Class 8: Breast Cancer Mini Project

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Before we get stuck into project work, we will have a quick look at applying PCA to some exampled RNAseq data (tail end of lab 7).

244 225 277 305 272 270 279

491 491 493 612 594 577 618 638

Read the data (detailed in lab 7)

```
url2 <- "https://tinyurl.com/expression-CSV"
rna.data <- read.csv(url2, row.names=1)
head(rna.data)

wt1 wt2 wt3 wt4 wt5 ko1 ko2 ko3 ko4 ko5
gene1 439 458 408 429 420 90 88 86 90 93
gene2 219 200 204 210 187 427 423 434 433 426
gene3 1006 989 1030 1017 973 252 237 238 226 210
gene4 783 792 829 856 760 849 856 835 885 894</pre>
```

Q: How many genes are in this dataset?

204

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

181 249

460 502

[1] 100

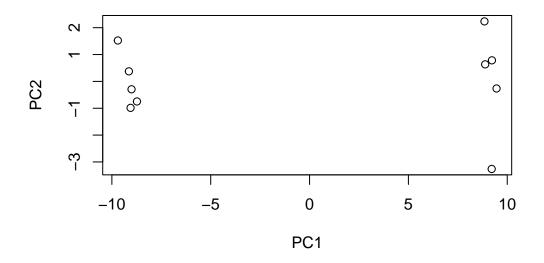
gene5

gene6

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</pre>
```



summary(pca)

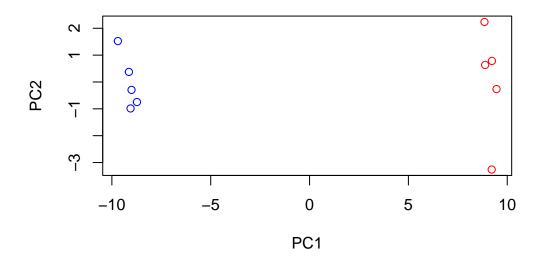
Importance of components:

```
PC2
                                         PC3
                                                 PC4
                         PC1
                                                         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
Standard deviation
                       0.62065 0.60342 3.457e-15
Proportion of Variance 0.00385 0.00364 0.000e+00
Cumulative Proportion 0.99636 1.00000 1.000e+00
```

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

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

```
\verb"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 Breast Cancer FNA Data

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

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

```
diagnosis radius_mean texture_mean perimeter_mean area_mean
842302
                          17.99
                                        10.38
                                                      122.80
                                                                 1001.0
                 М
                 М
                          20.57
                                        17.77
842517
                                                      132.90
                                                                 1326.0
84300903
                 Μ
                          19.69
                                        21.25
                                                      130.00
                                                                 1203.0
84348301
                 Μ
                          11.42
                                        20.38
                                                       77.58
                                                                  386.1
84358402
                 Μ
                          20.29
                                        14.34
                                                      135.10
                                                                 1297.0
843786
                 Μ
                          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
                                                                        0.12790
                                                    0.1974
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
                       0.006399
842302
          153.40
                                        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
           27.23
84348301
                       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
                                                   24.99
842517
             0.01389
                                  0.003532
                                                                  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
	<pre>fractal_dimension_worst</pre>		
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 α is a pathologist provided expert diagnosis.

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

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

	radius_mean text	ture_mean	perimet	er_mean	area_	mean sı	noothr	ness_mean
842302	17.99	10.38		122.80	10	01.0		0.11840
842517	20.57	17.77		132.90	13	26.0		0.08474
84300903	19.69	21.25		130.00	12	03.0		0.10960
84348301	11.42	20.38		77.58	3	86.1		0.14250
84358402	20.29	14.34		135.10	12	97.0		0.10030
843786	12.45	15.70		82.57	4	77.1		0.12780
	compactness_mean	n concavi	ty_mean	concave.	point	s_mean	symme	etry_mean
842302	0.27760)	0.3001		0	.14710		0.2419
842517	0.07864	1	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	on_mean ra	adius_se	texture	e_se p	erimet	er_se	area_se
842302	(0.07871	1.0950	0.9	9053	8	3.589	153.40
842517	(0.05667	0.5435	0.7	7339	;	3.398	74.08
84300903	(0.05999	0.7456	0.7	7869	4	4.585	94.03
84348301	(0.09744	0.4956	1.1	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
              0.006399
                               0.04904
                                             0.05373
842302
                                                                0.01587
842517
              0.005225
                               0.01308
                                             0.01860
                                                                0.01340
84300903
                                             0.03832
              0.006150
                               0.04006
                                                                0.02058
84348301
              0.009110
                               0.07458
                                             0.05661
                                                                0.01867
84358402
              0.011490
                               0.02461
                                             0.05688
                                                                0.01885
              0.007510
                               0.03345
                                             0.03672
843786
                                                                0.01137
         symmetry_se fractal_dimension_se radius_worst texture_worst
842302
             0.03003
                                   0.006193
                                                    25.38
                                                                   17.33
             0.01389
                                  0.003532
                                                    24.99
                                                                   23.41
842517
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
                                                         0.2364
84358402
                   0.4000
                                         0.1625
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.df)

[1] 569

There are 569 observations in this dataset.

Q2: How many observations have a malignant diagnosis?

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

B M 357 212

There are 212 observations with a malignant diagnosis.

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

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

[1] 10

There are 10 variables 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 to decide whether to use the scale=TRUE argument when we run prcomp().

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

```
# Check column means and standard deviations
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	${\tt smoothness_worst}$
2.721885e-01	2.542650e-01	1.323686e-01
<pre>fractal_dimension_worst</pre>	symmetry_worst	<pre>concave.points_worst</pre>
8.394582e-02	2.900756e-01	1.146062e-01

apply(wisc.data, 2, sd)

perimeter_mean	texture_mean	radius_mean
2.429898e+01	4.301036e+00	3.524049e+00
compactness_mean	${\tt smoothness_mean}$	area_mean
5.281276e-02	1.406413e-02	3.519141e+02
symmetry_mean	concave.points_mean	concavity_mean
2.741428e-02	3.880284e-02	7.971981e-02
texture_se	radius_se	fractal_dimension_mean
5.516484e-01	2.773127e-01	7.060363e-03
smoothness_se	area_se	perimeter_se
3.002518e-03	4.549101e+01	2.021855e+00
concave.points_se	concavity_se	compactness_se
6.170285e-03	3.018606e-02	1.790818e-02
radius_worst	${\tt fractal_dimension_se}$	symmetry_se
4.833242e+00	2.646071e-03	8.266372e-03
area_worst	perimeter_worst	texture_worst
5.693570e+02	3.360254e+01	6.146258e+00
concavity_worst	compactness_worst	${\tt smoothness_worst}$
2.086243e-01	1.573365e-01	2.283243e-02
${\tt fractal_dimension_worst}$	symmetry_worst	concave.points_worst
1.806127e-02	6.186747e-02	6.573234e-02

These are very different so we should scale=TRUE

```
#Perform PCA on wisc.data by completing the following code
wisc.pr <- prcomp(wisc.data, scale=TRUE)</pre>
```

Importance of components:

```
PC1
                                 PC2
                                         PC3
                                                  PC4
                                                          PC5
                                                                  PC6
                                                                          PC7
                       3.6444 2.3857 1.67867 1.40735 1.28403 1.09880 0.82172
Standard deviation
Proportion of Variance 0.4427 0.1897 0.09393 0.06602 0.05496 0.04025 0.02251
                       0.4427 0.6324 0.72636 0.79239 0.84734 0.88759 0.91010
Cumulative Proportion
                           PC8
                                  PC9
                                         PC10
                                                 PC11
                                                         PC12
                                                                 PC13
                       0.69037 0.6457 0.59219 0.5421 0.51104 0.49128 0.39624
Standard deviation
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
                       0.99749 0.99830 0.9989 0.99942 0.99969 0.99992 0.99997
Cumulative Proportion
                          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 the original variance is captured by the first principal components (PC1).

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

3 principal components (PC3) are required. They capture **72.64**% of the original variance in the data.

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

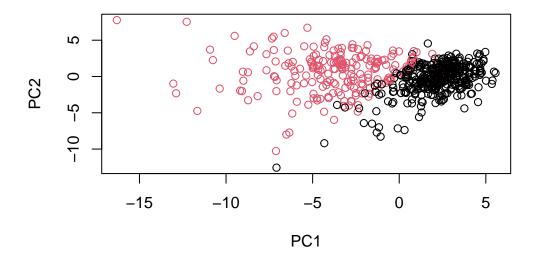
7 principal components (PC7) are required. They capture 91.01% of the original variance in the data.

Plotting the PCA Results

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

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

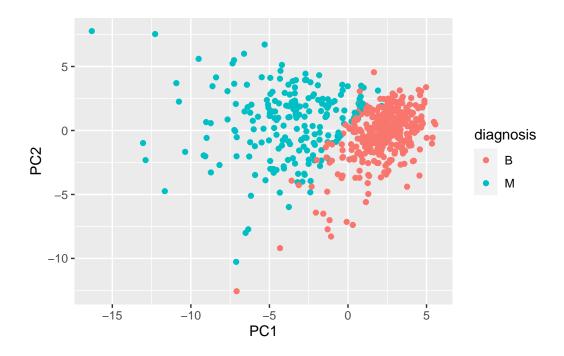
This plot is very difficult to understand, as the data is too large to neatly observe in a plot of this nature.



```
library(ggplot2)

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

ggplot(pc) +
   aes(PC1, PC2, col=diagnosis) +
   geom_point()</pre>
```

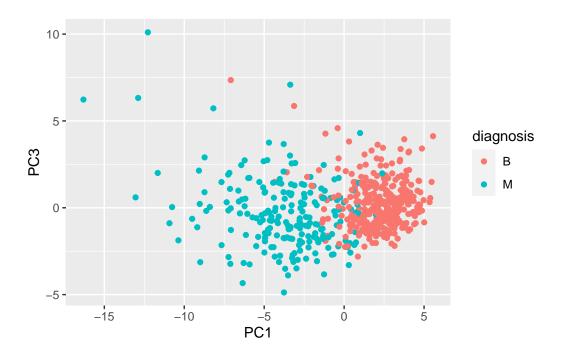


Q8: Generate a similar plot for principal components 1 and 3. What do you notice about these plots?

```
library(ggplot2)

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

ggplot(pc) +
   aes(PC1, PC3, col=diagnosis) +
   geom_point()</pre>
```



The PC1 vs PC2 plot has a cleaner separation of the two diagnoses, which suggests that PC1 captures this separation more strongly. This is supported by the fact the PC1 alone captures the bulk of the original variance.

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

The component of the loading vector is -0.26.

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

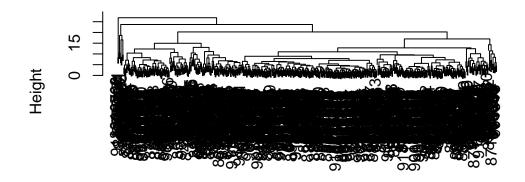
 ${f 5}$ principal components(PC5) are required. They capture ${f 84.73\%}$ of the original variance in the data.

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)</pre>
```

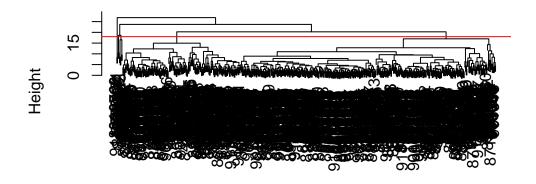
Cluster Dendrogram



d hclust (*, "complete")

```
plot(wisc.hclust)
abline(h=18, col="red")
```

Cluster Dendrogram



d hclust (*, "complete")

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

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



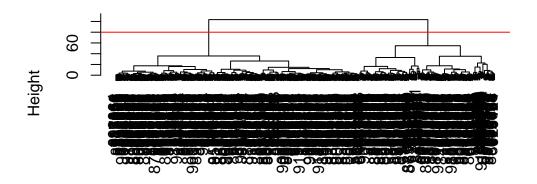
Combining Methods

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!

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

Cluster Dendrogram



d.pc hclust (*, "ward.D2")

```
grps <- cutree(wisc.pr.hsclust, h=80)
table(grps)</pre>
```

grps 1 2 203 366

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

```
table(diagnosis)

diagnosis
B M
357 212

table(grps, diagnosis)

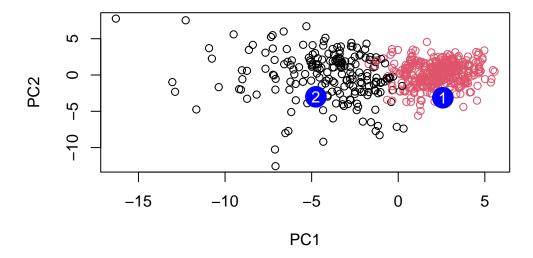
diagnosis
grps B M
```

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

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

Prediction

```
#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
                                                     PC12
                                                                PC13
                                                                         PC14
                                           PC11
[1,] -0.2307350 0.1029569 -0.9272861 0.3411457 0.375921 0.1610764 1.187882
[2,] -0.3307423 0.5281896 -0.4855301 0.7173233 -1.185917 0.5893856 0.303029
          PC15
                     PC16
                                  PC17
                                              PC18
                                                           PC19
                                                                      PC20
[1,] 0.3216974 -0.1743616 -0.07875393 -0.11207028 -0.08802955 -0.2495216
[2,] 0.1299153 0.1448061 -0.40509706 0.06565549 0.25591230 -0.4289500
           PC21
                      PC22
                                  PC23
                                             PC24
                                                         PC25
                                                                       PC26
[1,] 0.1228233 0.09358453 0.08347651
                                       0.1223396
                                                   0.02124121
                                                               0.078884581
[2,] -0.1224776 0.01732146 0.06316631 -0.2338618 -0.20755948 -0.009833238
             PC27
                         PC28
                                       PC29
                                                    PC30
[1,] 0.220199544 -0.02946023 -0.015620933
                                            0.005269029
[2,] -0.001134152  0.09638361  0.002795349 -0.019015820
And plot this up!
  plot(wisc.pr$x[,1:2], col=grps)
  points(npc[,1], npc[,2], col="blue", pch=16, cex=3)
  text(npc[,1], npc[,2], c(1,2), col="white")
```



Q17: SKIP

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

Patients from cluster 2.