# Class 8: Breast Cancer Mini Project

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Before we dive into the project lets try applying PCA to RNASeq data.

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

wt1 wt2 wt3 wt4 wt5 ko1 ko2 ko3 ko4 ko5
gene1 439 458 408 429 420 90 88 86 90 93
gene2 219 200 204 210 187 427 423 434 433 426
gene3 1006 989 1030 1017 973 252 237 238 226 210
gene4 783 792 829 856 760 849 856 835 885 894
gene5 181 249 204 244 225 277 305 272 270 279
gene6 460 502 491 491 493 612 594 577 618 638
```

Number of genes:

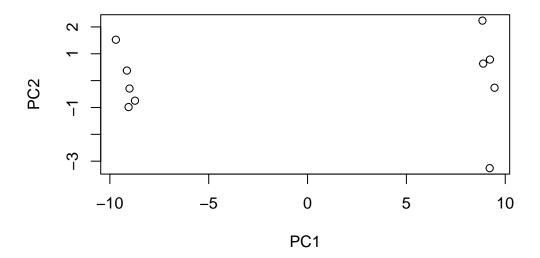
```
nrow(rna.data)
```

[1] 100

#### Run PCA

```
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")</pre>
```



### 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
```

Together, PC1 and PC2 capture almost 95% of all variance in the data. Great!

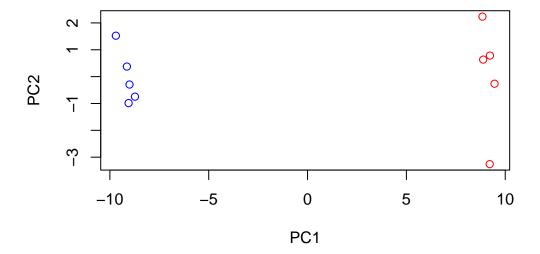
```
## Variance captured per PC
pca.var <- pca$sdev^2

## Percent variance is often more informative to look at
pca.var.per <- round(pca.var/sum(pca.var)*100, 1)
pca.var.per</pre>
```

### [1] 92.6 2.3 1.1 1.1 0.8 0.7 0.6 0.4 0.4 0.0

# Scree Plot PC1 PC3 PC5 PC7 PC9 Principal Component

```
# We have 5 wt + 5 ko samples
mycols <- c(rep('blue', 5), rep('red', 5)) # use to color the PCA plot points
plot(pca$x[,1], pca$x[,2], xlab="PC1", ylab="PC2", col=mycols)</pre>
```



I could examine which genes contribute most to PC1...

```
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**

FNA (fine needle aspiration): biopsy method

# **Exploratory Data Analysis**

Gathering the data

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

# Complete the following code to input the data and store as wisc.df</pre>
```

# wisc.df <- read.csv(fna.data, row.names=1)</pre>

head(wisc.df)

	diagnosis radiu	g mean	texture mean r	erimeter 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	
010700	smoothness_mean					nts mean
842302	0.11840	_	0.27760	0.3001	onouvo.poi	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 f				ture se p	
842302	0.2419	_	0.07871		0.9053	8.589
842517	0.1812		0.05667		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 smoothn	ess_se	compactness_se	concavity_se	concave.p	oints_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 fra	ctal_di	mension_se rad	lius_worst text	ture_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	_		_	_	
842302	184.60	20	019.0	0.1622	0.66	56

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.poin	ts_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	on_worst			
842302		0.11890			
842517		0.08902			
84300903		0.08758			
84348301		0.17300			
84358402		0.07678			
843786		0.12440			

 $\verb|wisc.df$diagnosis| is our expert medical diagnosis|$ 

Let's make a dataframe that excludes this diagnosis column

```
wisc.data <- wisc.df[,-1]
head(wisc.data)</pre>
```

	radius_mean tex	ture_mean p	perimeter_mean	area_mean s	moothness_mean
842302	17.99	10.38	122.80	1001.0	0.11840
842517	20.57	17.77	132.90	1326.0	0.08474
84300903	19.69	21.25	130.00	1203.0	0.10960
84348301	11.42	20.38	77.58	386.1	0.14250
84358402	20.29	14.34	135.10	1297.0	0.10030
843786	12.45	15.70	82.57	477.1	0.12780
	compactness_mea	n concavity	y_mean concave	.points_mean	symmetry_mean
842302	0.2776	0 (	0.3001	0.14710	0.2419
842517	0.0786	4 (	0.0869	0.07017	0.1812
84300903	0.1599	0 (	0.1974	0.12790	0.2069
84348301	0.2839	0 (	0.2414	0.10520	0.2597
84358402	0.1328	0 (	0.1980	0.10430	0.1809
843786	0.1700	0 (	0.1578	0.08089	0.2087
	<pre>fractal_dimensi</pre>	on_mean rad	dius_se textur	e_se perimet	er_se area_se

040000	,	07074	4 0050	0.0050	0 500	450.40
842302			1.0950	0.9053	8.589	
842517			0.5435	0.7339	3.398	
84300903			0.7456	0.7869	4.585	
84348301			0.4956	1.1560	3.445	
84358402			0.7572		5.438	
843786			0.3345	0.8902	2.217	
0.40000	smoothness_se co	-		•	-	
842302	0.006399	0.049		0.05373	0.01	
842517	0.005225	0.013		0.01860	0.01	
84300903		0.040		0.03832	0.02	
84348301	0.009110	0.074		0.05661	0.01	
84358402	0.011490	0.024		0.05688	0.01	
843786	0.007510	0.033		0.03672	0.01	
	symmetry_se frac					
842302	0.03003		006193	25.38		.33
842517	0.01389		003532	24.99		3.41
84300903			004571	23.5		.53
84348301	0.05963		009208	14.9		5.50
84358402			005115	22.54		6.67
843786	0.02165	0.0	005082	15.4	7 23	.75
	<pre>perimeter_worst</pre>	_	smoothne	_	${\tt compactness}$	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.po	ints_wors	st symmetry	y_worst	
842302	0.7119		0.265	54	0.4601	
842517	0.2416		0.186	30	0.2750	
84300903	0.4504		0.243	30	0.3613	
84348301	0.6869		0.257	75	0.6638	
84358402	0.4000		0.162	25	0.2364	
843786	0.5355		0.174	11	0.3985	
	fractal_dimension	on_worst				
842302		0.11890				
842517		0.08902				
84300903		0.08758				
84348301		0.17300				
84358402		0.07678				
843786		0.12440				

We should also store the diagnoses as a factor

```
diagnosis <- factor(wisc.df[,1])</pre>
  head(diagnosis)
[1] M M M M M M
Levels: B M
     Q1. How many observations are in this dataset? 569 observations
  nrow(wisc.data)
[1] 569
569 observations are in this dataset.
     Q2. How many of the observations have a malignant diagnosis? 212 malignant
     diagnoses
   table(diagnosis)
diagnosis
  В
      Μ
357 212
212 observations have a malignant diagnosis.
     Q3. How many variables/features in the data are suffixed with _mean? 10
  length(grep('_mean', names(wisc.data)))
[1] 10
  # use ,value=T, to find the names ending in _mean
10 variables in the data are suffixed with _mean.
```

## **PCA**

Looks like we need to scale the data. Here we will use prcomp() on the wisc.data object.

First we have to decide whether to use the scale=T or scale=F argument.

We can look at the means and sd of each column. If they are similar then we are all good to go.

Let's look at column means to see whether scaling is needed.

## colMeans(wisc.data)

radius_mean	texture_mean	perimeter_mean
1.412729e+01	1.928965e+01	9.196903e+01
area_mean	${\tt smoothness\_mean}$	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	<pre>fractal_dimension_worst</pre>
1.146062e-01	2.900756e-01	8.394582e-02

# apply(wisc.data,2,sd)

${\tt radius\_mean}$	texture_mean	perimeter_mean
3.524049e+00	4.301036e+00	2.429898e+01
area_mean	${\tt smoothness\_mean}$	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
                                                            3.002518e-03
                                   4.549101e+01
        compactness se
                                   concavity se
                                                       concave.points se
          1.790818e-02
                                   3.018606e-02
                                                            6.170285e-03
                           fractal dimension se
                                                            radius worst
           symmetry_se
          8.266372e-03
                                   2.646071e-03
                                                            4.833242e+00
         texture_worst
                                perimeter_worst
                                                              area_worst
          6.146258e+00
                                   3.360254e+01
                                                            5.693570e+02
                              compactness_worst
      smoothness_worst
                                                         concavity_worst
                                   1.573365e-01
          2.283243e-02
                                                            2.086243e-01
  concave.points_worst
                                 symmetry_worst fractal_dimension_worst
                                   6.186747e-02
                                                            1.806127e-02
          6.573234e-02
```

We need to scale the data because the means and sd vary heavily in magnitude.

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

#### Importance of components:

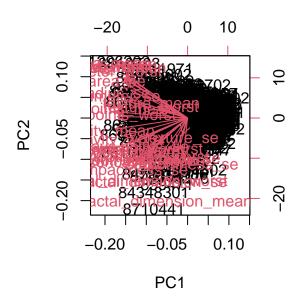
```
PC1
                                 PC2
                                         PC3
                                                 PC4
                                                          PC5
                                                                  PC6
                                                                          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
                                  PC16
                                          PC17
                                                  PC18
                                                          PC19
                                                                   PC20
                          PC15
                                                                          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
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 (much lower than earlier). This makes sense because data from patient samples expectedly have a lot of noise.

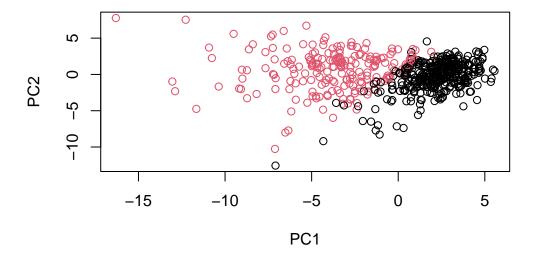
- Q5. How many principal components (PCs) are required to describe at least 70% of the original variance in the data?
- 3 PCs. PC1-3 account for 72.6% of variance in the data.
  - Q6. How many principal components (PCs) are required to describe at least 90% of the original variance in the data?
- 7 PCs. PC1-7 account for 91% of variance in the data.

biplot(wisc.pr)



What a mess!

Let's try another plot of PC1 vs PC2.

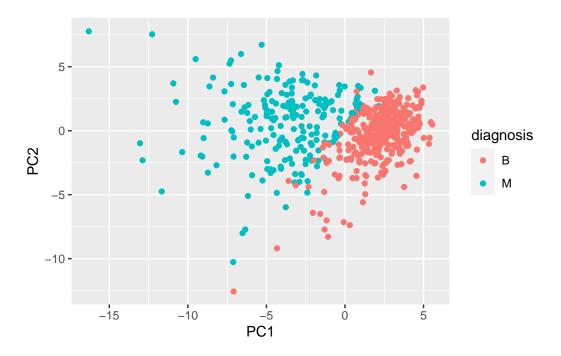


This looks much more readable.

```
library(ggplot2)

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

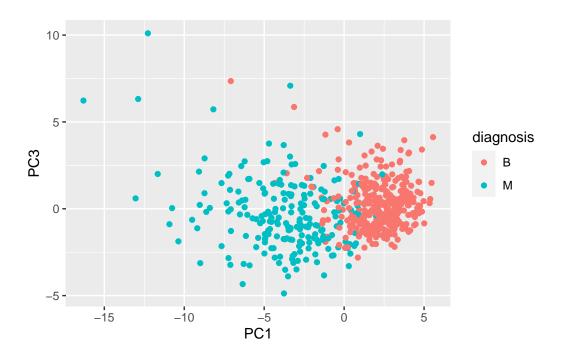
ggplot(pc) +
   aes(PC1, PC2, col=diagnosis) +
   geom_point()</pre>
```



This is even better.

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

```
ggplot(pc) +
  aes(PC1, PC3, col=diagnosis) +
  geom_point()
```



These plots have as many datapoints as initial observations. This is because each observation is given a score based on the PCs.

Q9. For the first principal component, what is the component of the loading vector (i.e. wisc.pr\$rotation[,1]) for the feature concave.points\_mean?

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

### [1] -0.2608538

- -0.2608538 is the component of the loading vector for the feature concave.points\_mean.
  - Q10. What is the minimum number of principal components required to explain 80% of the variance of the data?
- 5 PCs explain 80% of the variance.

```
tbl <- summary(wisc.pr)
tbl$importance[3,]</pre>
```

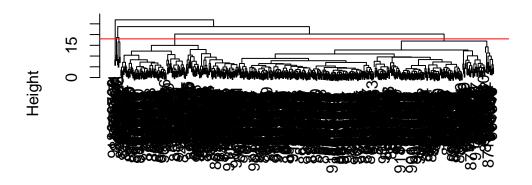
```
PC1
            PC2
                     PC3
                              PC4
                                      PC5
                                               PC6
                                                       PC7
                                                                PC8
                                                                         PC9
                                                                                PC10
0.44272 0.63243 0.72636 0.79239 0.84734 0.88759 0.91010 0.92598 0.93988 0.95157
   PC11
           PC12
                    PC13
                            PC14
                                     PC15
                                              PC16
                                                      PC17
                                                               PC18
                                                                       PC19
                                                                                PC20
0.96137\ 0.97007\ 0.97812\ 0.98335\ 0.98649\ 0.98915\ 0.99113\ 0.99288\ 0.99453\ 0.99557
           PC22
   PC21
                    PC23
                            PC24
                                     PC25
                                              PC26
                                                      PC27
                                                               PC28
                                                                       PC29
                                                                                PC30
0.99657 0.99749 0.99830 0.99890 0.99942 0.99969 0.99992 0.99997 1.00000 1.00000
```

## **Hierarchical Clustering**

The main function for hierarchical clustering is called hclust() which takes a distance matrix as input.

```
d <- dist(scale(wisc.data))
wisc.hclust <- hclust(d)
plot(wisc.hclust)
abline(h=18, col="red")</pre>
```

# **Cluster Dendrogram**



d hclust (\*, "complete")

```
grps <- cutree(wisc.hclust, h=18)</pre>
```

Let's see how our cluster grps correspond to malignancy (M or B diagnosis groups).

```
table(grps)

grps

1 2 3 4 5
177 5 383 2 2
```

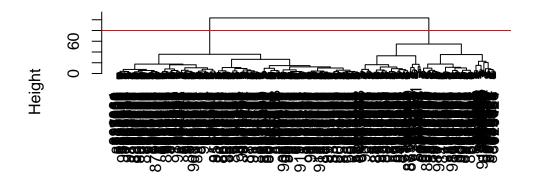
### 5. Combining methods

Here we will perform clustering on PCA results rather than the original data.

In other words, we will cluster using wisc.pr\$x - our new better variables of PCs. We can choose as many or as few PCs to use as we like.

```
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')</pre>
```

# **Cluster Dendrogram**



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

We can use the table() function to make a cross-table.

```
table(diagnosis)

diagnosis
    B    M
357 212

table(grps, diagnosis)

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

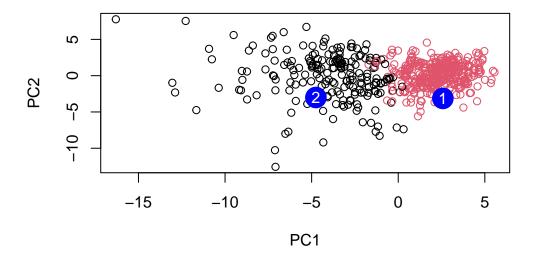
From group 1, 179 were malignant and only 24 were benign. From group 2, 333 were benign and 33 were malignant. Cluster 1 mostly captures malignant (M) tumors while cluster 2 captures mainly benign masses/healthy patients (B).

#### 7. Predict

```
url <- "https://tinyurl.com/new-samples-CSV"
new <- read.csv(url)
npc <- predict(wisc.pr, newdata=new)
npc</pre>
```

```
PC1
                     PC2
                                PC3
                                            PC4
                                                      PC5
                                                                 PC6
                                                                             PC7
     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
```

```
 \begin{smallmatrix} [1,] & -0.2307350 & 0.1029569 & -0.9272861 & 0.3411457 & 0.375921 & 0.1610764 & 1.187882 \end{smallmatrix} 
[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
                 0.1448061 -0.40509706
[2,] 0.1299153
                                          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
      0.220199544 -0.02946023 -0.015620933
[1,]
                                               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?

We should prioritize group 2, which is likely to contain patients with malignancies that were missed in the initial diagnosis.