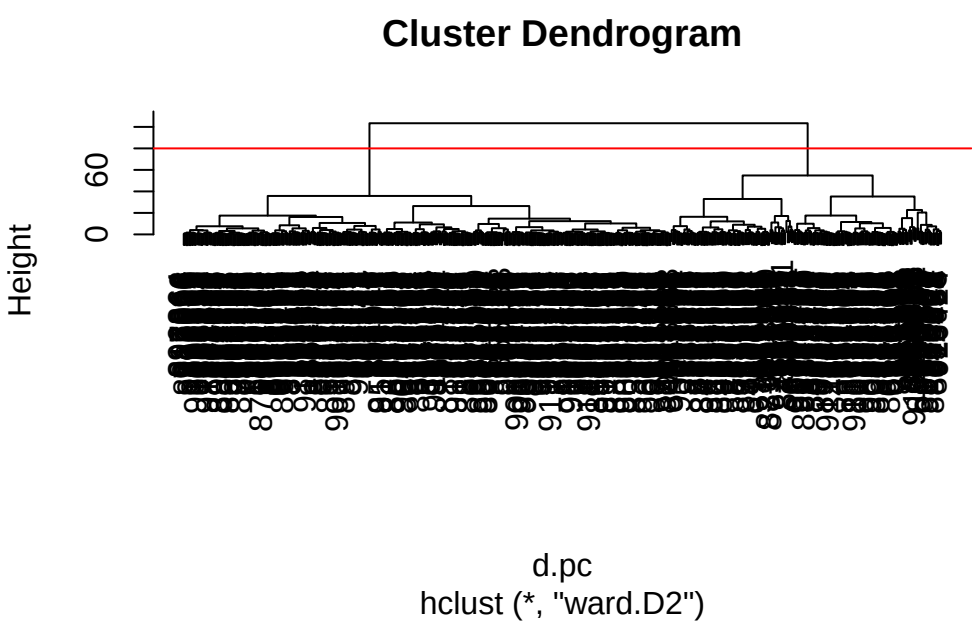
2 clusters have overlap of both diagnosis (B:M = 357:210) and small left overs (0:2) that it fails to seperate diagnosis. 3 Clusters shows same trend, where each clusters are (355:205), (2:5), and (0:2). Five or more clusters have main groups with majority of B or M, and other small groups with (0:1~5) or (1~4:0) that grouping creates unnecessary meaningless data. Therefore, 4 clusters, where two groups contains majority of one type of diagnosis with minimal amount of small group, is the best.

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 as 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 tableas well as just a count table.

```
table(diagnosis)
```

```
diagnosis
  B   M
357 212
```

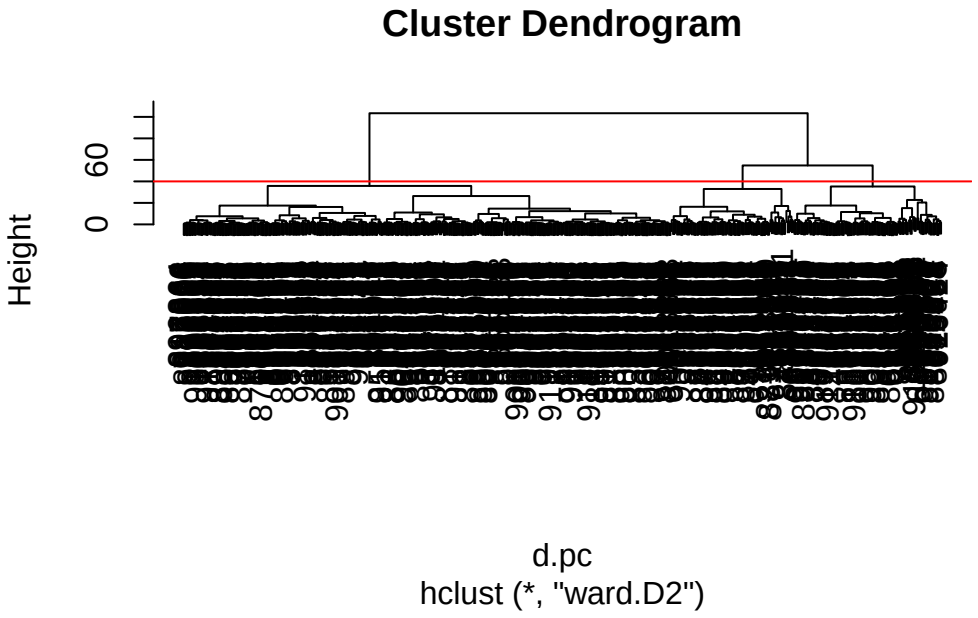
```
table(grps, diagnosis)
```

```
    diagnosis
grps   B   M
```

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

Q13. Which method gives your favorite results for the same data.dist dataset? Explain your reasoning.

```
d.pc <- dist(wisc.pr$x[ ,1:3])
wisc.pr.hclust <- hclust(d.pc, method = "ward.D2")
plot(wisc.pr.hclust)
abline(h = 40, col = "red")
```




```
grps <- cutree(wisc.pr.hclust, h = 40)
table(grps, diagnosis)
```

```
    diagnosis
grps   B   M
   1   0 111
   2  24  68
   3 333  33
```

Single link considers distance between closest elemt between clusters, complete link considers furthest elements between clusters, average link considers average distance of all pairs, and ward link considers total distance from centroids. I prefer ward method because it minimizes variance of elements within the cluster that each group has minimal mixing (with element in other cluster) while maximizing seperation among groups. That said, Ward's method gives highest precision thus my favorite method.

Q14. How well does k-means separate the two diagnoses? How does it compare to your hclust results?

```
wisc.km <- kmeans(wisc.data, centers= 2, nstart= 20)
table(wisc.km$cluster, diagnosis)
```

```
   diagnosis
      B   M
  1   1 130
  2 356  82
```

k-means does seperate two diagnose well as one diagnosis is majority of elements in each group. Group 1 has mostly benign while group 2 has mostly malignant. While k-means does excellent job in detecting malignant data, only one false positive in group 2, it shows high false negative in group 1. Compared to hclust, k-mean's result is more inaccurate in seperating two diagnosis due to higher inaccuracy in group 1.

```
table(wisc.hclust.clusters, wisc.km$cluster)
```

```
wisc.hclust.clusters   1   2
                   1 109  68
                   2   2   5
                   3  18 365
                   4   2   0
```

```
library(rgl)
plot3d(wisc.pr$x[,1:3], xlab="PC 1", ylab="PC 2", zlab="PC 3", cex=1.5, size=1, type="s",
```

```
dist1 <- dist(wisc.pr$x[ ,1:7])
wisc.pr.hclust1 <- hclust(dist1, method="ward.D2")
wisc.pr.hclust.clusters <- cutree(wisc.pr.hclust1, k=2)
```

```
table(wisc.pr.hclust.clusters, diagnosis)
```

```
                     diagnosis
wisc.pr.hclust.clusters   B   M
                      1  28 188
                      2 329  24
```

New model has higher count difference in each diagnosis (compared to previous ones), seperating two diagnosis well.

Q16. How well do the k-means and hierarchical clustering models you created in previous sections (i.e. before PCA) do in terms of separating the diagnoses? Again, use the table() function to compare the output of each model (wisc.km$cluster and wisc.hclust.clusters) with the vector containing the actual diagnoses.

```
table(wisc.km$cluster, diagnosis)
```

```
   diagnosis
      B   M
  1   1 130
  2 356  82
```

```
table(wisc.hclust.clusters, diagnosis)
```

```
                    diagnosis
wisc.hclust.clusters   B   M
                   1  12 165
                   2   2   5
                   3 343  40
                   4   0   2
```

Both models does decent job in seperating diagnosis. However, combining both method maximize differentiation between groups that it's more accurate.

Q17. Which of your analysis procedures resulted in a clustering model with the best specificity? How about sensitivity?

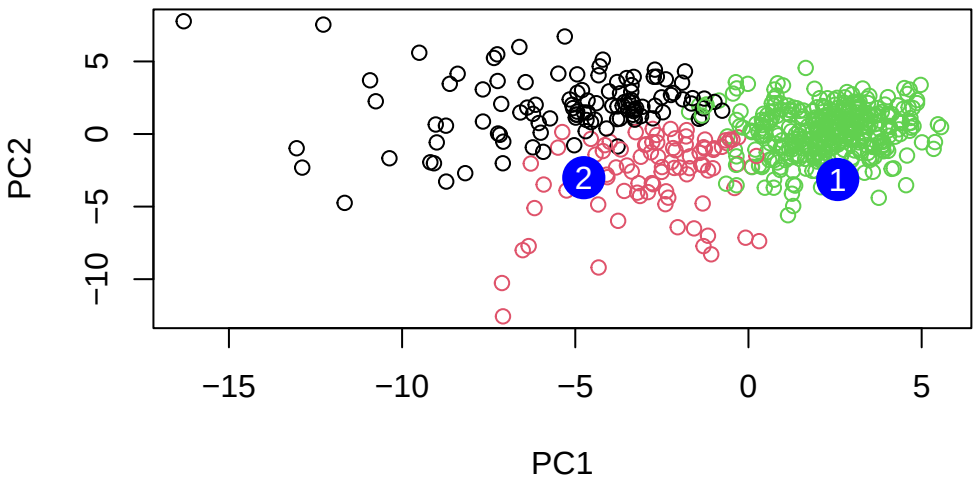
k-means showed highest sensitivity (only one false positive), and combining two methods showed highest specificity (highest true negative rate)

Section 7.

```
#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
```

```
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?

Patients with malignant diagnosis should be prioritized for follow up appointment. That said, group 2 should be prioritized.