Class 9 Mini-Project: Unsupervised Learning Analysis of Human Breast Cancer Cells

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Background

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.

The data itself comes from the Wisconsin Breast Cancer Diagnostic Data Set first reported by K. P. Benne and O. L. Mangasarian: "Robust Linear Programming Discrimination of Two Linearly Inseparable Sets".

Values in this data set describe characteristics of the cell nuclei present in digitized images of a fine needle aspiration (FNA) of a breast mass.

FNA is a type of biopsy procedure where a very thin needle is inserted into an area of abnormal tissue or cells with a guide of CT scan or ultrasound monitors (Figure 1). The collected sample is then transferred to a pathologist to study it under a microscope and examine whether cells in the biopsy are normal or not.

For example radius (i.e. mean of distances from center to points on the perimeter), texture (i.e. standard deviation of gray-scale values), and smoothness (local variation in radius lengths). Summary information is also provided for each group of cells including diagnosis (i.e. benign (not cancerous) and and malignant (cancerous)).

1. Exploratory data analysis

Getting organized.

Before analyzing any dataset we need to get ourselves organized by following the steps below:

First open a new RStudio Project for your work called mini-project (i.e. click: File > New Project > New Directory > New Project, making sure to save this along with the rest of your course work for this class).

Then open a new Quarto document (File > New File > Quarto document) for saving your code and accompanying narrative notes. We will use this to generate our final project report later.

In your new Quarto document be sure to keep the YAML header but remove the boilerplate example text and code (i.e. delete from line 6 onward) so you have a clean document to work in and add your content to.

Test if you can add some content and generate a PDF or HTML report (i.e. Use the Render button/option in RStudio) as we will need this for project submission to gradescope later.

Note that your report should clearly indicate your answers for all Questions. Question 11 and 15 are optional extensions and are not required in your report.

Preparing the data.

Before we can begin our analysis we first have to download and import our data correctly into our R session.

For this we can use the read.csv() function to read the CSV (comma-separated values) file containing the data (available from our class website: WisconsinCancer.csv)

Assign the result to an object called wisc.df.

```
# 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)
wisc.df <- wisc.df[, 1: length(colnames(wisc.df)) - 1]</pre>
```

Examine your input data to ensure column names are set correctly. The id and diagnosis columns will not be used for most of the following steps (you can use the View() or head() functions here).

Note that the first column here wisc.df\$diagnosis is a pathologist provided expert diagnosis. We will not be using this for our unsupervised analysis as it is essentially the "answer" to the question which cell samples are malignant or benign.

To make sure we don't accidentally include this in our analysis, lets create a new data.frame that omits this first column

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

Finally, setup a separate new vector called diagnosis that contains the data from the diagnosis column of the original dataset. We will store this as a factor (useful for plotting) and use this later to check our results.

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

Exploratory data analysis

The first step of any data analysis, unsupervised or supervised, is to familiarize yourself with the data.

Explore the data you created before (wisc.data and diagnosis) to answer the following questions:

Q1. How many observations are in this dataset? 569 observations

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

[1] 569

Q2. How many of the observations have a malignant diagnosis? 212 malignant diagnosis

```
sum(wisc.df$diagnosis == "M")
[1] 212
```

Q3. How many variables/features in the data are suffixed with $_$ mean? *10 features with $'_$ mean' suffix*

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

[1] 10

2. Principal Component Analysis.

Performing PCA.

The next step in your analysis is to perform principal component analysis (PCA) on wisc.data.

It is important to check if the data need to be scaled before performing PCA. Recall two common reasons for scaling data include:

The input variables use different units of measurement. The input variables have significantly different variances.

Check the mean and standard deviation of the features (i.e. columns) of the wisc.data to determine if the data should be scaled. Use the colMeans() and apply() functions like you've done before.

```
# Check column means and standard deviations
print("### mean ###")
```

[1] "### mean ###"

colMeans(wisc.data)

perimeter_mean	texture_mean	radius_mean
9.196903e+01	1.928965e+01	1.412729e+01
compactness_mean	${\tt smoothness_mean}$	area_mean
1.043410e-01	9.636028e-02	6.548891e+02
symmetry_mean	concave.points_mean	concavity_mean
1.811619e-01	4.891915e-02	8.879932e-02
texture_se	radius_se	<pre>fractal_dimension_mean</pre>
1.216853e+00	4.051721e-01	6.279761e-02
smoothness_se	area_se	perimeter_se
7.040979e-03	4.033708e+01	2.866059e+00
concave.points_se	concavity_se	compactness_se
1.179614e-02	3.189372e-02	2.547814e-02
radius_worst	fractal_dimension_se	symmetry_se
1.626919e+01	3.794904e-03	2.054230e-02
area_worst	perimeter_worst	texture_worst
8.805831e+02	1.072612e+02	2.567722e+01
concavity_worst	compactness_worst	smoothness_worst
2.721885e-01	2.542650e-01	1.323686e-01

```
concave.points_worst symmetry_worst fractal_dimension_worst
1.146062e-01 2.900756e-01 8.394582e-02

print("### Standard Deviation ###")
```

[1] "### Standard Deviation ###"

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

texture_mean	radius_mean
4.301036e+00	3.524049e+00
${\tt smoothness_mean}$	area_mean
1.406413e-02	3.519141e+02
concave.points_mean	concavity_mean
3.880284e-02	7.971981e-02
radius_se	fractal_dimension_mean
2.773127e-01	7.060363e-03
area_se	perimeter_se
4.549101e+01	2.021855e+00
concavity_se	compactness_se
3.018606e-02	1.790818e-02
fractal_dimension_se	symmetry_se
2.646071e-03	8.266372e-03
perimeter_worst	texture_worst
3.360254e+01	6.146258e+00
${\tt compactness_worst}$	smoothness_worst
1.573365e-01	2.283243e-02
symmetry_worst	concave.points_worst
6.186747e-02	6.573234e-02
	4.301036e+00 smoothness_mean 1.406413e-02 concave.points_mean 3.880284e-02 radius_se 2.773127e-01 area_se 4.549101e+01 concavity_se 3.018606e-02 fractal_dimension_se 2.646071e-03 perimeter_worst 3.360254e+01 compactness_worst 1.573365e-01 symmetry_worst

Execute PCA with the prcomp() function on the wisc.data, scaling if appropriate, and assign the output model to wisc.pr.

```
wisc.pr <- prcomp( wisc.data , scale=TRUE)</pre>
```

Inspect a summary of the results with the summary() function.

```
# Look at summary of results
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
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
                       0.30681 0.28260 0.24372 0.22939 0.22244 0.17652 0.1731
Standard deviation
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
```

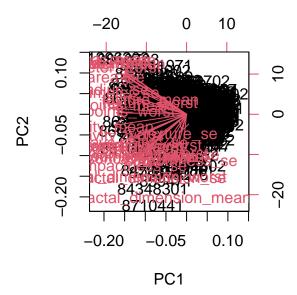
- Q4. From your results, what proportion of the original variance is captured by the first principal components (PC1)? 44.27% of variance.
- Q5. How many principal components (PCs) are required to describe at least 70% of the original variance in the data? At least the 3 principal components
- Q6. How many principal components (PCs) are required to describe at least 90% of the original variance in the data? At least the first 7 principal components

Interpreting PCA results.

Now you will use some visualizations to better understand your PCA model. A common visualization for PCA results is the so-called biplot.

However, you will often run into some common challenges with using biplots on real-world data containing a non-trivial number of observations and variables. Here we will need to look at some alternative visualizations. You are encouraged to experiment with additional visualizations before moving on to the next section

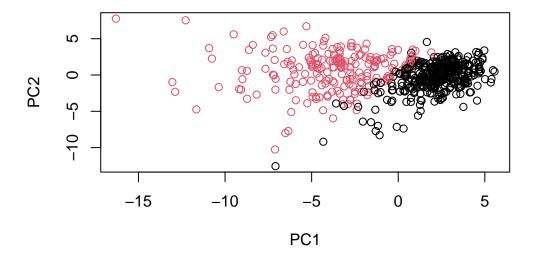
Create a biplot of the wisc.pr using the biplot() function.



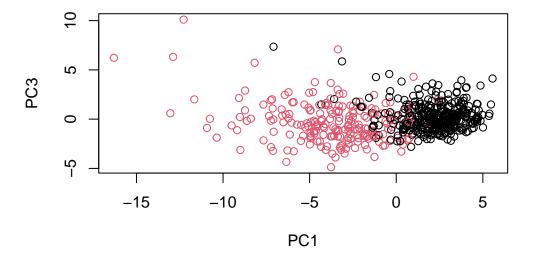
Q7. What stands out to you about this plot? Is it easy or difficult to understand? Why? It is imposible to understand, all labels overlap each other making data interpretation difficult.

Rownames are used as the plotting character for biplots like this one which can make trends rather hard to see. In fact, this plot is very poor. So lets generate a more standard scatter plot of each observation along principal components 1 and 2 (i.e. a plot of PC1 vs PC2 available as the first two columns of wisc.pr\$x) and color the points by the diagnosis (available in the diagnosis vector you created earlier).

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



Q8. Generate a similar plot for principal components 1 and 3. What do you notice about these plots? The first plot seems to do a better job at separating the two diagnosis groups



Because principal component 2 explains more variance in the original data than principal component 3, you can see that the first plot has a cleaner cut separating the two subgroups.

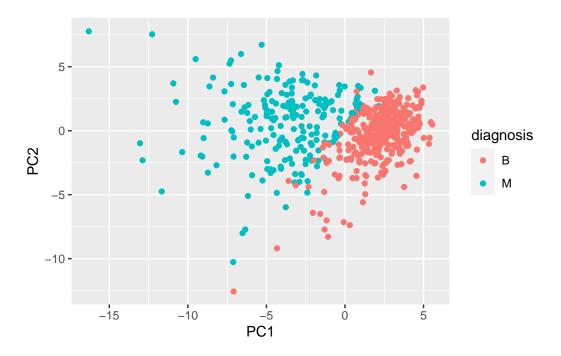
Overall, the plots indicate that principal component 1 is capturing a separation of malignant (red) from benign (black) samples. This is an important and interesting result worthy of further exploration - as we will do in the next sections!

As this is such a striking result let's see if we can use the ggplot2 package to make a more fancy figure of these results. Remember that ggplot requires a data.frame as input and we will also need to add our diagnosis vector as a column if we want to use it for mapping to the plot color aesthetic.

```
# Create a data.frame for ggplot
df <- as.data.frame(wisc.pr$x)
df$diagnosis <- diagnosis

# Load the ggplot2 package
library(ggplot2)

# Make a scatter plot colored by diagnosis
ggplot(df) +
   aes(PC1, PC2, col=diagnosis) +
   geom_point()</pre>
```



Variance explained.

In this exercise, you will produce scree plots showing the proportion of variance explained as the number of principal components increases. The data from PCA must be prepared for these plots, as there is not a built-in function in base R to create them directly from the PCA model.

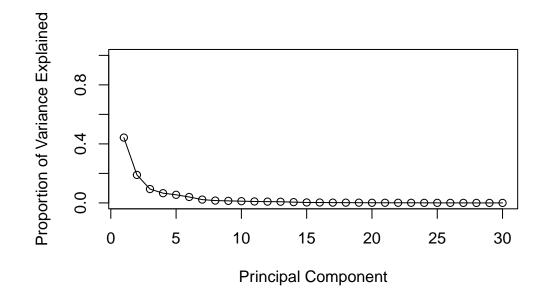
As you look at these plots, ask yourself if there's an 'elbow' in the amount of variance explained that might lead you to pick a natural number of principal components. If an obvious elbow does not exist, as is typical in some real-world datasets, consider how else you might determine the number of principal components to retain based on the scree plot.

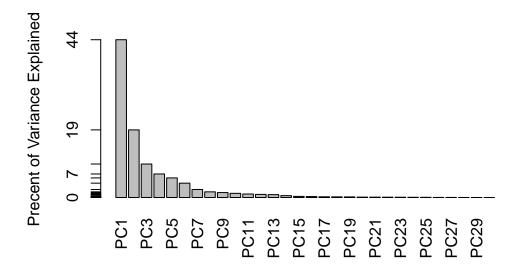
Calculate the variance of each principal component by squaring the sdev component of wisc.pr (i.e. wisc.pr\$sdev^2). Save the result as an object called pr.var.

```
# Calculate variance of each component
pr.var <- wisc.pr$sdev^2
head(pr.var)</pre>
```

[1] 13.281608 5.691355 2.817949 1.980640 1.648731 1.207357

Calculate the variance explained by each principal component by dividing by the total variance explained of all principal components. Assign this to a variable called pve and create a plot of variance explained for each principal component.





OPTIONAL: There are quite a few CRAN packages that are helpful for PCA. This includes the factoextra package. Feel free to explore this package. For example:

```
## ggplot based graph
#install.packages("factoextra")
library(factoextra)
```

Welcome! Want to learn more? See two factoextra-related books at https://goo.gl/ve3WBa

```
fviz_eig(wisc.pr, addlabels = TRUE)
```



Communicating PCA results.

In this section we will check your understanding of the PCA results, in particular the loadings and variance explained. The loadings, represented as vectors, explain the mapping from the original features to the principal components. The principal components are naturally ordered from the most variance explained to the least variance explained.

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. -0.2608538

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

[1] -0.2608538

3. Hierarchical clustering

The goal of this section is to do hierarchical clustering of the original data. Recall from class that this type of clustering does not assume in advance the number of natural groups that

exist in the data (unlike K-means clustering).

As part of the preparation for hierarchical clustering, the distance between all pairs of observations are computed. Furthermore, there are different ways to link clusters together, with single, complete, and average being the most common "linkage methods".

First scale the wisc.data data and assign the result to data.scaled.

```
# Scale the wisc.data data using the "scale()" function
data.scaled <- scale(wisc.data)</pre>
```

Calculate the (Euclidean) distances between all pairs of observations in the new scaled dataset and assign the result to data.dist.

```
data.dist <- dist(data.scaled)</pre>
```

Create a hierarchical clustering model using complete linkage. Manually specify the method argument to hclust() and assign the results to wisc.hclust.

```
wisc.hclust <- hclust(data.dist, method = "complete")</pre>
```

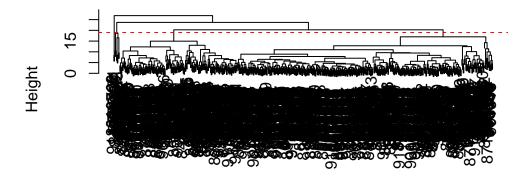
Results of hierarchical clustering

Let's use the hierarchical clustering model you just created to determine a height (or distance between clusters) where a certain number of clusters exists.

Q10. Using the plot() and abline() functions, what is the height at which the clustering model has 4 clusters? 19

```
plot(wisc.hclust)
abline(a=19, b=0, col="red", lty=2)
```

Cluster Dendrogram



data.dist hclust (*, "complete")

Selecting number of clusters

In this section, you will compare the outputs from your hierarchical clustering model to the actual diagnoses. Normally when performing unsupervised learning like this, a target variable (i.e. known answer or labels) isn't available. We do have it with this dataset, however, so it can be used to check the performance of the clustering model.

When performing supervised learning - that is, when you're trying to predict some target variable of interest and that target variable is available in the original data - using clustering to create new features may or may not improve the performance of the final model.

This exercise will help you determine if, in this case, hierarchical clustering provides a promising new feature.

Use cutree() to cut the tree so that it has 4 clusters. Assign the output to the variable wisc.hclust.clusters.

```
wisc.hclust.clusters <- cutree(wisc.hclust, k = 4)</pre>
```

We can use the table() function to compare the cluster membership to the actual diagnoses.

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

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

Here we picked four clusters and see that cluster 1 largely corresponds to malignant cells (with diagnosis values of 1) whilst cluster 3 largely corresponds to benign cells (with diagnosis values of 0).

Before moving on, explore how different numbers of clusters affect the ability of the hierarchical clustering to separate the different diagnoses.

Q11. OPTIONAL: Can you find a better cluster vs diagnoses match by cutting into a different number of clusters between 2 and 10? How do you judge the quality of your result in each case?

Using different methods.

As we discussed in our last class videos there are number of different "methods" we can use to combine points during the hierarchical clustering procedure. These include "single", "complete", "average" and (my favorite) "ward.D2".

Q12. Which method gives your favorite results for the same data.dist dataset? Explain your reasoning. I prefer ward.D2 because it chooses the clustering with less variance within each cluster.

One of the problems with Cluster Analysis is that different methods may produce different results – There is generally no universally accepted "best" method. The good news is that if your data really has clear groups all methods will likely find them and give you similar results. However, in more challenging cases like this one it is best to try multiple algorithms and see what groups logically make sense. A common approach is use a smaller dummy dataset with pre-determined groups that you can use to see which algorithm best recreates what you expect.

4. Combining methods

Clustering on PCA results.

In this final section, you will put together several steps you used earlier and, in doing so, you will experience some of the creativity and open endedness that is typical in unsupervised learning.

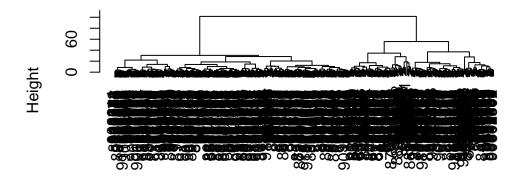
Recall from earlier sections that the PCA model required significantly fewer features to describe 70%, 80% and 95% of the variability of the data. In addition to normalizing data and potentially avoiding over-fitting, PCA also uncorrelates the variables, sometimes improving the performance of other modeling techniques.

Let's see if PCA improves or degrades the performance of hierarchical clustering.

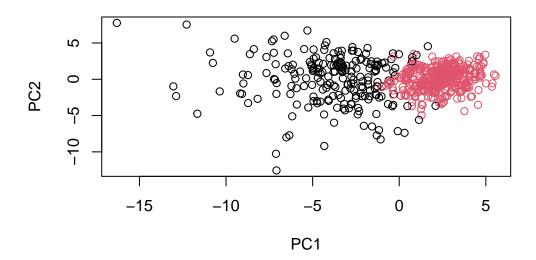
Using the minimum number of principal components required to describe at least 90% of the variability in the data, create a hierarchical clustering model with the linkage method="ward.D2". We use Ward's criterion here because it is based on multidimensional variance like principal components analysis. Assign the results to wisc.pr.hclust.

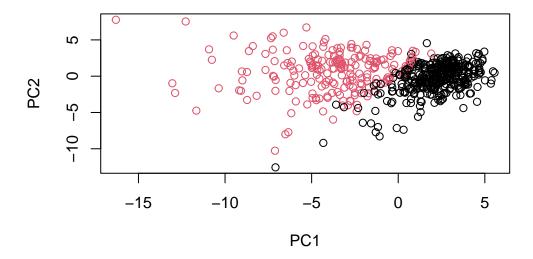
```
wisc.pr.hclust <- hclust(dist(wisc.pr$x[, 1:7]), method="ward.D2")
plot(hclust(dist(wisc.pr$x[, 1:7]), method="ward.D2"))</pre>
```

Cluster Dendrogram



dist(wisc.pr\$x[, 1:7]) hclust (*, "ward.D2") This looks much more promising than our previous clustering results on the original scaled data. Note the two main branches of or dendrogram indicating two main clusters - maybe these are malignant and benign. Let's find out!





OPTIONAL: Note the color swap here as the hclust cluster 1 is mostly "M" and cluster 2 is mostly "B" as we saw from the results of calling table(grps, diagnosis). To match things up we can turn our groups into a factor and reorder the levels so cluster 2 comes first and thus gets the first color (black) and cluster 1 gets the second color (red).

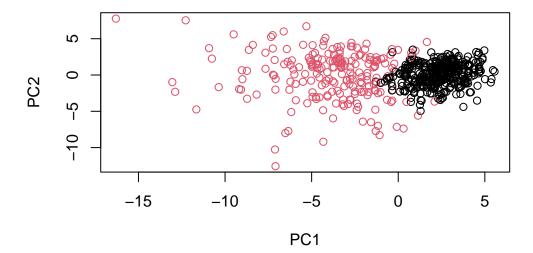
```
g <- as.factor(grps)
levels(g)

[1] "1" "2"

g <- relevel(g,2)
levels(g)

[1] "2" "1"

# Plot using our re-ordered factor
plot(wisc.pr$x[,1:2], col=g)</pre>
```



OPTIONAL: We can be fancy and look in 3D with the rgl or plotly packages. Note that this output will not work well with PDF format reports yet so feel free to skip this optional step for your PDF report. If you have difficulty installing the rgl package on mac then you will likely need to install the XQuartz package from here: https://www.xquartz.org. There are also lots of other packages (like plotly) that can make interactive 3D plots.

```
#install.packages('rgl')
#library(rgl)
#plot3d(wisc.pr$x[,1:3], xlab="PC 1", ylab="PC 2", zlab="PC 3", cex=1.5, size=1, type="s"
#rglwidget(width = 400, height = 400)
```

To include the interactive rgl plot in your HTML renderd lab report (not PDF) you can add the R code rglwidget(width = 400, height = 400) after you call the plot3d() function. It will look just like the plot above. Try rotating and zooming on this 3D plot.

```
## Use the distance along the first 7 PCs for clustering i.e. wisc.prx[, 1:7] wisc.pr.hclust <- hclust(dist(wisc.prx[, 1:7]), method="ward.D2")
```

Cut this hierarchical clustering model into 2 clusters and assign the results to wisc.pr.hclust.clusters.

```
wisc.pr.hclust.clusters <- cutree(wisc.pr.hclust, k=2)</pre>
```

Using table(), compare the results from your new hierarchical clustering model with the actual diagnoses.

Q13. How well does the newly created model with four clusters separate out the two diagnoses? It does a nice job, around 90% of the data is separated into 2 different group corresponding to the diagnosis type and 2 more groups that we can think of them as unclassified. See below

```
# Compare to actual diagnoses
  table(wisc.pr.hclust.clusters, diagnosis)
                      diagnosis
wisc.pr.hclust.clusters
                        В
                      1 28 188
                     2 329 24
  table(wisc.hclust.clusters, diagnosis)
                   diagnosis
wisc.hclust.clusters
                      В
                   1 12 165
                   2
                    2
                   3 343 40
                     0
                          2
```

Q14. How well do the 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. In my opinion, the hierarchical clustering with four groups does a good job, it reduces the number of false positive at the cost of creating two new groups of unclassified data. However, the PCA has a better TP rate so probably I would prefer the PCA.

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

diagnosis
wisc.hclust.clusters B M
```

```
1 12 165
                        2
                            5
                    3 343
                           40
                        0
                            2
  wisc.km <- kmeans(wisc.data, centers = 2)</pre>
  table(wisc.km$cluster, diagnosis)
   diagnosis
      В
          М
      1 130
  2 356 82
  # Compare to actual diagnoses
  table(wisc.pr.hclust.clusters, diagnosis)
                        diagnosis
                           В
wisc.pr.hclust.clusters
                         28 188
                       2 329 24
```

5. Sensitivity/Specificity.

Sensitivity refers to a test's ability to correctly detect ill patients who do have the condition. In our example here the sensitivity is the total number of samples in the cluster identified as predominantly malignant (cancerous) divided by the total number of known malignant samples. In other words: TP/(TP+FN).

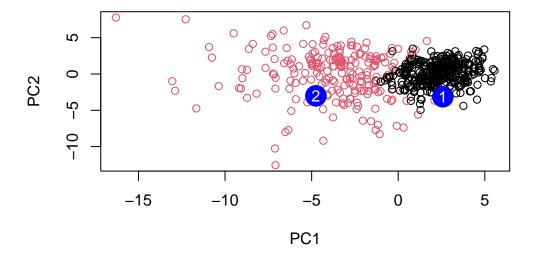
Specificity relates to a test's ability to correctly reject healthy patients without a condition. In our example specificity is the proportion of benign (not cancerous) samples in the cluster identified as predominantly benign that are known to be benign. In other words: TN/(TN+FN).

Q15. OPTIONAL: Which of your analysis procedures resulted in a clustering model with the best specificity? How about sensitivity?

6. Prediction

We will use the predict() function that will take our PCA model from before and new cancer cell data and project that data onto our PCA space.

```
#url <- "new_samples.csv"</pre>
  url <- "https://tinyurl.com/new-samples-CSV"</pre>
  new <- read.csv(url)</pre>
  npc <- predict(wisc.pr, newdata=new)</pre>
  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
PC22
                               PC23
          PC21
                                          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=g)
  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? Patient number two since looks like it has malignant characteristics. It clusters with the malignant group.