High-Throughput Sequencing Course

Unsupervised Learning

Biostatistics and Bioinformatics

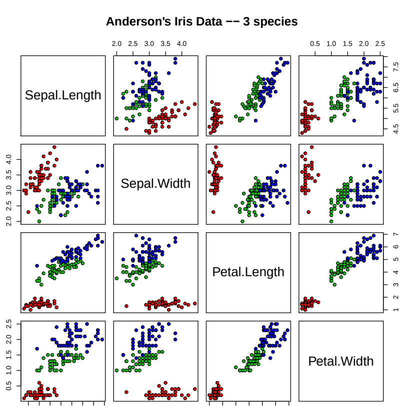
Summer 2017

Duke University School of Medicine

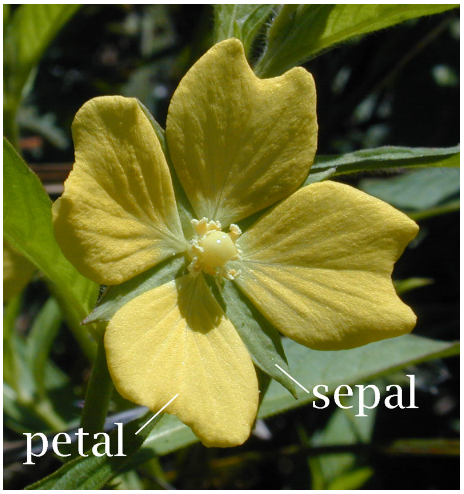
Scope

- Let X denote the genetic/genomic profile of a sample
- Often we would like to discover groups, clusters or outliers based on the genetic profiles of the samples
- These are *unsupervised* methods in the sense that the algorithm knows nothing about the grouping/clustering
- The method is only aware of the genetic profile (X) and not the outcome Y

Fisher's Iris Data

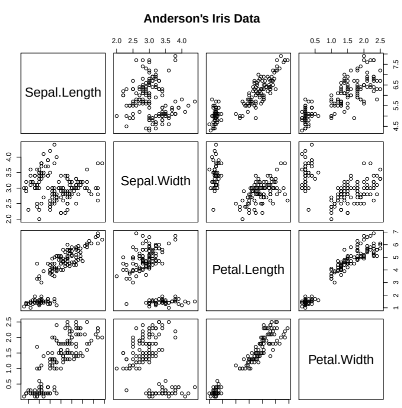


On Petals and Sepals

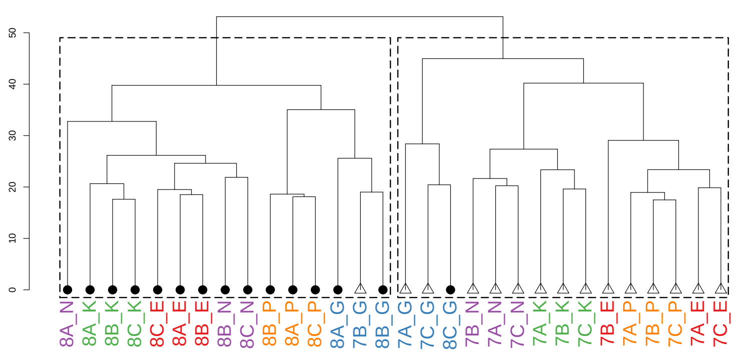


https://en.wikipedia.org/wiki/Sepal

Fisher's Iris Data



2015 Data: Agglomerative Hierarchical Clustering



A Self-fulfilling Prophecy

- Statistical methods for unsupervised learning guarantee one thing
- They will return a clustering of your data
- What they do not guarantee and are invariably unable to verify, is the biological relevance or reproducibility of the clustering
- In light of this Self-fulfilling Prophecy, these methods should be used with utmost care

Methods to be Discussed

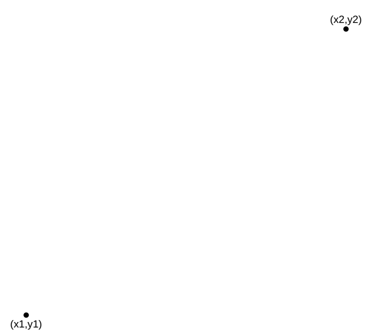
- There are many methods for unsupervised class discovery.
- We will consider three types of methods:
  - Hierarchical Clustering
  - k-means Clustering
  - Ordination Methods (e.g., Multi-Dimensional Scaling (MDS) and Principal Components (PC))
- Note that there are many variations of these methods
- Most mathematical details will be left out
- We focus on discovering classes among samples (not genes)

Distance between Two Points

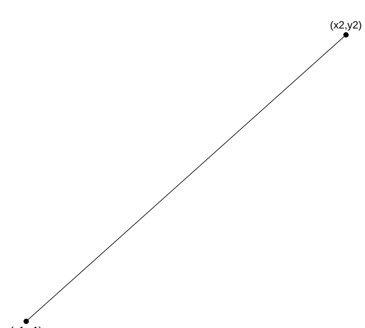
- Many class discover methods aim to quantify the similarity (or dissimilarity) among patients
- For each patient, the vector of gene expression can be thought of a "point" in an m-dimensional space
- For many class discovery methods, one has to be able to quantify the "distance" between two points (the expression profiles between two individuals)
- A common distance measure is the Euclidean distance

Distance (Two points on the plane)

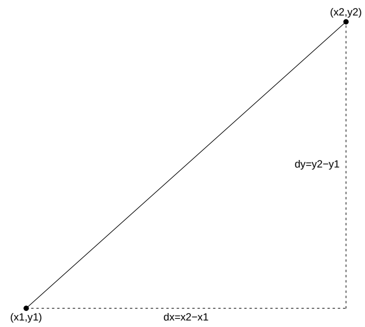
Distance (Coordinates)



Distance



Distance (horizontal/vertical shifts)



Pythagorean Theorem (on the plane)

- According to the Pythagorean theorem

$$h^2 = \mathrm{d}x^2 + \mathrm{d}y^2 = (x_2 - x_1)^2 + (y_2 - y_1)^2$$

- h is called the hypotenuse
- The distance between (x_1, y_1) and (x_2, y_2) is given by

$$h = \sqrt{\mathrm{d}x^2 + \mathrm{d}y^2} = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$$

Pythagorean Theorem (on the plane)

- Can be extended to higher dimensions
- In a three-dimensional space the distance between (x_1, y_1, z_1) and (x_2, y_2, z_2) is given by

$$\sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2 + (z_1 - z_2)^2}$$

- For any given dimension, the distance is obtained as the square root of the sum of the square of the coordinate-wise differences

Golub *et al* Leukemia Data

- 47 patients with acute lymphoblastic leukemia (ALL)
- 25 patients with acute myeloid leukemia (AML)
- Platform: Affymetrix Hgu6800
- 7129 probe sets
- Golub *et al.* (1999). Molecular classification of cancer: class discovery and class prediction by gene expression monitoring, Science, Vol. 286:531-537.

Golub *et al* Leukemia Data

Expression data from first three features and 5 patients

```
dim(exprs(Golub_Merge))

## [1] 7129   72

exprs(Golub_Merge)[1:3, 1:5]

##                  39   40   42   47   48
## AFFX-BioB-5_at -342  -87   22 -243 -130
## AFFX-BioB-M_at -200 -248 -153 -218 -177
## AFFX-BioB-3_at   41  262   17 -163  -28
```

Golub *et al* Leukemia Data: Distance

Expression vector for patients 39 and 40

```
x <- exprs(Golub_Merge)[, "39"]
y <- exprs(Golub_Merge)[, "40"]
```

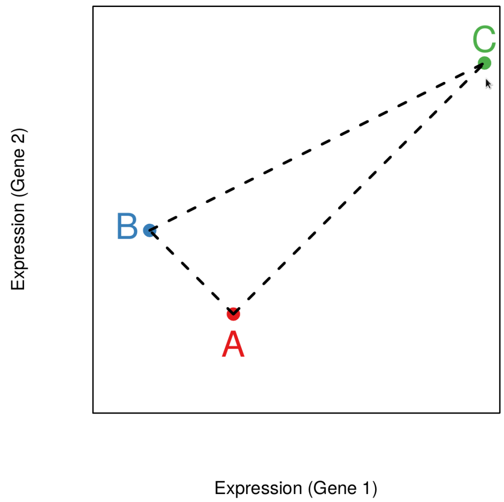
Lengths of these vectors

```
length(x)

## [1] 7129

length(y)

## [1] 7129
```

Distance between these two vectors

```
sqrt(sum((x - y)^2))

## [1] 101530.8
```

Relative Distance (From CST 2011 Paper)



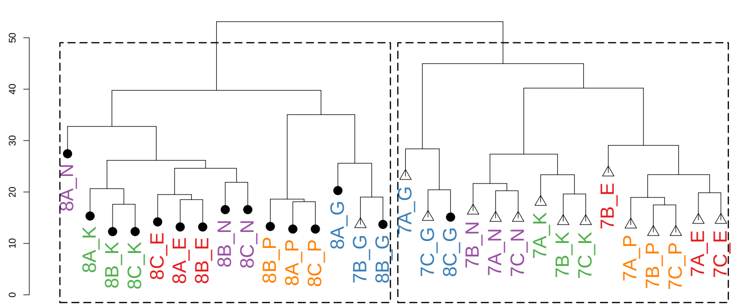
Dissimilarity matrix

- Use pairwise distances to quantify similarity (or dissimilarity) among patients
- Construct a matrix containing all pairwise distances
- Take the first three patients in the Golub data set

```
dist(t(exprs(Golub_Merge[, 1:3])))

##           39        40
## 40 101530.75
## 42  94405.04  89502.29
```

- Patient 42 is more similar (closer) to patient 39 than patient 40 (distance of 94405.04 vs 101530.75)
- Patient 39 is more similar (closer) to 42 than patient 40 (distance of 94405.04 vs 101530.75)

2015 Data: Agglomerative Hierarchical Clustering



Clusters

- Let $c_1, c_2, \ldots, c_n$ denote the n samples
- Define a cluster to be a set of patients
  - (c_1) is a cluster with one member: c_1
  - (c_1, c_3) is a cluster of two members: c_1 and c_3
  - (c_1, c_2, c_3) is a cluster of three members of c_1, c_2 and c_3
- Note that c_1 and (c_1) are different entities

Notion of a Linkage

- The distance measure quantified the distance between two points
- In clustering, you need to think about the criterion to link (merge) the clusters
- maximum distance (aka complete linkage)
- average distance (aka average linkage)
- minimum distance (aka single linkage)

Agglomerative Hierarchical Clustering

- Agglomerate: To form clusters
- Let each of the n points be its own cluster (n clusters each with one single member)
- Find the pair of clusters that is most similar
- Merge these two
- Now you have $n-1$ clusters (1 cluster with two members and $n-2$ clusters each with a single member)
- Compute the similarities between the $n-2$ "old" clusters with the new cluster
- Repeat the last two steps until all members have been merged into a single cluster.

Clustering Cities by Distances

	ATL	BOS	ORD	DCA
ATL	0	934	585	542
BOS	934	0	853	392
ORD	585	853	0	598
DCA	542	392	598	0

Clustering Cities by Distances (Single Linkage)

	ATL	BOS	ORD	DCA
ATL	0	934	585	542
BOS	934	0	853	392
ORD	585	853	0	598
DCA	542	392	598	0

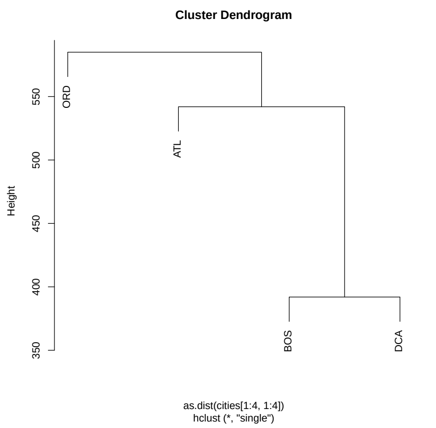
	DCA-BOS	ATL	ORD
DCA-BOS	0	542	598
ATL	542	0	585
ORD	598	585	0

Clustering Cities by Distances (Single Linkage)

	DCA-BOS	ATL	ORD
DCA-BOS	0	542	598
ATL	542	0	585
ORD	598	585	0

	DCA-BOS-ATL	ORD
DCA-BOS-ATL	0	585
ORD	585	0

Four Airports (Single linkage)



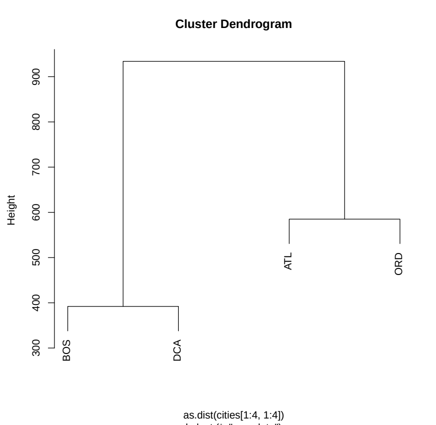
Clustering Cities by Distances (complete linkage)

	ATL	BOS	ORD	DCA
ATL	0	934	585	542
BOS	934	0	853	392
ORD	585	853	0	598
DCA	542	392	598	0

	DCA-BOS	ATL	ORD
DCA-BOS	0	934	853
ATL	934	0	585
ORD	853	585	0

	DCA-BOS	ATL-ORD
DCA-BOS	0	934
ATL-ORD	934	0

Four Airports (complete linkage)



Four Airports (side by side)

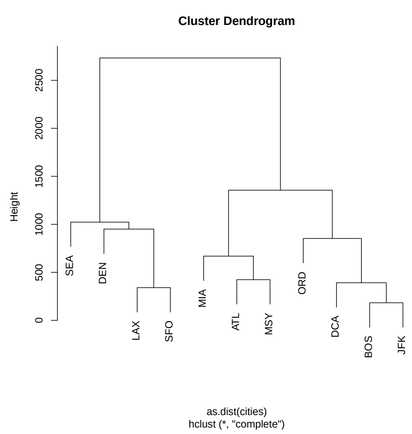
	ATL	BOS	ORD	DCA
ATL	0	934	585	542
BOS	934	0	853	392
ORD	585	853	0	598
DCA	542	392	598	0
	DCA-BOS	ATL	ORD	
DCA-BOS	0	934	853	
ATL	934	0	585	
ORD	853	585	0	
	DCA-BOS	ATL-ORD		
DCA-BOS	0	934		
ATL-ORD	934	0		

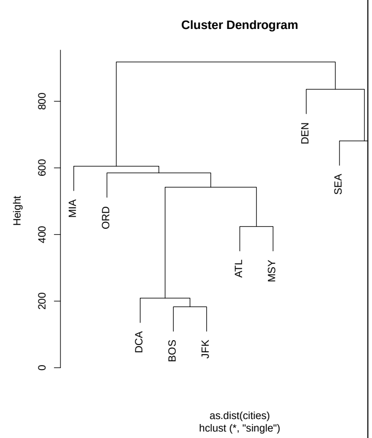
Table: Complete Linkage

	ATL	BOS	ORD	DCA
ATL	0	934	585	542
BOS	934	0	853	392
ORD	585	853	0	598
DCA	542	392	598	0
	DCA-BOS	ATL	ORD	
DCA-BOS	0	542	598	
ATL	542	0	585	
ORD	598	585	0	
	DCA-BOS-ATL	ORD		
DCA-BOS-ATL	0	585		
ORD	585	0		

Table: Single Linkage

All Airports (comparison)



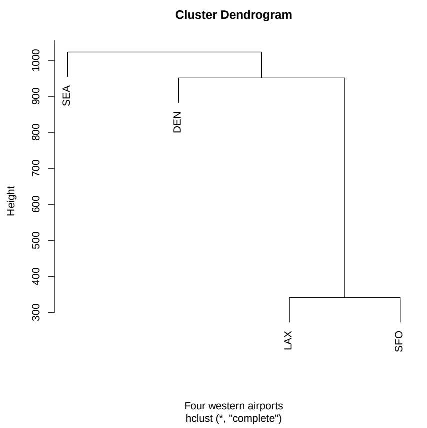


Western Airports: Exercise

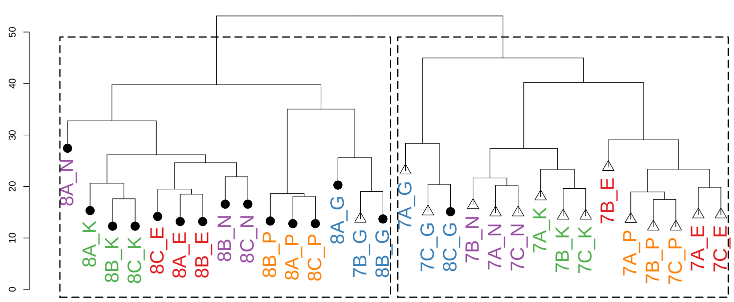
Carry out hierarchical clustering with complete linkage

```
##      DEN LAX  SEA SFO
## DEN    0 836 1023 951
## LAX  836   0  957 341
## SEA 1023 957    0 681
## SFO  951 341  681   0
```

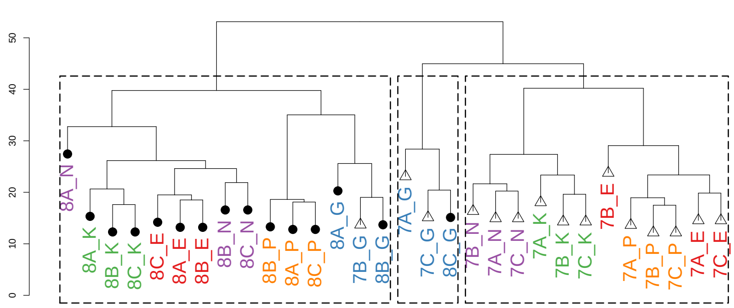
Western Airports: Solution



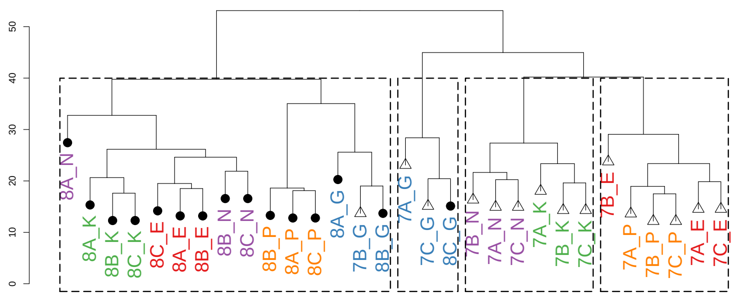
2015 Data: Agglomerative Hierarchical Clustering Complete Linkage



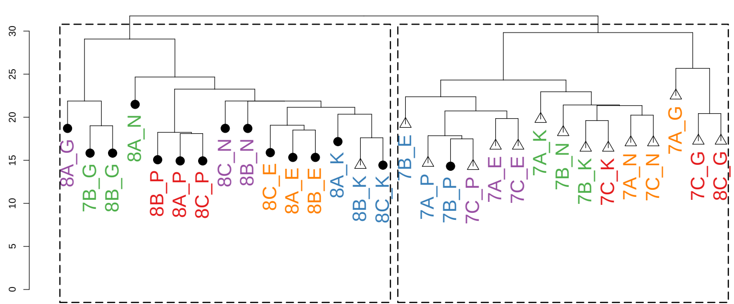
2015 Data: Agglomerative Hierarchical Clustering Complete Linkage



2015 Data: Agglomerative Hierarchical Clustering Complete Linkage



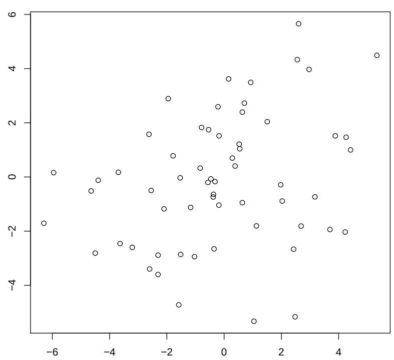
2015 Data: Agglomerative Hierarchical Clustering Single Linkage



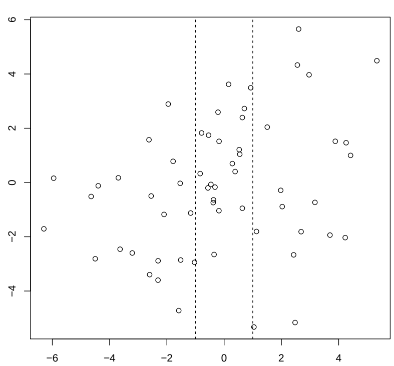
k-means Clustering

- Specify a number of potential clusters (k)
- Split of the data (either randomly or based on some previous results) into k partitions
- Compute the mean (aka centroid) for each partition
- For the first point (sample) determine the *nearest* centroid
- The closeness is typically quantified using the Euclidean distance
- Assign that point to that center
- Repeat for points 2 through n
- Assess the fit using the intra-cluster variance
- Repeat as needed.

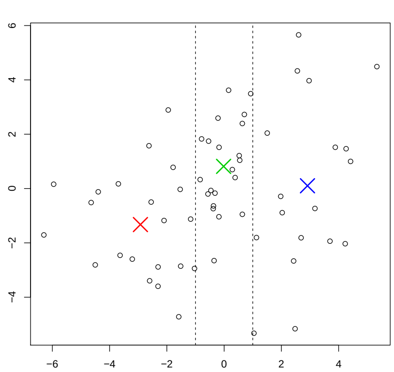
k-means clustering: Data



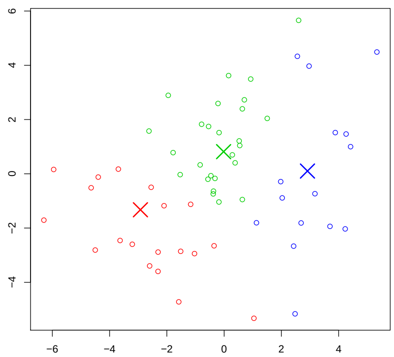
k-means clustering: Initial Clusters



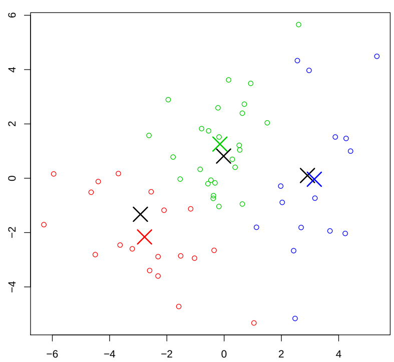
k-means clustering: Initial Centers



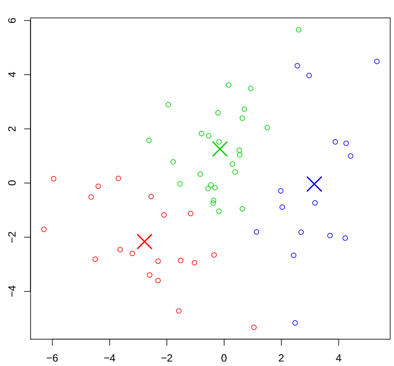
k-means clustering: Label points according to centers



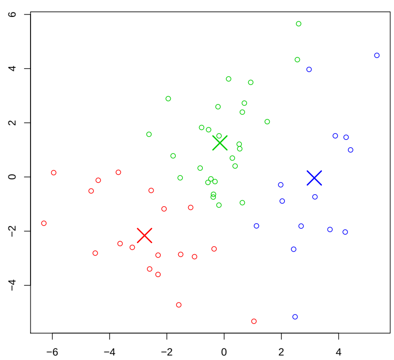
k-means clustering: Update Centers



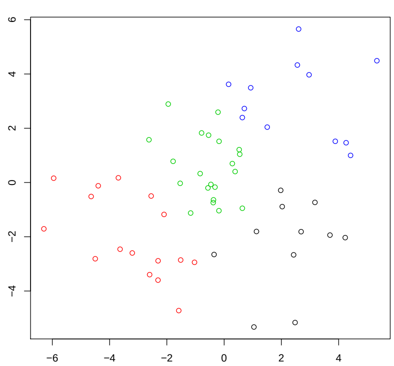
k-means clustering: Update Centers



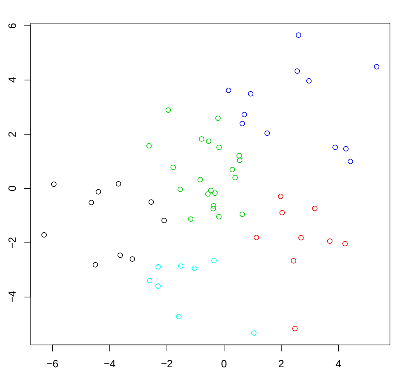
k-means clustering: Update Points



Why not 4 clusters?



Why not 5 clusters?



k-means

- This is an example of *non-hierarchical* clustering
- Need to specify the number of clusters up front
- Need to specify (deterministically or randomly) the centers of the clusters up front
- Results are sensitive to the choice of k and initial partitions
- Note: All the data points were simulated from a single cluster!

Dimension reduction

- Genome-wide profiling platforms are high-dimensional (m is large)
- Visualization beyond $m = 3$ not possible (for mortals)
- Representing the data by a lower dimensional format without losing too much information is desired.
- Two guiding principles:
  - Keep variables with highest variability
  - Reduce redundancy

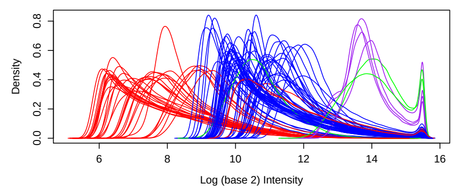
Multi-Dimensional Scaling (MDS)

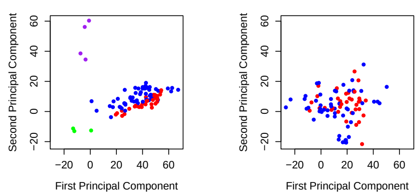
- Compute the dissimilarity matrix based on a distance measure
- Project the points into a lower dimensional space (say 2D or 3D) while preserving the similarity matrix
- PCA is a related (and in a sense equivalent method to MDS)
- Project the points into a lower dimensional space where the new variables are linear combinations of the original variables
- The new variables are chosen so as to have maximum variance and to be uncorrelated.

Batch Effect Discovery

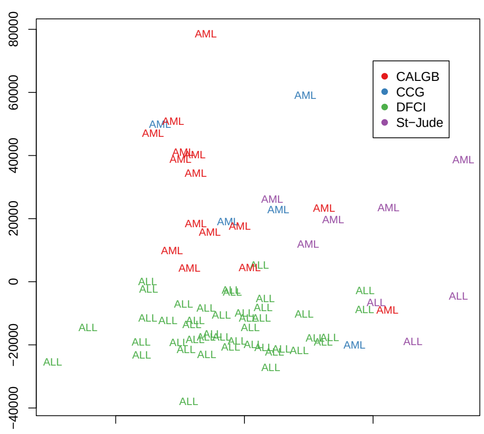
- The MDS method is very useful for detecting batch effects
- Batch effects tend to be stronger that biological effects
- They also affect most probe sets (the biological effect may only be captured by a few)
- This can be an effective weapon in your QC arsenal (this is how I start any new analysis)

From CCR 2008 Paper





ALL/AML Data



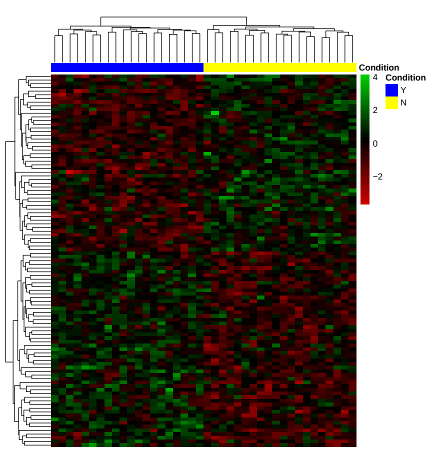
Semi-supervised Learning

- Heatmap illustration:
  - Select a panel of probe-sets based on the two-sample t-test
  - Carry out hierarchical clustering with respect to the patients (the columns)
  - Carry out hierarchical clustering with respect to the probe sets in the panel (the rows)
  - Present the results using a heatmap
- Some consider this an *unsupervised* analysis as the hierarchical clustering algorithm is unaware of the classes
- This is not an accurate assessment: It is semi-supervised in the sense that we are picking genes based on the phenotype
- A procedure is *unsupervised* if the class info is only used for annotation

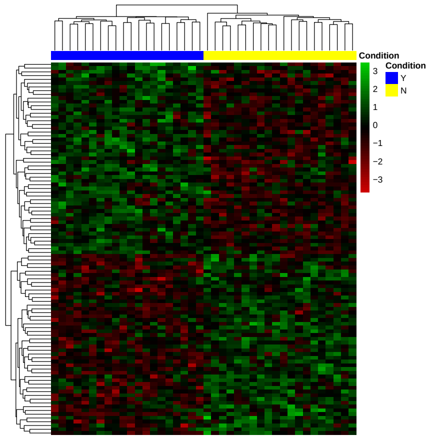
R Code to simulate Heatmap

```r
simulate.noise.heatmap = function(n, m, alpha) {
    # Simulate Expression Matrix
    EXPRS = matrix(rnorm(2 * n * m), m, 2 * n)
    grp = factor(rep(0:1, c(n, n)))
    rownames(EXPRS) = paste("Gene", 1:m, sep = "")
    colnames(EXPRS) = paste("patient id", 1:(2 * n), sep = "")

    # Get the two sample t-statistics
    pvals = rowttests(EXPRS, grp)$p.value
    topgenes = which(pvals < alpha)
    EXPRS = EXPRS[topgenes, ]
    annodat = data.frame(Condition = ifelse(grp == 0, "N", "Y"), row.names = colnames(EXPRS))
    pheatmap(EXPRS, border_color = NA, show_rownames = FALSE, show_colnames = FALSE,
        annotation_col = annodat, color = colorRampPalette(c("red3", "black",
            "green3"))(50), annotation_colors = list(Condition = c(Y = "blue",
            N = "yellow")))
    return(length(topgenes))
}
```

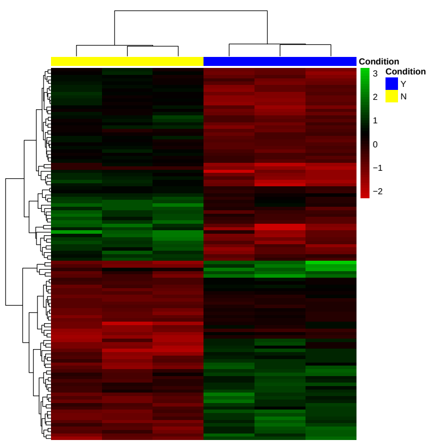
Heatmap Example: $m = 20,000, n = 20,\ \alpha = 0.005$



Heatmap Example: $m = 40,000, n = 20,\ \alpha = 0.0025$



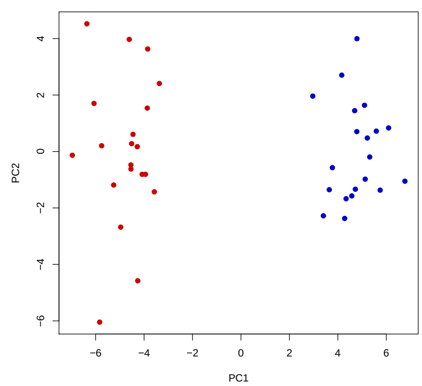
Heatmap Example: $m = 20,000, n = 3,\ \alpha = 0.005$



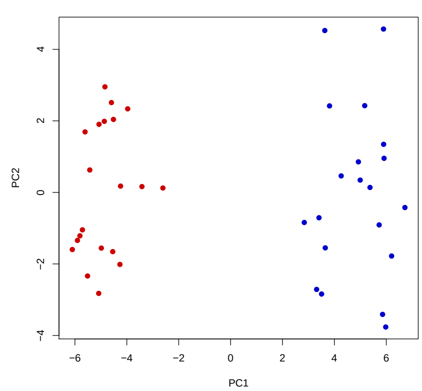
R Code to simulate PC

```
simulate.noise.PC = function(n, m, alpha) {
    # Simulate Expression Matrix
    EXPRS = matrix(rnorm(2 * n * m), m, 2 * n)
    grp = factor(rep(0:1, c(n, n)))
    # Get the two sample t-statistics
    pvals = rowttests(EXPRS, grp)$p.value
    topgenes = which(pvals < alpha)
    EXPRS = EXPRS[topgenes, ]
    annodat = data.frame(Condition = ifelse(grp == 0, "N", "Y"), row.names = colnames(EXPRS))
    PC = cmdscale(dist(t(EXPRS)))
    plot(PC, xlab = "PC1", ylab = "PC2", col = ifelse(grp == 0, "red3", "blue3"),
        pch = 19)
    return(length(topgenes))
}
```

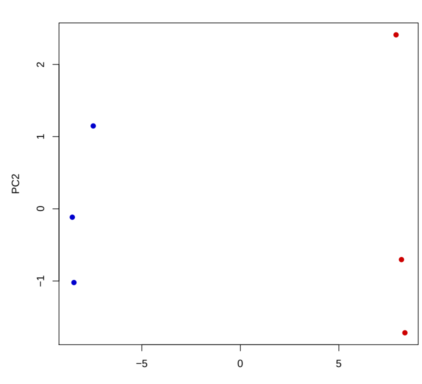
Heatmap Example: $K = 20000, n = 20, \ \alpha = 0.005$



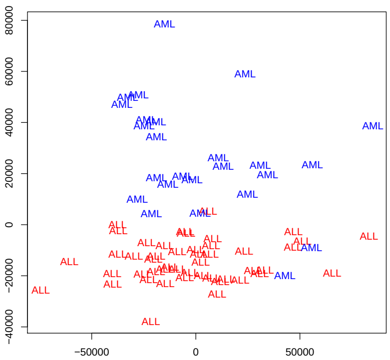
Heatmap Example: $K = 40000, n = 20, \ \alpha = 0.0025$



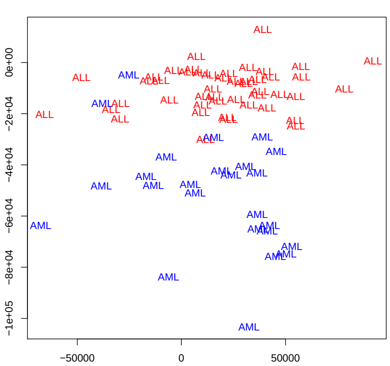
Heatmap Example: $K = 20000, n = 3, \ \alpha = 0.005$



MDS for Golub Data



PCA for Golub Data



Preserving The Distances

- Extract and standardize expression matrix for Golub data set

```
scexpdat = scale(t(exprs(Golub_Merge)))
dim(scexpdat)

## [1]   72 7129
```

- Check means for the first 4 genes

```
apply(scexpdat[, 1:4], 2, mean)

## AFFX-BioB-5_at AFFX-BioB-M_at AFFX-BioB-3_at AFFX-BioC-5_at
##  -7.841417e-17  -4.460287e-18   1.491832e-17  -5.051177e-17
```

- Check standard deviations for the first 4 genes

```
apply(scexpdat[, 1:4], 2, sd)

## AFFX-BioB-5_at AFFX-BioB-M_at AFFX-BioB-3_at AFFX-BioC-5_at
##              1              1              1              1
```

Preserving The Distances

- Check distance among the first three patients

```
dist(scexpdat[1:3, ])

##          39       40
## 40 125.3402
## 42 118.1911 125.0390
```

- Calculate MDS $d = 2$

```
MDS = cmdscale(dist(scexpdat), 2)
dist(MDS[1:3, ])

##           39        40
## 40  4.644939
## 42 29.665656 34.287630
```

- Calculate MDS $d = 3$

```
MDS = cmdscale(dist(scexpdat), 3)
dist(MDS[1:3, ])

##           39        40
## 40  9.293559
## 42 45.719192 54.869668
```

Preserving The Distances

- Check distance among the first three patients

```
dist(scexpdat[1:3, ])

##          39       40
## 40 125.3402
## 42 118.1911 125.0390
```

- Calculate MDS $d = 20$

```
MDS = cmdscale(dist(scexpdat), 3)
dist(MDS[1:3, ])

##           39        40
## 40  9.293559
## 42 45.719192 54.869668
```

- Calculate MDS $d = 45$

```
MDS = cmdscale(dist(scexpdat), 45)
dist(MDS[1:3, ])

##          39       40
## 40 124.9860
## 42 113.3668 121.7808
```

Reminder: A Self-fulfilling Prophecy

- Statistical method for unsupervised learning guarantee one thing
- They will return a clustering of your data
- What they do not guarantee and are invariably unable to verify, is the biological relevance or reproducibility of the clustering
- In light of this Self-fulfilling Prophecy, these methods should be used with utmost care