

Class 8: Mini Project

AUTHOR

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

Read the 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

Q. How many genes are in this dataset?

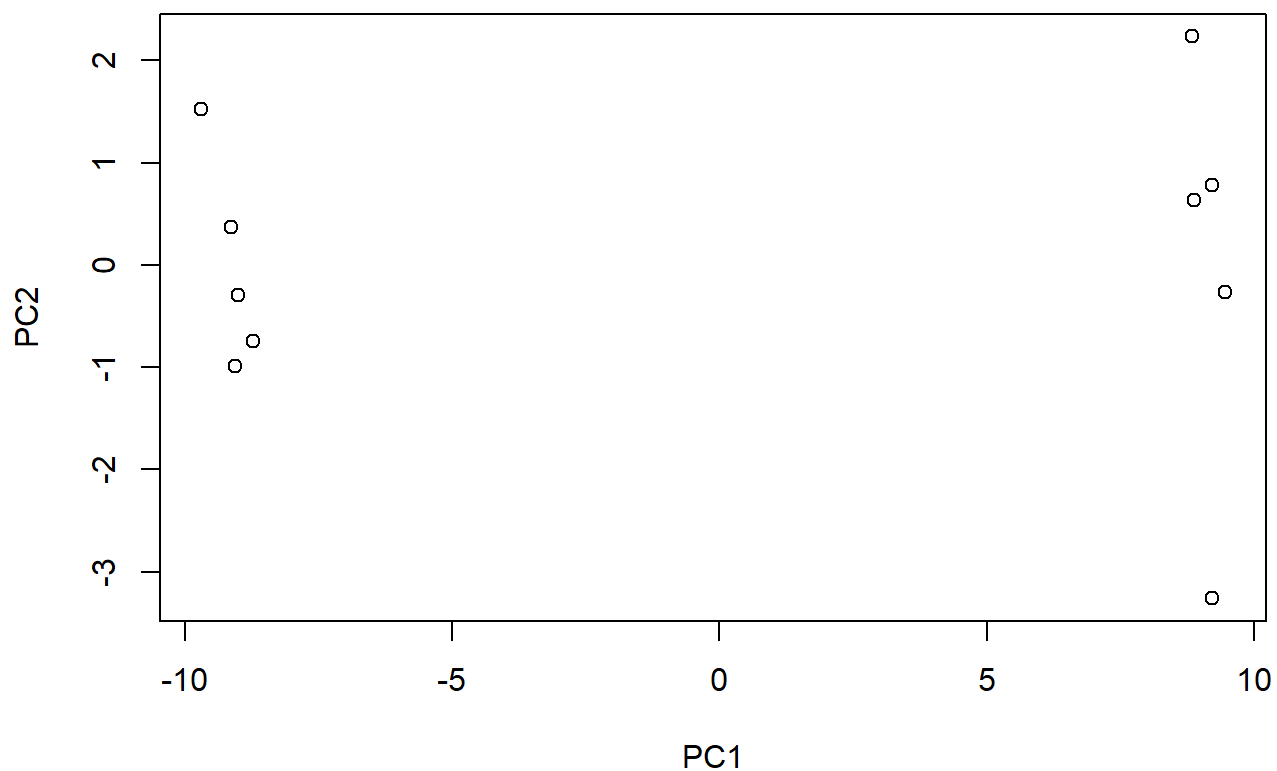
```
nrow(rna.data)
```

```
[1] 100
```

Run PCA

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

## Simple un polished plot of pc1 and pc2
plot(pca$x[,1], pca$x[,2], xlab="PC1", ylab="PC2")
```



```
summary(pca)
```

Importance of components:

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Standard deviation	9.6237	1.5198	1.05787	1.05203	0.88062	0.82545	0.80111
Proportion of Variance	0.9262	0.0231	0.01119	0.01107	0.00775	0.00681	0.00642
Cumulative Proportion	0.9262	0.9493	0.96045	0.97152	0.97928	0.98609	0.99251

	PC8	PC9	PC10
Standard deviation	0.62065	0.60342	3.457e-15
Proportion of Variance	0.00385	0.00364	0.000e+00
Cumulative Proportion	0.99636	1.00000	1.000e+00

```
pca$x
```

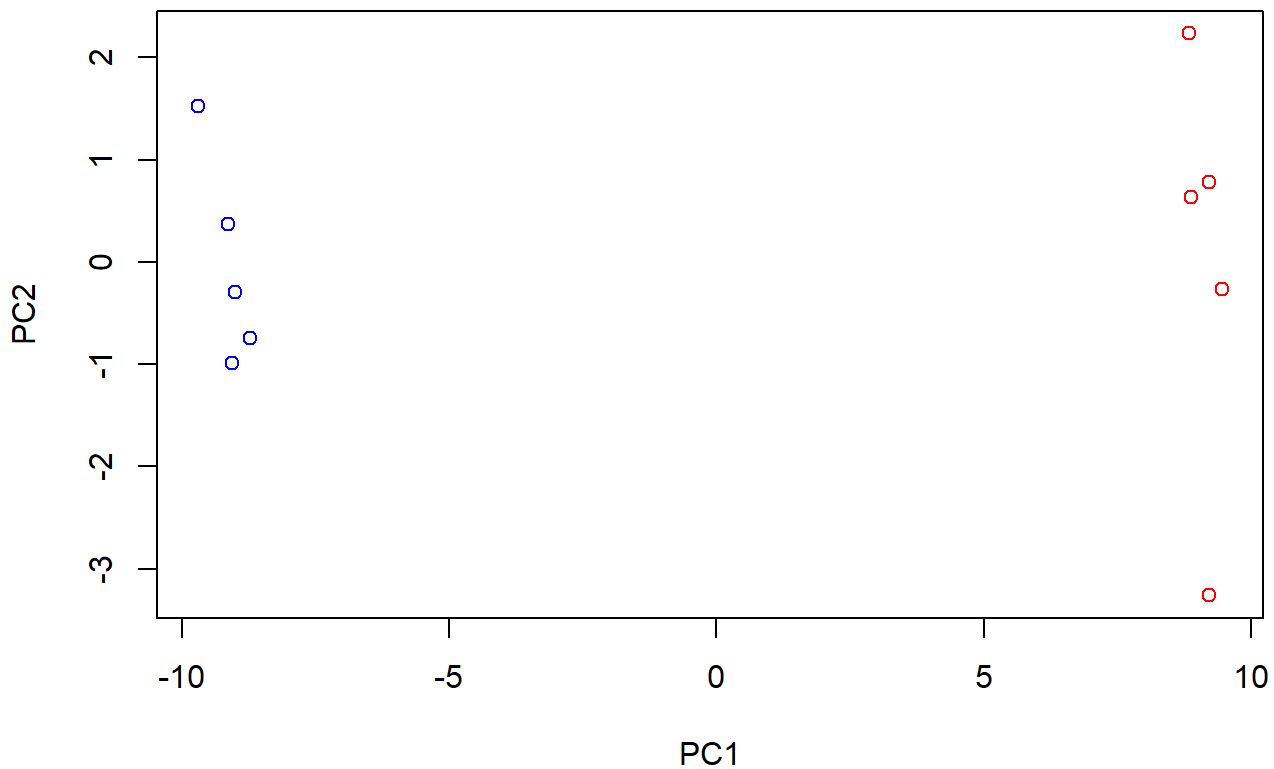
	PC1	PC2	PC3	PC4	PC5	PC6
wt1	-9.697374	1.5233313	-0.2753567	0.7322391	-0.6749398	1.1823860
wt2	-9.138950	0.3748504	1.0867958	-1.9461655	0.7571209	-0.4369228
wt3	-9.054263	-0.9855163	0.4152966	1.4166028	0.5835918	0.6937236
wt4	-8.731483	-0.7468371	0.5875748	0.2268129	-1.5404775	-1.2723618
wt5	-9.006312	-0.2945307	-1.8498101	-0.4303812	0.8666124	-0.2496025
ko1	8.846999	2.2345475	-0.1462750	-1.1544333	-0.6947862	0.7128021
ko2	9.213885	-3.2607503	0.2287292	-0.7658122	-0.4922849	0.9170241
ko3	9.458412	-0.2636283	-1.5778183	0.2433549	0.3654124	-0.5837724

	PC7	PC8	PC9	PC10
ko4	8.883412	0.6339701	1.5205064	0.7760158
ko5	9.225673	0.7845635	0.0103574	0.9017667
wt1	-0.24446614	1.03519396	0.07010231	3.073930e-15
wt2	-0.03275370	0.26622249	0.72780448	1.963707e-15
wt3	-0.03578383	-1.05851494	0.52979799	2.893519e-15
wt4	-0.52795595	-0.20995085	-0.50325679	2.872702e-15
wt5	0.83227047	-0.05891489	-0.81258430	1.693090e-15
ko1	-0.07864392	-0.94652648	-0.24613776	4.052314e-15
ko2	0.30945771	0.33231138	-0.08786782	3.268219e-15
ko3	-1.43723425	0.14495188	0.56617746	2.636780e-15
ko4	-0.35073859	0.30381920	-0.87353886	3.615164e-15
ko5	1.56584821	0.19140827	0.62950330	3.379241e-15

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

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

```
plot(pca$x[,1], pca$x[,2], xlab="PC1", ylab="PC2", col=mycols)
```



```
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

```
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	

Note that the first column here `wisc$diagnosis` is a pathologist

```
[1] 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
[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 M M B B B M B 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 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 B M B B M M B M B B B B M 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 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 B M M M B B B B B B B B B B M 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
[408] B M B B B B B M B B M 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 M B B M B M M M
[519] B B B M 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 M M M M M M B
```

Levels: B M

	radius_mean	texture_mean	perimeter_mean	area_mean	smoothness_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_mean	concavity_mean	concave.points_mean	symmetry_mean	
842302	0.27760	0.3001		0.14710	0.2419
842517	0.07864	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_mean	radius_se	texture_se	perimeter_se	area_se
842302	0.07871	1.0950	0.9053	8.589	153.40
842517	0.05667	0.5435	0.7339	3.398	74.08
84300903	0.05999	0.7456	0.7869	4.585	94.03
84348301	0.09744	0.4956	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	
842302	0.006399	0.04904	0.05373	0.01587	
842517	0.005225	0.01308	0.01860	0.01340	
84300903	0.006150	0.04006	0.03832	0.02058	
84348301	0.009110	0.07458	0.05661	0.01867	
84358402	0.011490	0.02461	0.05688	0.01885	
843786	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

Q1. How many observations are in this dataset?

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

```
[1] 569
```

A1. 569 observations in this dataset.

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

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

```
  B    M  
357 212
```

A2. 212 have a malignant diagnosis.

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

```
colnames(wisc.data)
```

```
[1] "radius_mean"      "texture_mean"  
[3] "perimeter_mean"   "area_mean"  
[5] "smoothness_mean"  "compactness_mean"  
[7] "concavity_mean"   "concave.points_mean"  
[9] "symmetry_mean"    "fractal_dimension_mean"  
[11] "radius_se"        "texture_se"  
[13] "perimeter_se"     "area_se"  
[15] "smoothness_se"    "compactness_se"  
[17] "concavity_se"     "concave.points_se"  
[19] "symmetry_se"      "fractal_dimension_se"  
[21] "radius_worst"     "texture_worst"  
[23] "perimeter_worst"  "area_worst"  
[25] "smoothness_worst" "compactness_worst"  
[27] "concavity_worst"  "concave.points_worst"  
[29] "symmetry_worst"   "fractal_dimension_worst"
```

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

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

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

```
[1] 10
```

A3. 10 variables in the data are suffixed with "_mean".

Principal Component Analysis

Here we will use "prcom()" on the 'wisc.data' object First, we have to decide whether to use the 'scale=TRUE'

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

```
colMeans(wisc.data)
```

radius_mean	texture_mean	perimeter_mean
1.412729e+01	1.928965e+01	9.196903e+01
area_mean	smoothness_mean	compactness_mean
6.548891e+02	9.636028e-02	1.043410e-01
concavity_mean	concave.points_mean	symmetry_mean
8.879932e-02	4.891915e-02	1.811619e-01
fractal_dimension_mean	radius_se	texture_se
6.279761e-02	4.051721e-01	1.216853e+00
perimeter_se	area_se	smoothness_se
2.866059e+00	4.033708e+01	7.040979e-03
compactness_se	concavity_se	concave.points_se
2.547814e-02	3.189372e-02	1.179614e-02
symmetry_se	fractal_dimension_se	radius_worst
2.054230e-02	3.794904e-03	1.626919e+01
texture_worst	perimeter_worst	area_worst
2.567722e+01	1.072612e+02	8.805831e+02
smoothness_worst	compactness_worst	concavity_worst
1.323686e-01	2.542650e-01	2.721885e-01
concave.points_worst	symmetry_worst	fractal_dimension_worst
1.146062e-01	2.900756e-01	8.394582e-02

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

radius_mean	texture_mean	perimeter_mean
3.524049e+00	4.301036e+00	2.429898e+01
area_mean	smoothness_mean	compactness_mean
3.519141e+02	1.406413e-02	5.281276e-02

concavity_mean	concave.points_mean	symmetry_mean
7.971981e-02	3.880284e-02	2.741428e-02
fractal_dimension_mean	radius_se	texture_se
7.060363e-03	2.773127e-01	5.516484e-01
perimeter_se	area_se	smoothness_se
2.021855e+00	4.549101e+01	3.002518e-03
compactness_se	concavity_se	concave.points_se
1.790818e-02	3.018606e-02	6.170285e-03
symmetry_se	fractal_dimension_se	radius_worst
8.266372e-03	2.646071e-03	4.833242e+00
texture_worst	perimeter_worst	area_worst
6.146258e+00	3.360254e+01	5.693570e+02
smoothness_worst	compactness_worst	concavity_worst
2.283243e-02	1.573365e-01	2.086243e-01
concave.points_worst	symmetry_worst	fractal_dimension_worst
6.573234e-02	6.186747e-02	1.806127e-02

These are very different so we should set scale=TRUE

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

Importance of components:

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Standard deviation	3.6444	2.3857	1.67867	1.40735	1.28403	1.09880	0.82172
Proportion of Variance	0.4427	0.1897	0.09393	0.06602	0.05496	0.04025	0.02251
Cumulative Proportion	0.4427	0.6324	0.72636	0.79239	0.84734	0.88759	0.91010
	PC8	PC9	PC10	PC11	PC12	PC13	PC14
Standard deviation	0.69037	0.6457	0.59219	0.5421	0.51104	0.49128	0.39624
Proportion of Variance	0.01589	0.0139	0.01169	0.0098	0.00871	0.00805	0.00523
Cumulative Proportion	0.92598	0.9399	0.95157	0.9614	0.97007	0.97812	0.98335
	PC15	PC16	PC17	PC18	PC19	PC20	PC21
Standard deviation	0.30681	0.28260	0.24372	0.22939	0.22244	0.17652	0.1731
Proportion of Variance	0.00314	0.00266	0.00198	0.00175	0.00165	0.00104	0.0010
Cumulative Proportion	0.98649	0.98915	0.99113	0.99288	0.99453	0.99557	0.9966
	PC22	PC23	PC24	PC25	PC26	PC27	PC28
Standard deviation	0.16565	0.15602	0.1344	0.12442	0.09043	0.08307	0.03987
Proportion of Variance	0.00091	0.00081	0.0006	0.00052	0.00027	0.00023	0.00005
Cumulative Proportion	0.99749	0.99830	0.9989	0.99942	0.99969	0.99992	0.99997
	PC29	PC30					
Standard deviation	0.02736	0.01153					
Proportion of Variance	0.00002	0.00000					
Cumulative Proportion	1.00000	1.00000					

Q4. From your results, what proportion of the original variance is captured by the first principal components (PC1)? > A4. 44.27%

Q5. How many principal components (PCs) are required to describe at least 70% of the original variance in the data? > A5. 3 PCs capture 72.6% of the original variance.

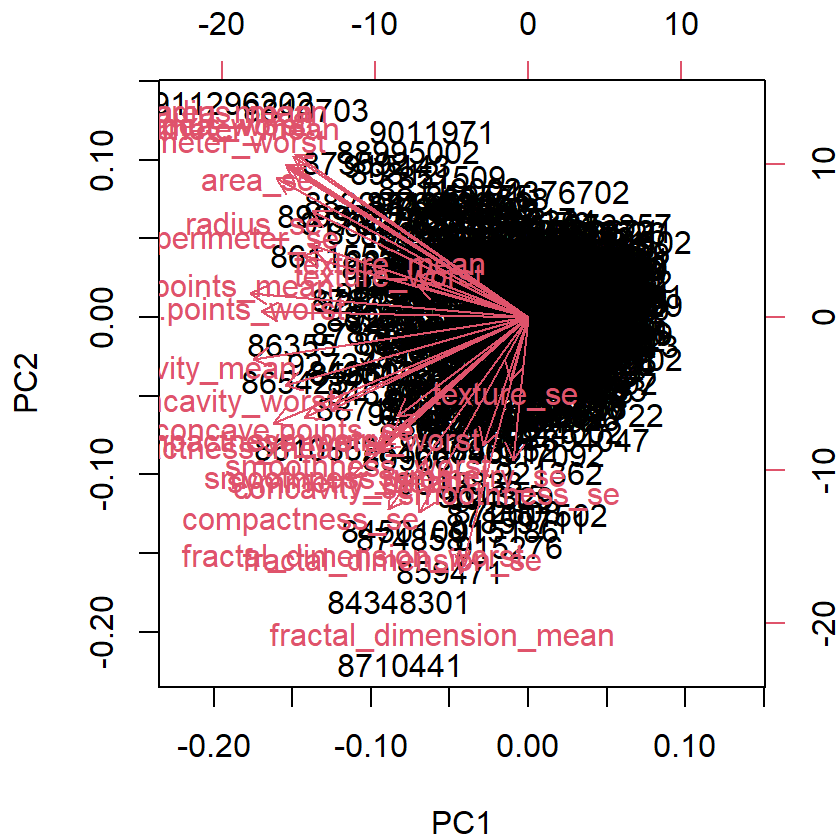
Q6. How many principal components (PCs) are required to describe at least 90% of the original variance in the data? > A6. 7 PCs capture 91% of the original variance.

Plotting the PCA results

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

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

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



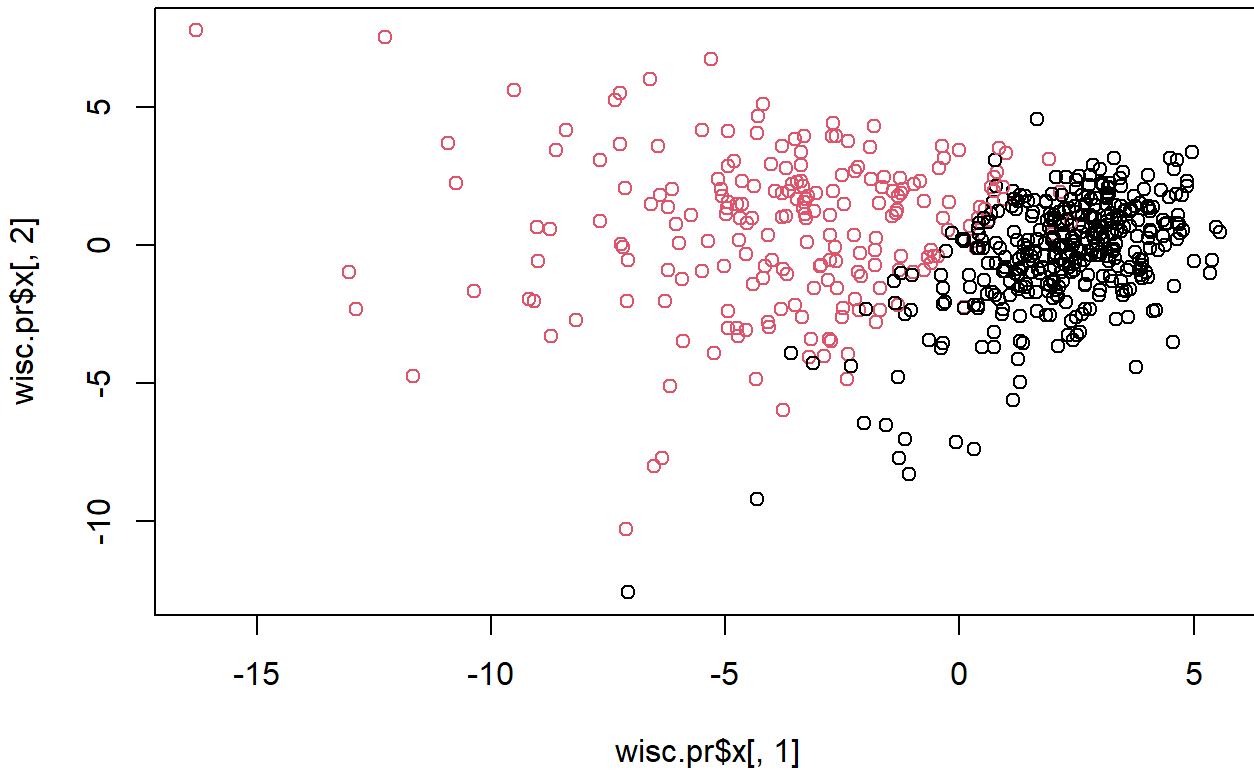
A7. It is difficult to understand because its so messy and impossible to read all the stacked numeric values.

We need to make our own plot.

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

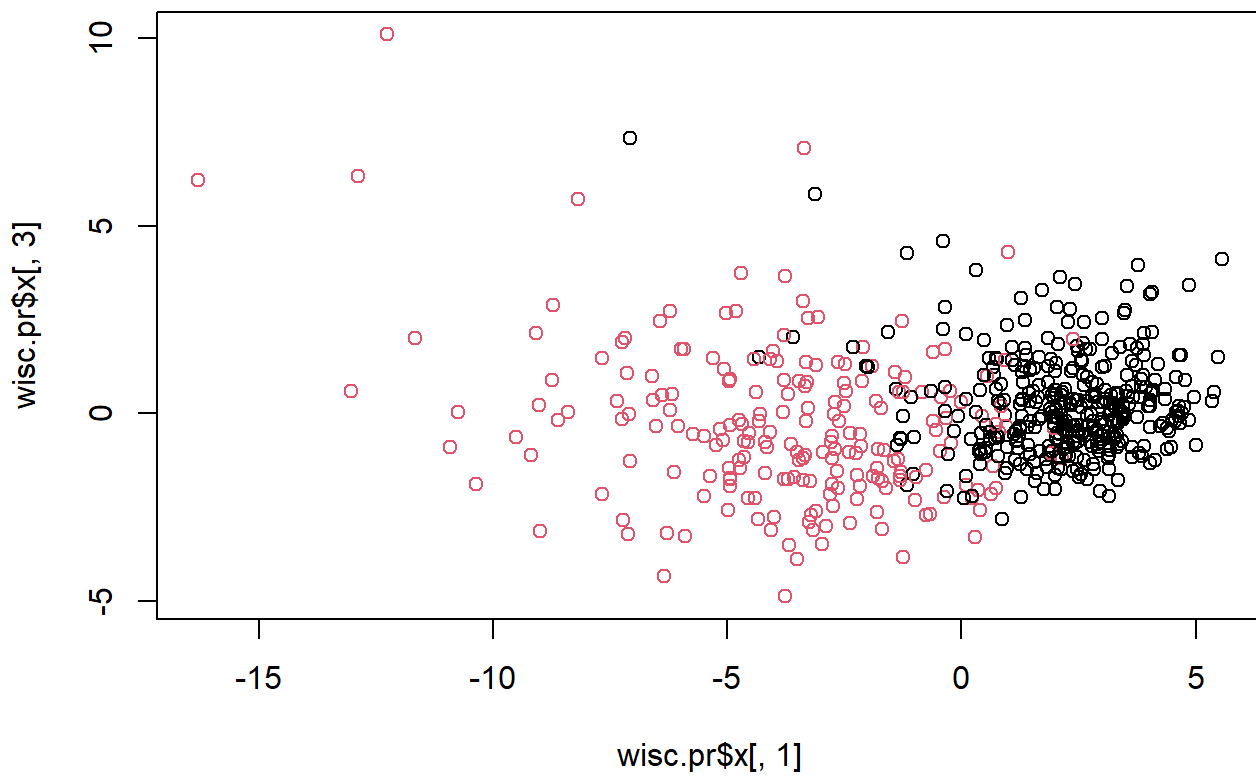
```
$names  
[1] "sdev"      "rotation" "center"    "scale"     "x"  
  
$class  
[1] "prcomp"
```

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



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

```
plot(wisc.pr$x[,1], wisc.pr$x[,3], col=diagnosis)
```

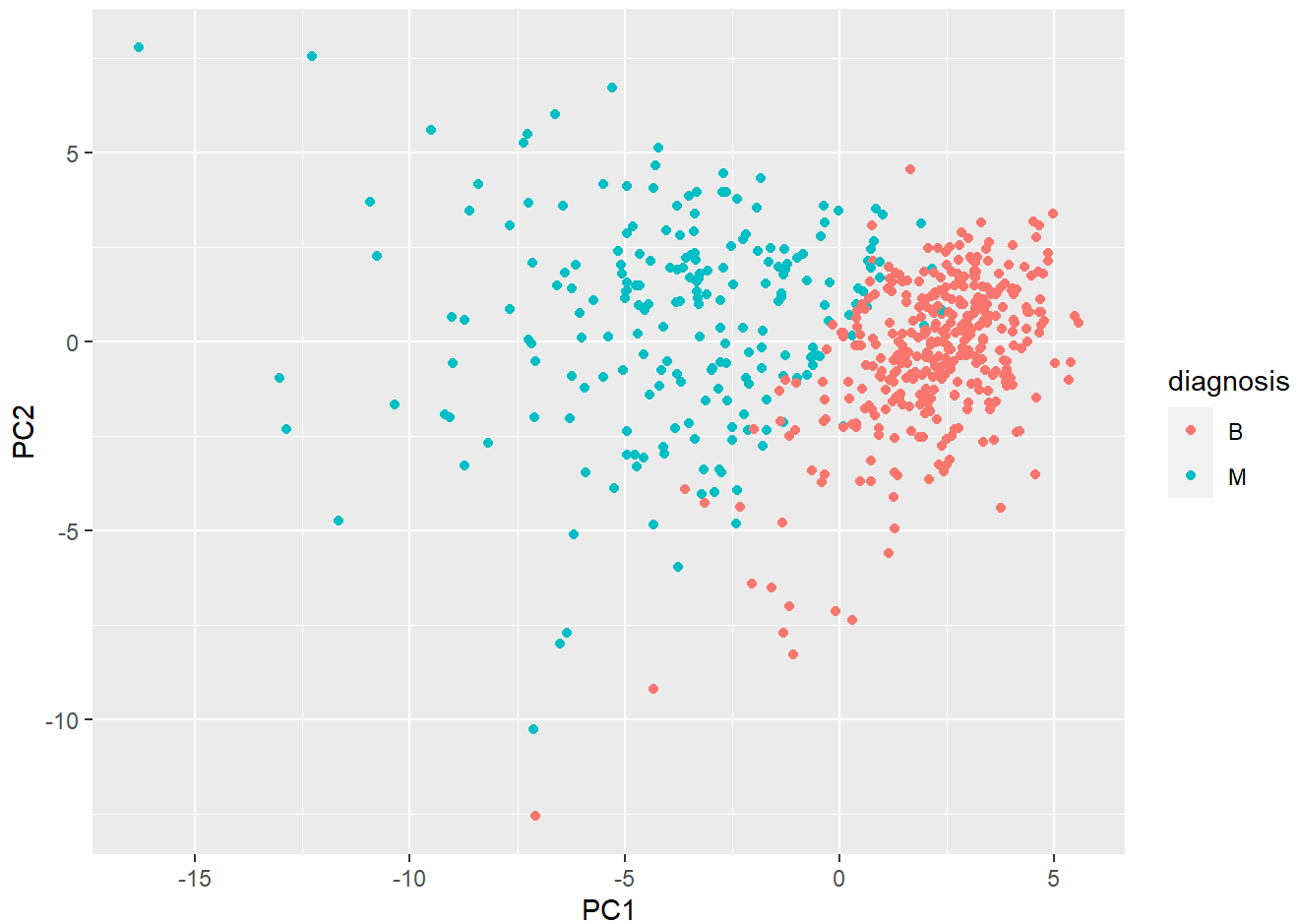


A8. The plots show that PC1 is capturing a separation of malignant from benign samples. PC2 shows more variance in the original data than PC3.

```
library(ggplot2)

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

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



```
ggplot
```

```
function (data = NULL, mapping = aes(), ..., environment = parent.frame())
{
  UseMethod("ggplot")
}
<bytecode: 0x000001af8a3057f0>
<environment: namespace:ggplot2>
```

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

```
[1] 13.281608  5.691355  2.817949  1.980640  1.648731  1.207357
```

Communicating PCA results

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
```

A9. -0.26 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?

```
tbl <- summary(wisc.pr)
tbl$importance[3,] > 0.8
```

PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13
FALSE	FALSE	FALSE	FALSE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
PC14	PC15	PC16	PC17	PC18	PC19	PC20	PC21	PC22	PC23	PC24	PC25	PC26
TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
PC27	PC28	PC29	PC30									
TRUE	TRUE	TRUE	TRUE									

```
which(tbl$importance[3,] > 0.8 )[1]
```

```
PC5
5
```

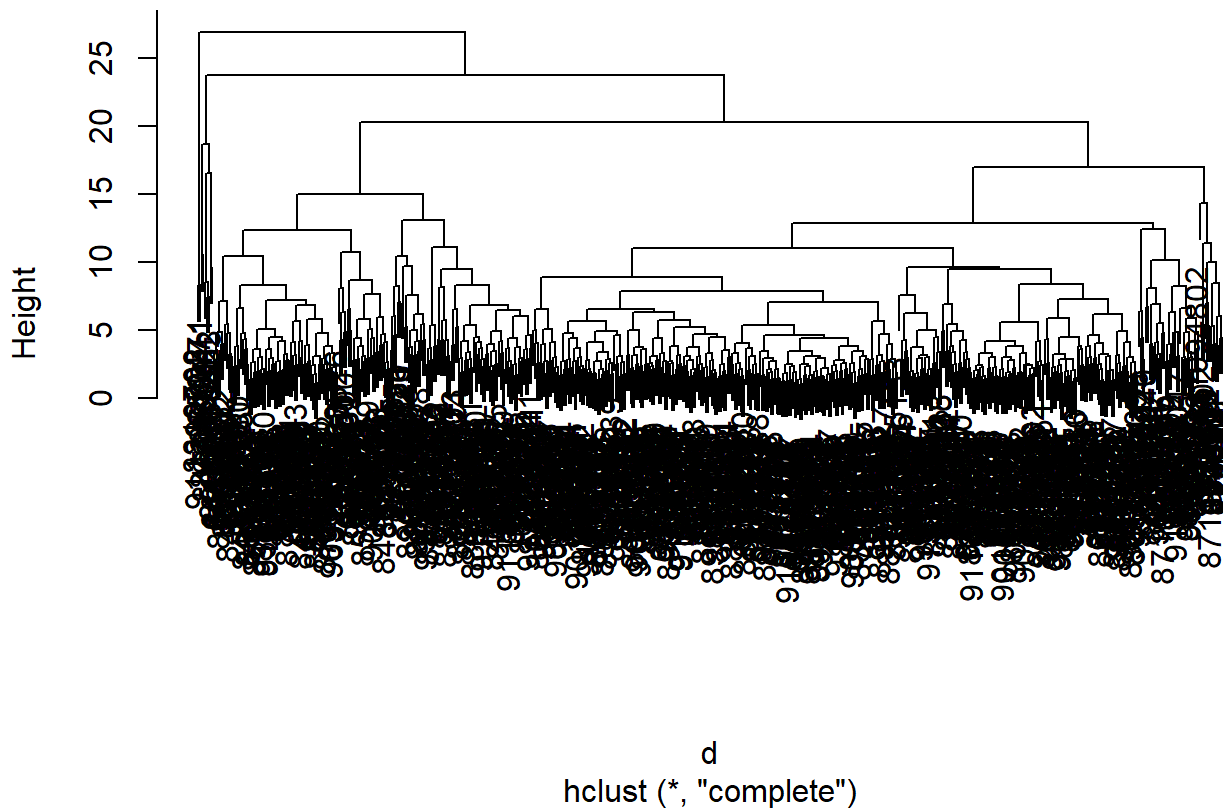
A10. PC5 is the minimum to explain 80% of the variance.

Hierarchical Clustering

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

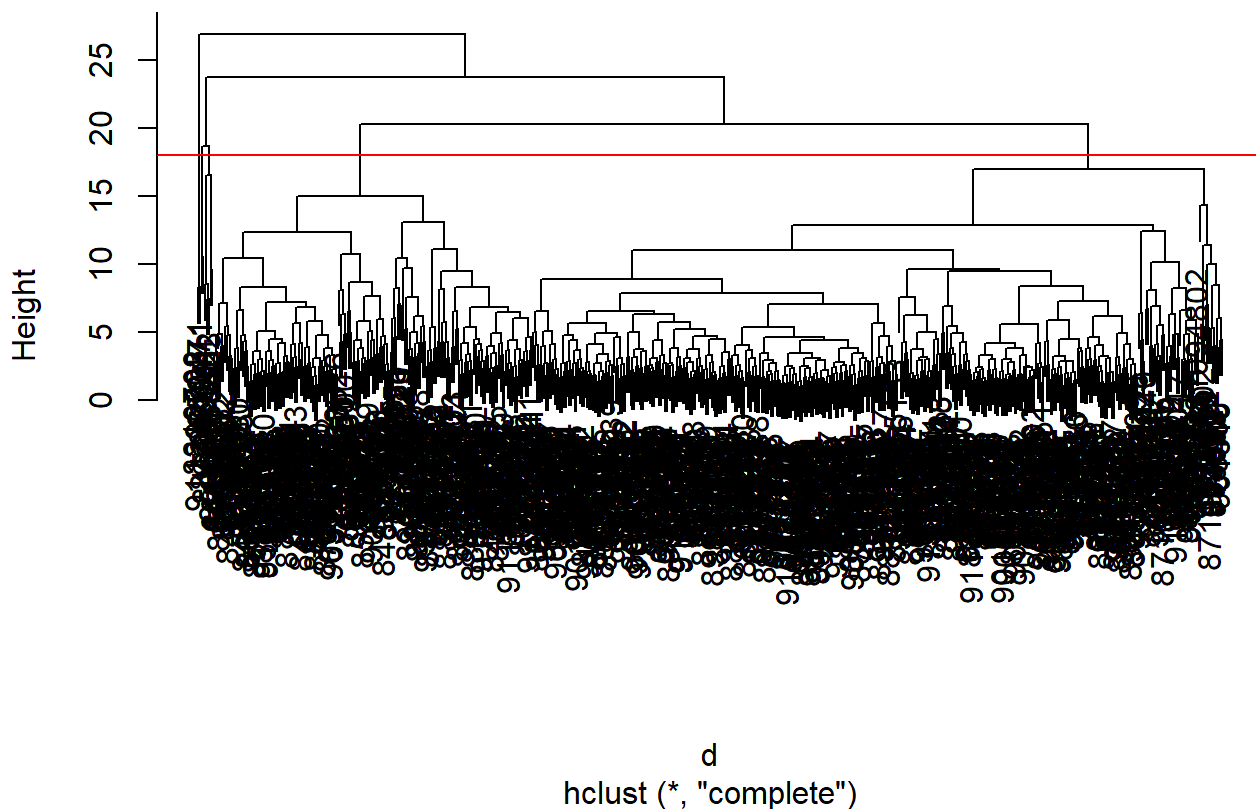
```
d <- dist( scale(wisc.data))
wisc.hclust <- hclust(d)
plot(wisc.hclust)
```

Cluster Dendrogram



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

Cluster Dendrogram



```
grps <- cutree(wisc.hclust, h=18)
table(grps)
```

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

A11. At a height of about 18 the clustering model has 4 clusters.

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

Jumping down to Section 5, skip 4.

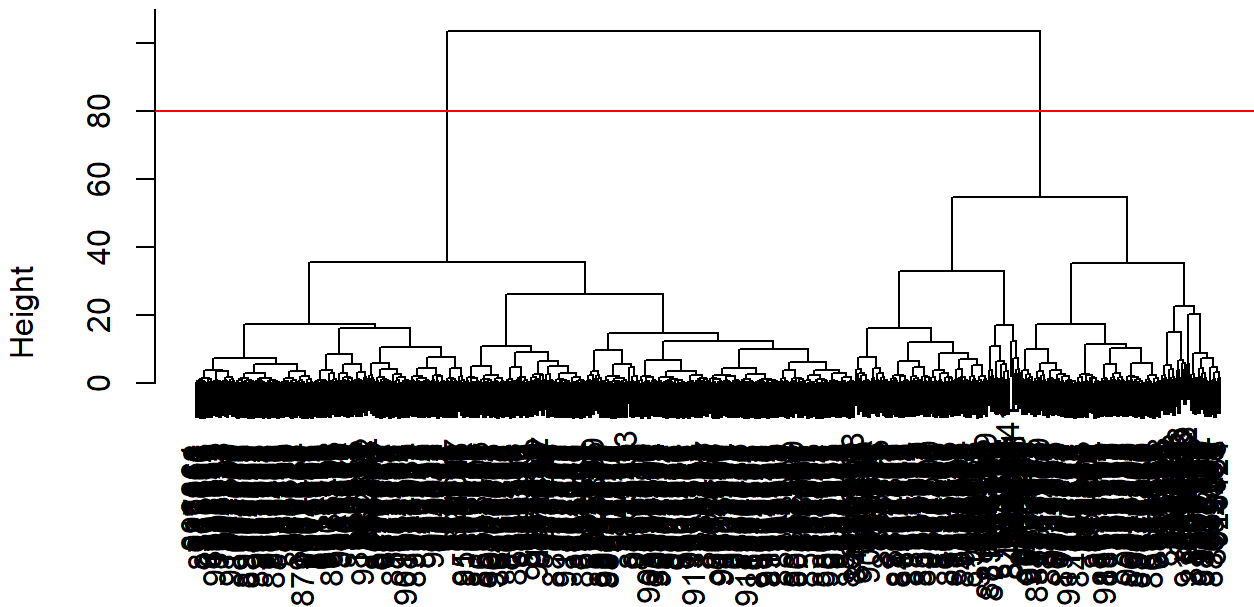
5. Combining methods

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

In other words we will cluster using 'wisc.pr\$x' - our new better variables or PCs. We can choose as many or as few PCs to use as we like. It is your call!


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

Cluster Dendrogram



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

```
grps <- cutree(wisc.pr.hclust, h=80)
table(grps)
```

```
grps
  1  2
203 366
```

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

```
table(diagnosis)
```

```
diagnosis
  B  M
357 212
```

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

```

diagnosis
grps   B   M
1    24 179
2   333  33

```

Write a note here about how to read this cross-table result. The results indicates that our cluster 1 mostly captures cancer (M) and our cluster 2 mostly captures healthy (B) samples/individuals.

7. Prediction

```

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

```

```

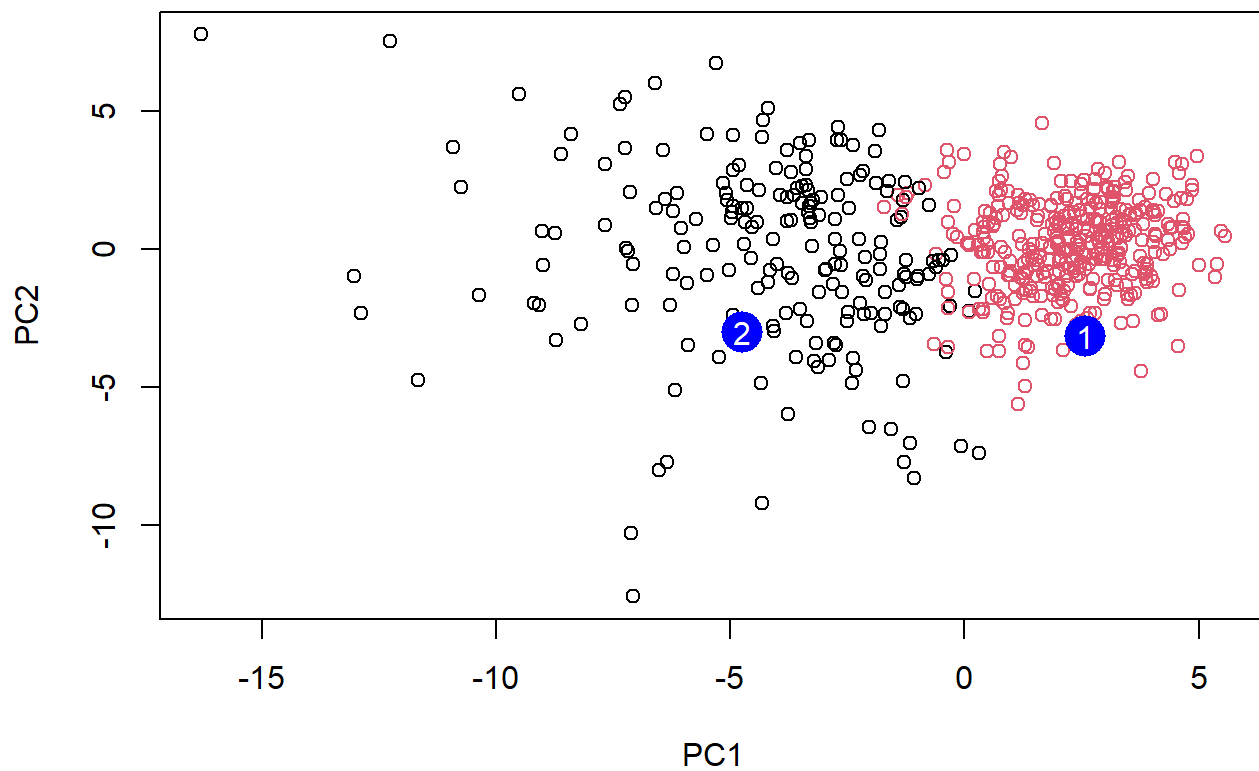
      PC1      PC2      PC3      PC4      PC5      PC6      PC7
[1,]  2.576616 -3.135913  1.3990492 -0.7631950  2.781648 -0.8150185 -0.3959098
[2,] -4.754928 -3.009033 -0.1660946 -0.6052952 -1.140698 -1.2189945  0.8193031
      PC8      PC9      PC10     PC11     PC12     PC13     PC14
[1,] -0.2307350 0.1029569 -0.9272861 0.3411457  0.375921 0.1610764 1.187882
[2,] -0.3307423 0.5281896 -0.4855301 0.7173233 -1.185917 0.5893856 0.303029
      PC15     PC16     PC17     PC18     PC19     PC20
[1,] 0.3216974 -0.1743616 -0.07875393 -0.11207028 -0.08802955 -0.2495216
[2,] 0.1299153  0.1448061 -0.40509706  0.06565549  0.25591230 -0.4289500
      PC21     PC22     PC23     PC24     PC25     PC26
[1,] 0.1228233 0.09358453 0.08347651 0.1223396 0.02124121 0.078884581
[2,] -0.1224776 0.01732146 0.06316631 -0.2338618 -0.20755948 -0.009833238
      PC27     PC28     PC29     PC30
[1,] 0.220199544 -0.02946023 -0.015620933 0.005269029
[2,] -0.001134152 0.09638361 0.002795349 -0.019015820

```

```

plot(wisc.pr$x[,1:2], col=grps)
points(npc[,1], npc[,2], col="blue", pch=16, cex=3)
text(npc[,1], npc[,2], c(1,2), col="white")

```



This principal analysis method can be very helpful for organizing data that is usually messy or harder to read.

Q18. Which of these new patients should we prioritize for follow up based on your results? > A18.
Patient 2 should be prioritized.