Class 8: Mini Project

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The goal of this mini-project is for you to explore a complete analysis using the unsupervised learning techniques covered in class. You'll extend what you've learned by combining PCA as a preprocessing step to clustering using data that consist of measurements of cell nuclei of human breast masses. This expands on our RNA-Seq analysis from last day.

Our data for today comes from FNA of breast tissue. Let's read this data into R.

First, save the csv file itself into the same directory as the R project. Second, read the CSV using `read.csv` Third, set `row.names=1`

```
wisc.df <- read.csv("WisconsinCancer.csv", 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
```

```
dim(wisc.df)
```

```
[1] 569  31
```

Q. How many observations/samples/rows are there?

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

```
[1] 569
```

There are 569 rows.

Can also check with 569 (shows the number directly in the rendered Quarto doc)

Q. What is in the $diagnosis column? How many of each type?

```
sum(wisc.df$diagnosis == "M")
```

```
[1] 212
```

```
sum(wisc.df$diagnosis == "B")
```

```
[1] 357
```

Or, use table. Table the function with the column, giving how many of each there are.

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

```
  B   M
357 212
```

There are 357 benign examples, and 212 malignant examples

Generally if we do machine learning, we want examples to be balanced, so that its learning is not skewed.

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

Can use `grep`. `grep(pattern,x)`

Pattern = character string containing a regular expression (or character string for fixed = TRUE) to be matched in the given character vector.

`length` gives the total sum of vectors.

```
length(grep("_mean", colnames(wisc.df), value=T))
```

```
[1] 10
```

There are 10 variables/features in the data suffixed with _mean.

Q. How many variables/dimensions do we have?

```
ncol(wisc.df)
```

```
[1] 31
```

Save the diagnosis variable for reference later.

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

```
  [1] M M M M M M M M M M M M M M M M M B B B M M M M M M M M M M M M M M
 [38] B M M M M M M M M B M B B B B B M M B M M B B B B M B B M B M M M
 [75] B M B M M B B B M M B M M M B B B M B B M M B B B M M B B B B M B B M B B
[112] B B B B B B M M M B M M B B B M M B M B M M B M M B B M B B M B B B B M B
[149] B B B B B B B B M B B B B M M B M B B M M B B M M B B B B M B B M M M B M
[186] B M B B B M B B M M B M M M M B M M M B M B M B B M B M M M M B B M M B B
[223] B M B B B B B M M B B M B B M M B M B B B B M B B B B B M B M M M M M M M
[260] M M M M M M M B B B B B B M B M B B M B B M B M M B B B B B B B B B B B
[297] B M B B M B M B B B B B B B B B B B B B B M B B B M B M B B B B M M M B B
[334] B B M B M B M B B B M B B B B B B B M M M B B B B B B B B B B M M B M M
[371] M B M M B B B B B M B B B B B M B B B M B B M M B B B B B B M B B B B B B
[408] B M B B B B B M B B M B B B B B B B B B B B B M B M M B M B B B B B M B B
[445] M B M B B M B M B B B B B B B B M M B B B B B B M B B B B B B B B B M B
[482] B B B B B B M B M B B M B B B B B M M B M B M B B B B B M B B M B M B M M
[519] B B B M B B B B B B B B B B B M B M M B B B B B B B B B B B B B B B B B
[556] B B B B B B B M M M M M M B
Levels: B M
```

Now we must remove the column before we do analysis on the numerical values in the other columns.

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

```
[1] 30
```

We can try hierarchical clustering first with `hclust()`. Let's try clustering this data. hclust requires "d", a distance matrix.

But the graph does not look promising at all (not printed).

#Principal Component Analysis

Let's try PCA on this data instead. Before doing any analysis like this we should check if our input data needs to be scaled first.

Side note:

```
head(mtcars)
```

```
                   mpg cyl disp  hp drat    wt  qsec vs am gear carb
Mazda RX4         21.0   6  160 110 3.90 2.620 16.46  0  1    4    4
Mazda RX4 Wag     21.0   6  160 110 3.90 2.875 17.02  0  1    4    4
Datsun 710        22.8   4  108  93 3.85 2.320 18.61  1  1    4    1
Hornet 4 Drive    21.4   6  258 110 3.08 3.215 19.44  1  0    3    1
Hornet Sportabout 18.7   8  360 175 3.15 3.440 17.02  0  0    3    2
Valiant           18.1   6  225 105 2.76 3.460 20.22  1  0    3    1
```

We cannot run PCA without scaling, because PCA will capture variance that is dominated by certain variables (in the case of cars, by horsepower). We need to treat the data fairly.

```
apply(mtcars, 2, mean)
```

```
      mpg       cyl      disp        hp      drat        wt      qsec 
20.090625  6.187500 230.721875 146.687500  3.596563  3.217250 17.848750 
       vs        am      gear      carb 
 0.437500  0.406250  3.687500  2.812500 
```

```
apply(mtcars, 2, sd)
```

```
      mpg         cyl        disp          hp        drat          wt
6.0269481   1.7859216 123.9386938  68.5628685   0.5346787   0.9784574
     qsec          vs          am        gear        carb
1.7869432   0.5040161   0.4989909   0.7378041   1.6152000
```

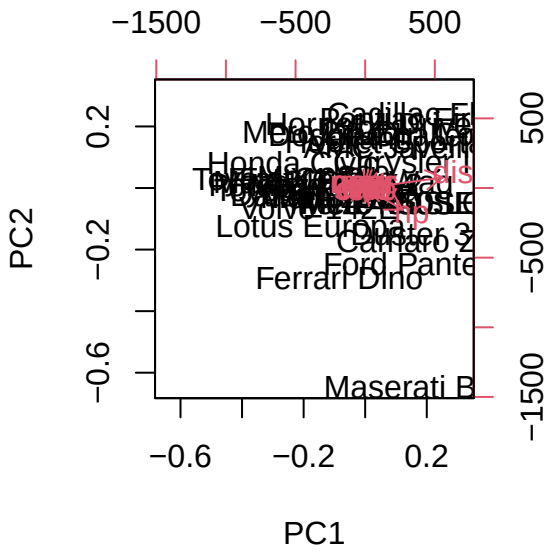
```
#In this case, the variance in displacement (disp) is massive, and will dominate the PCA
```

Let's try PCA on this car dataset.

```
pc <- prcomp(mtcars)
summary(pc)
```

```
Importance of components:
                          PC1      PC2     PC3     PC4     PC5     PC6    PC7
Standard deviation     136.533 38.14808 3.07102 1.30665 0.90649 0.66354 0.3086
Proportion of Variance   0.927  0.07237 0.00047 0.00008 0.00004 0.00002 0.0000
Cumulative Proportion    0.927  0.99937 0.99984 0.99992 0.99996 0.99998 1.0000
                          PC8    PC9   PC10   PC11
Standard deviation     0.286 0.2507 0.2107 0.1984
Proportion of Variance 0.000 0.0000 0.0000 0.0000
Cumulative Proportion  1.000 1.0000 1.0000 1.0000
```

```
biplot(pc)
```

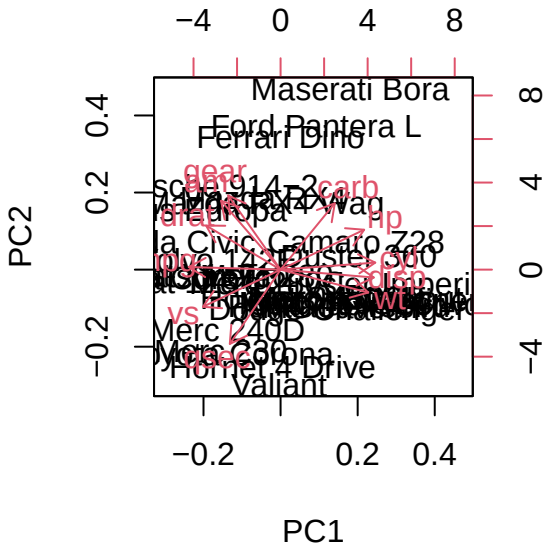



```
#Note that this captures plenty of variance, but it likely arises from difference in horse
```

```
pr.scale <-prcomp(mtcars, scale=T)
summary(pr.scale)
```

```
Importance of components:
                          PC1    PC2     PC3     PC4     PC5     PC6     PC7
Standard deviation     2.5707 1.6280 0.79196 0.51923 0.47271 0.46000 0.3678
Proportion of Variance 0.6008 0.2409 0.05702 0.02451 0.02031 0.01924 0.0123
Cumulative Proportion  0.6008 0.8417 0.89873 0.92324 0.94356 0.96279 0.9751
                           PC8    PC9    PC10   PC11
Standard deviation     0.35057 0.2776 0.22811 0.1485
Proportion of Variance 0.01117 0.0070 0.00473 0.0020
Cumulative Proportion  0.98626 0.9933 0.99800 1.0000
```

```
biplot(pr.scale)
```



Again, scaling is important so that the variance is not dominated by non-scaled data. So be sure to check the SD and means, and if they are drastically different, then remember to scale.

##Back to our cancer dataset.

We will need to scale this dataset, because the spread is very 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
```

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

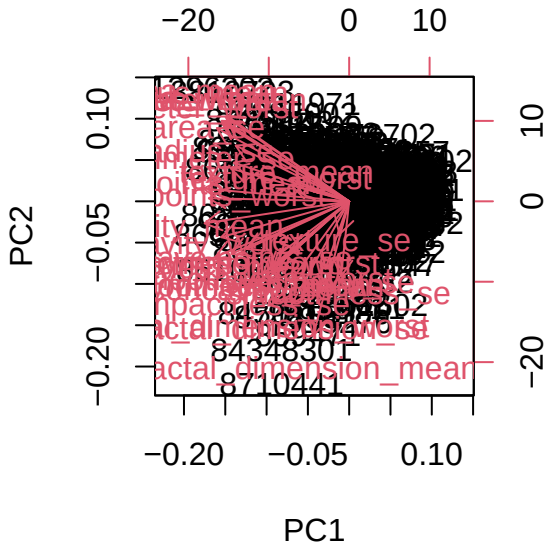
```
            radius_mean            texture_mean          perimeter_mean
           1.412729e+01            1.928965e+01            9.196903e+01
              area_mean         smoothness_mean        compactness_mean
           6.548891e+02            9.636028e-02            1.043410e-01
         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
```

```
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
```

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



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

Using mtcars dataset as an example, there is little clarity afforded by this graph. It is very difficult to understand and anothing stands out.

How well do the PCs capture the variance in the original data?

```
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)?

PC1 captures 44.27% of the variance.

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

3 PCs are required to describe at least 70% of the variance.

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

7 PCs are required to describe at least 90% of the variance.

Cumulatively, PC1 and PC2 capture 63.24% of the variance.

Our main PC score plot (AKA PC plot, PC1 vs PC2, ordination plot).

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

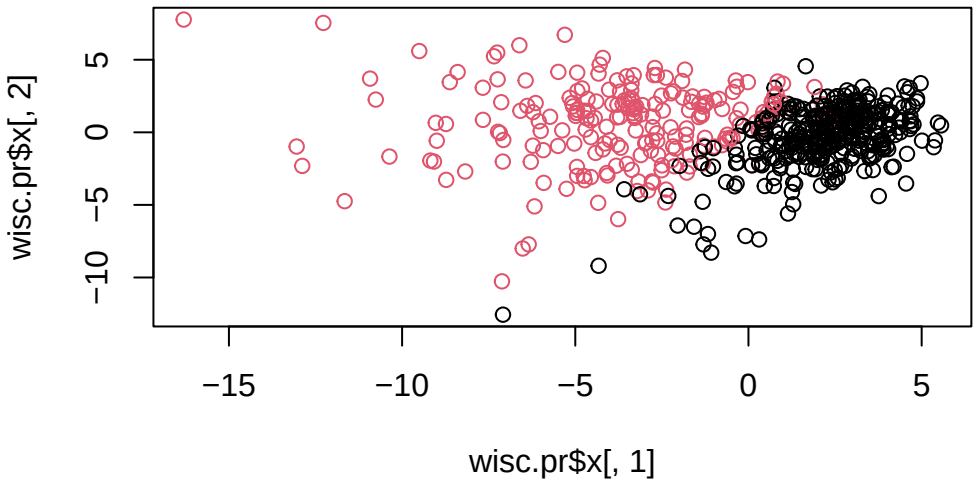
```
$names
[1] "sdev"     "rotation" "center"   "scale"    "x"

$class
[1] "prcomp"
```

```
#`wisc.pr$x` prints out the PC values for all patients.
#We want to plot PC1 vs PC2 for all patients.
```

We need to build our own plot here:

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



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

PCA reveals a striking difference between the malignant and benign points.

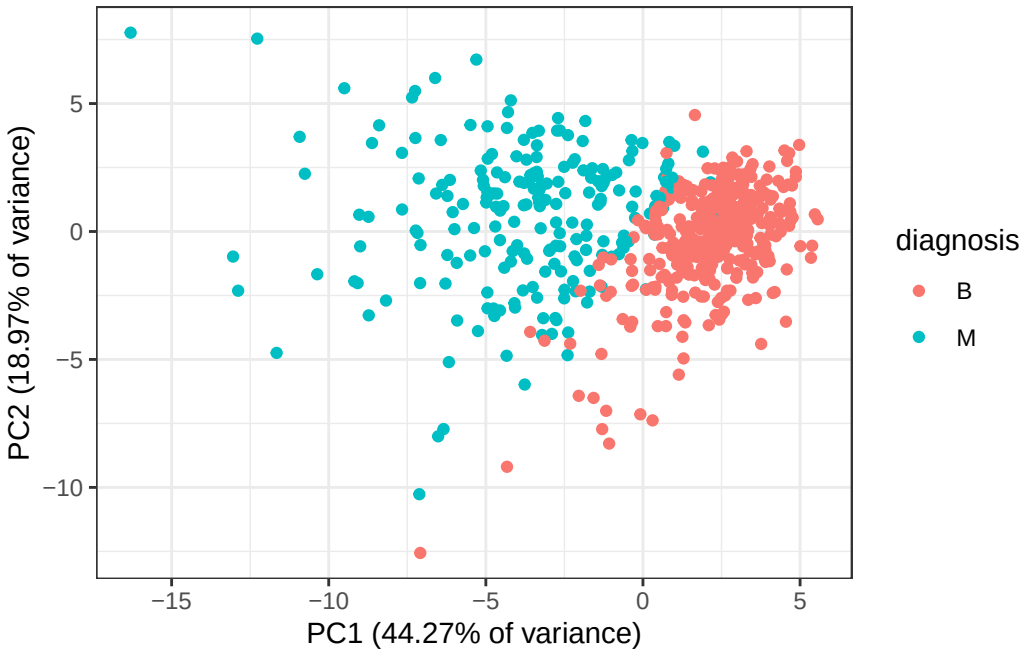
The points lying next to one another should have similar cell characteristics.

Now let's try to make a nice ggplot figure.

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

```
library(ggplot2)
```

```
ggplot(pc) +
aes(PC1, PC2, col=diagnosis) +
geom_point() +
xlab ("PC1 (44.27% of variance)") +
ylab ("PC2 (18.97% of variance)") +
theme_bw()
```



Each point represents a sample and its measured cell characteristics in the dataset. The general idea is that cells with similar characteristics should cluster.

What's actually going on in this analysis?

Recall that PCA is a method for compressing data into something that captures the essence of the original data.

- Takes a dataset with lots of dimensions and flattens it to 2-3 dimensions so we can look

at it
- Takes lines of best fit (first best line = PC1, second best line = PC2) to create the new axes; rotates the axis and dispenses with the original axes.

The length and direction of PC1 is mostly dictated by the endpoint points.

We can therefore score points based on how much they influence PC1.

- With the centerpoint of the PC1 line being 0, can arbitrarily set numerical values to reflect the influence of points on either side of tthe 0 midpoint.

We can take the original data and combine them with influence scores.

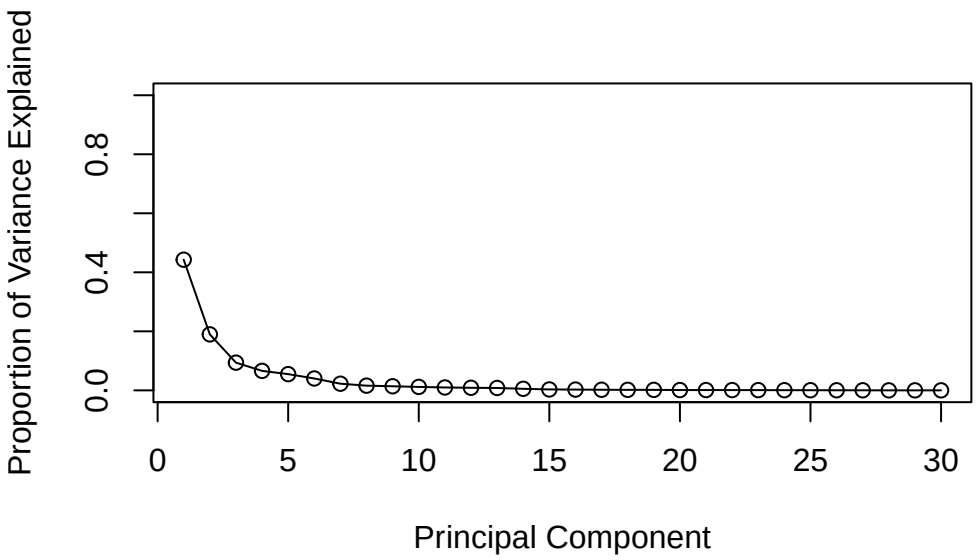
For example: Cell1 PC1 score = (read count * influence)…for all cells. Cell1 PC2 score = (read count * influence)…for all cells.

In this way, all data is compressed to one point.

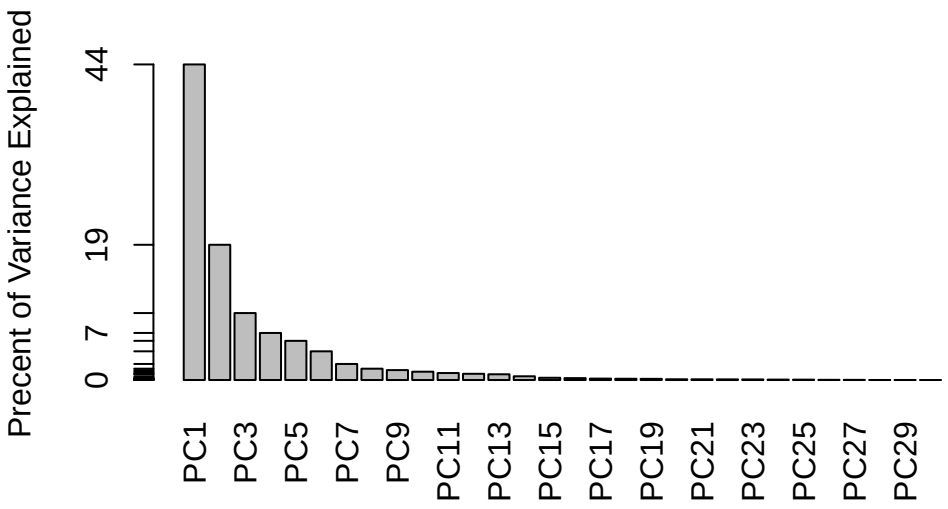
```
v <- summary(wisc.pr)
pr.var <- v$importance[2,]
head(pr.var)
```

```
    PC1     PC2     PC3     PC4     PC5     PC6
0.44272 0.18971 0.09393 0.06602 0.05496 0.04025
```

```
plot(pr.var, xlab = "Principal Component",
     ylab = "Proportion of Variance Explained",
     ylim = c(0, 1), type = "o")
```




```
barplot(pr.var, ylab = "Precent of Variance Explained",
     names.arg=paste0("PC",1:length(pr.var)), las=2, axes = FALSE)
axis(2, at=pr.var, labels=round(pr.var,2)*100 )
```



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? This tells us how much this original feature contributes to the first PC.

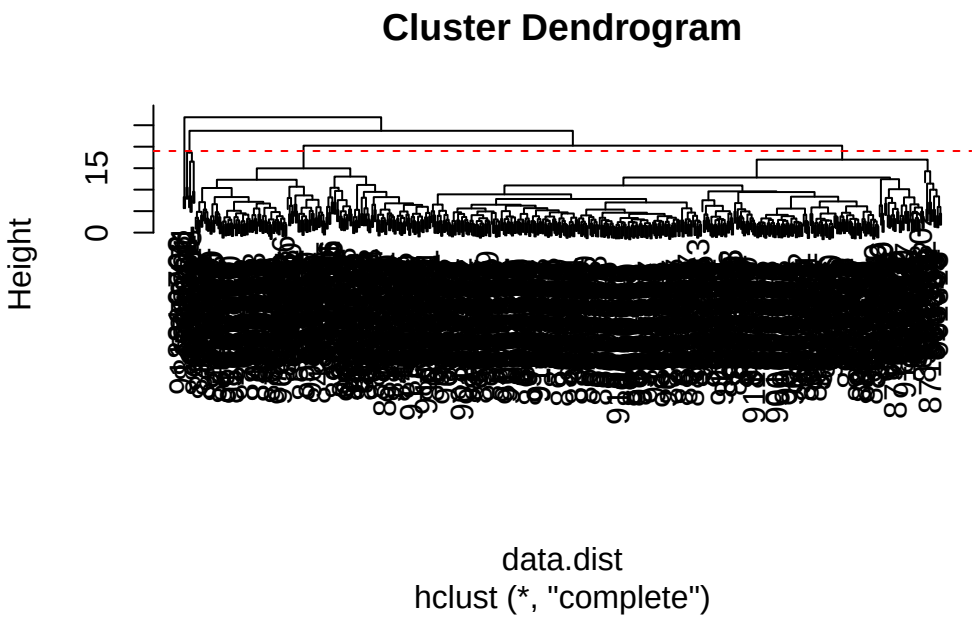
```
data.scaled <- scale(wisc.data)
data.dist <- dist(data.scaled)
```

```
wisc.hclust <- hclust(data.dist)
wisc.hclust
```

```
Call:
hclust(d = data.dist)

Cluster method   : complete
Distance         : euclidean
Number of objects: 569
```

```
plot(wisc.hclust)
abline(h=19, col="red", lty=2)
```



Q10. Using the plot() and abline() functions, what is the height at which the clustering model has 4 clusters?

There are four clusters at height=19.

```
wisc.hclust.clusters <- cutree(wisc.hclust, k=4)
table(wisc.hclust.clusters, diagnosis)
```

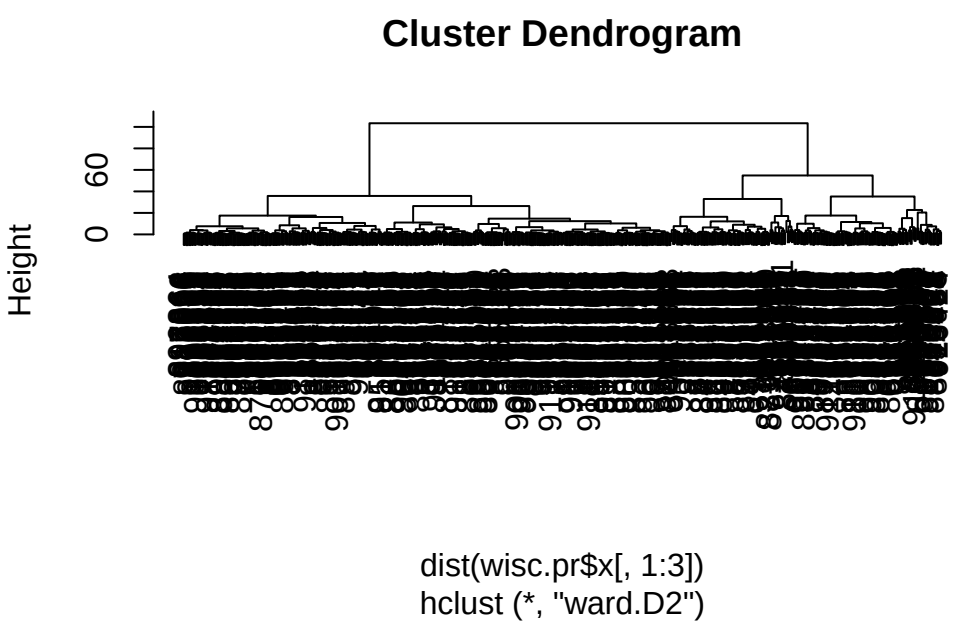
```
                    diagnosis
wisc.hclust.clusters   B   M
                   1  12 165
                   2   2   5
                   3 343  40
                   4   0   2
```

##4. Combining Methods

Here we will use the results of PCA as the input of a clustering analysis.

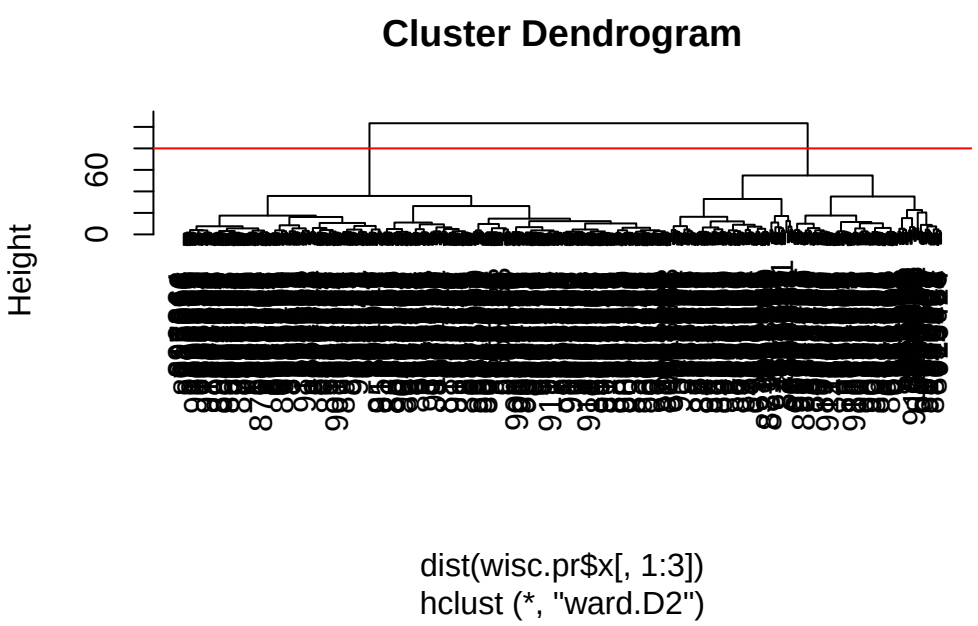
We start with using 3 PCs

```
wisc.pr.hclust <- hclust(dist(wisc.pr$x[,1:3]), method="ward.D2")
plot(wisc.pr.hclust)
```



With this PCA data, there are now two clear branches. The pattern is quite different.

```
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 created a comined table.

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

```
         grps
diagnosis   1   2
        B  24 333
        M 179  33
```

From this, we gather that most malignant examples are in cluster 1, while most benign examples are in cluster 2. Cluster 1 is enriched in malignant, while Cluster 2 is enriched in benign.

- True positives: 179

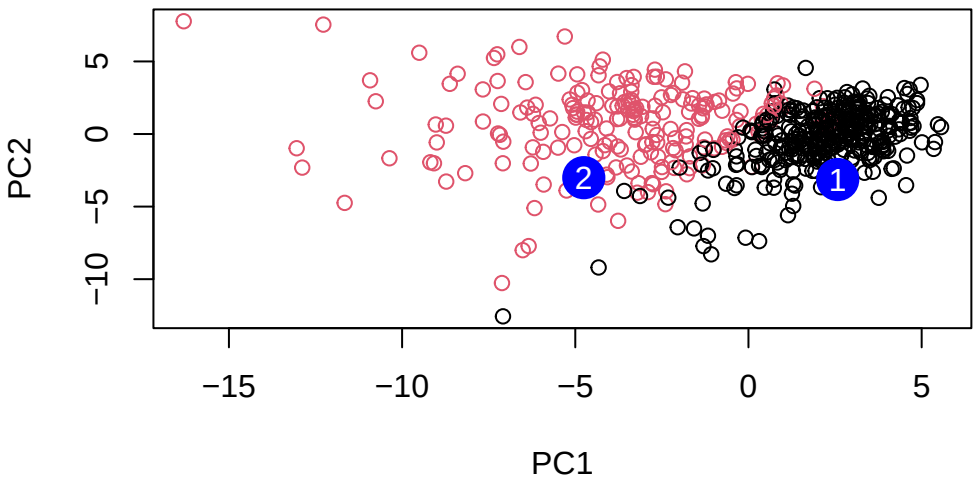
- False positives: 33
- True negatives: 333
- False negatives: 24

We can now use our existing PCA model by projecting new cell samples, to see where they land. It is a common strategy to first create a model and then project data upon them to do predictions.

```
#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=diagnosis)
points(npc[,1], npc[,2], col="blue", pch=16, cex=3)
text(npc[,1], npc[,2], c(1,2), col="white")
```



Q16. Which of these new patients should we prioritize for follow up based on your results?

Based on our PCA model, patient 2's cells more closely resemble malignant cells. This patient should be afforded higher priority and urgency regarding treatment.

In summary: PCA is an extremely useful method for reducing and visualizing data with many dimensions. We can also combine data to see where data overlaps.