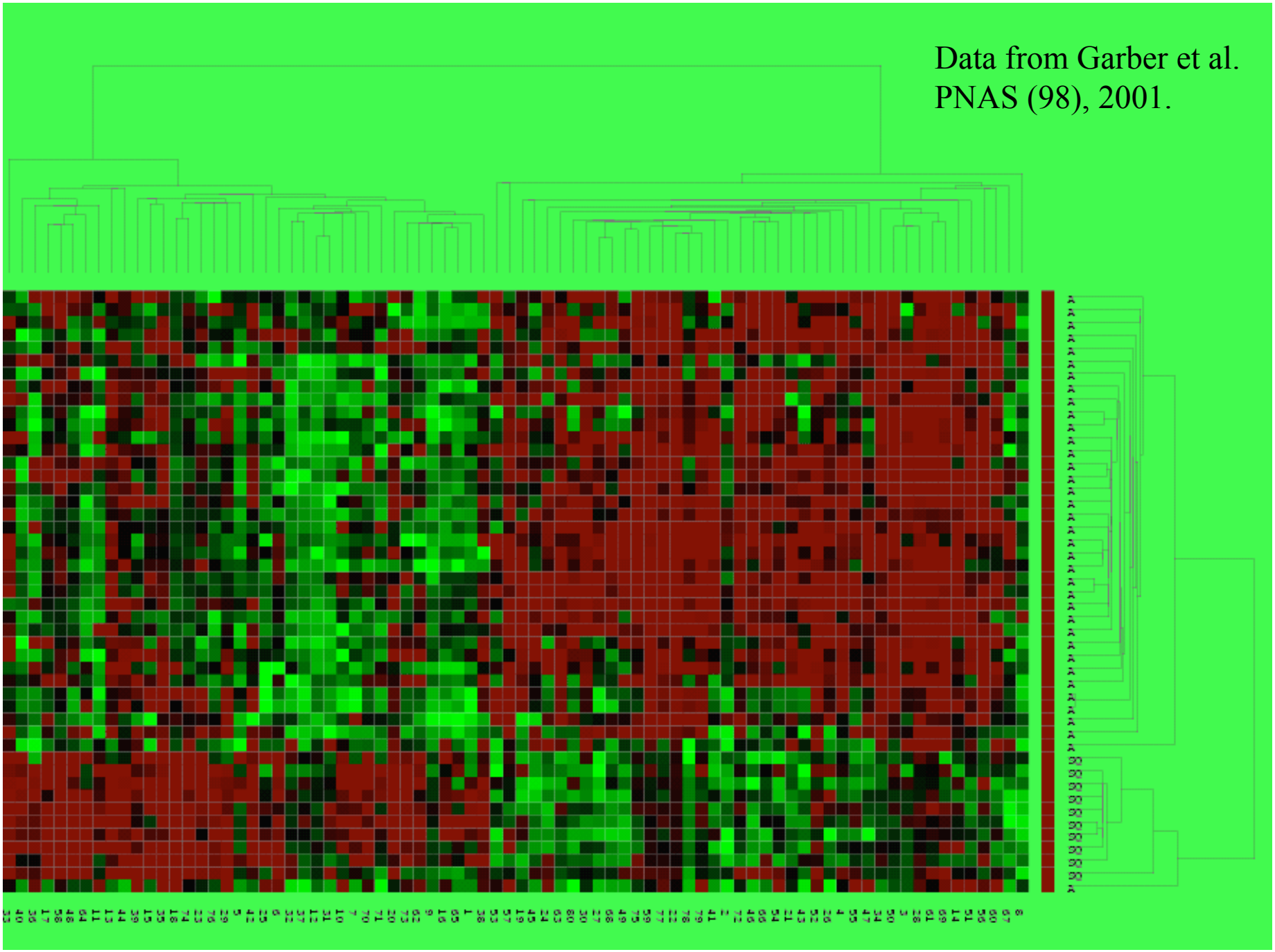
Clustering Gene Expression Data:
The Good, The Bad, and The Misinterpreted

Elizabeth Garrett-Mayer
April 19, 2004
Oncology Biostatistics
Johns Hopkins University
esg@jhu.edu

Acknowledgements: Giovanni Parmigiani, David Madigan, Kevin Coombs


Data from Garber et al.
PNAS (98), 2001.

Clustering

- Clustering is an exploratory tool for looking at associations within gene expression data
- It is good for visualization, hypothesis generation, selection of genes for further consideration
- **Clustering is not an inferential method** and includes no natural measure of "strength of evidence" or "strength of clustering structure"

More specifically….

- Cluster analysis arranges samples and genes into groups based on their expression levels.
- This arrangement is determined purely by the measured distance between samples and genes.
- Arrangements are sensitive to choice of distance
- In hierarchical clustering, the VISUALIZTION of the arrangement (the dendrogram) is not unique!

A Misconception

- Clustering is not a classification method
- Clustering is ‘unsupervised:’
  - We don’t use any information about what class the samples belong to (e.g. AML vs. ALL) (Golub et al.) to determine cluster structure
  - We don’t use any information about which genes are functionally or otherwise related to determine cluster structure
  - ***Clustering finds groups in the data***
- Classification methods are ‘supervised:’
  - By definition, we use phenotypic data to help us find out which genes best classify genes
  - LDA, KNN, CART, SVM
  - ***Classification methods finds ‘classifiers’***

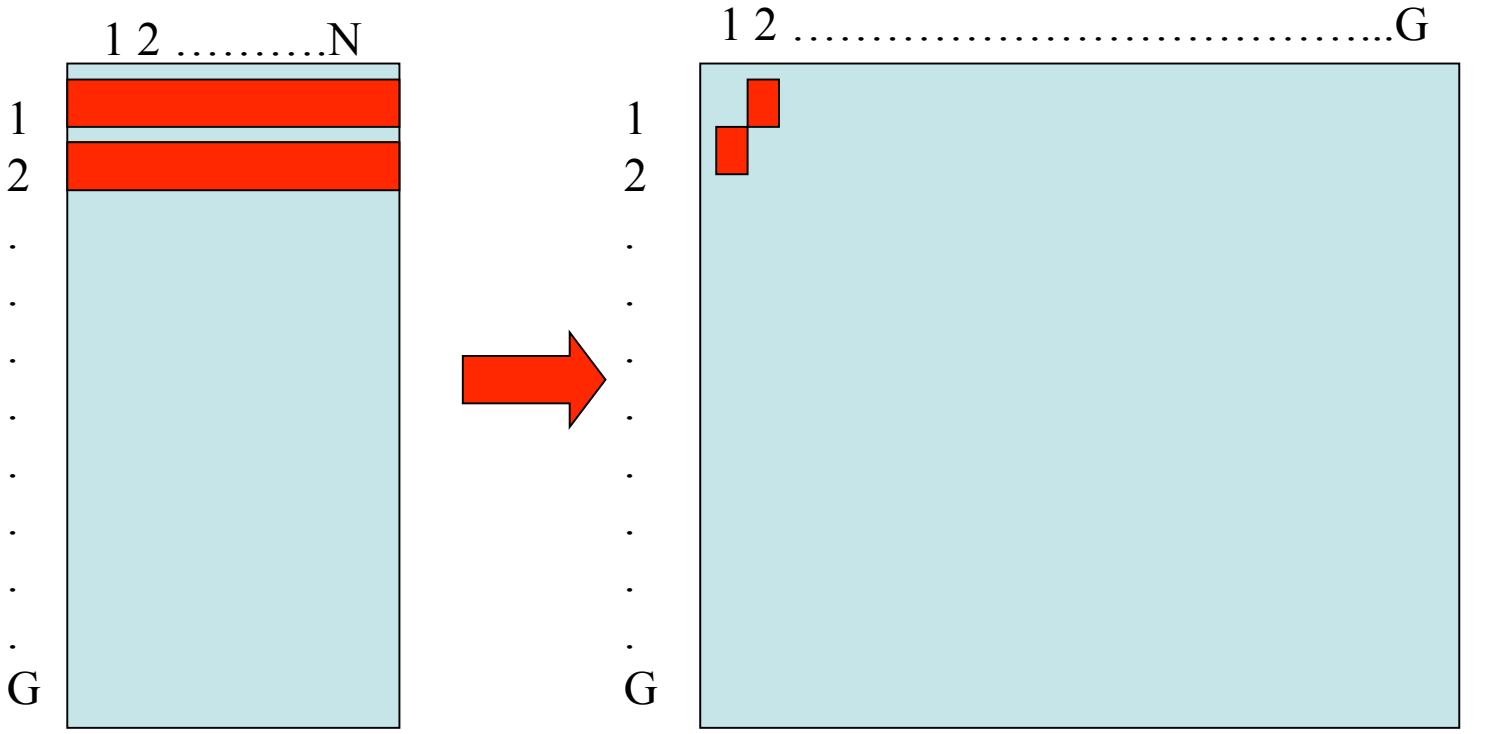
Distance and Similarity

- Every clustering method is based **solely** on the measure of distance or similarity.
- E.g. correlation: measures linear association between two samples or genes.
  - What if data are not properly transformed?
  - What if there are outliers?
  - What if there are saturation effects?
- Even with large number of samples, bad measure of distance or similarity will not be helped.

Commonly Used Measures of Similarity and Distance

- Euclidean distance
- Correlation (similarity)
  - Absolute value of correlation
  - Uncentered correlation
- Spearman correlation
- Categorical measures

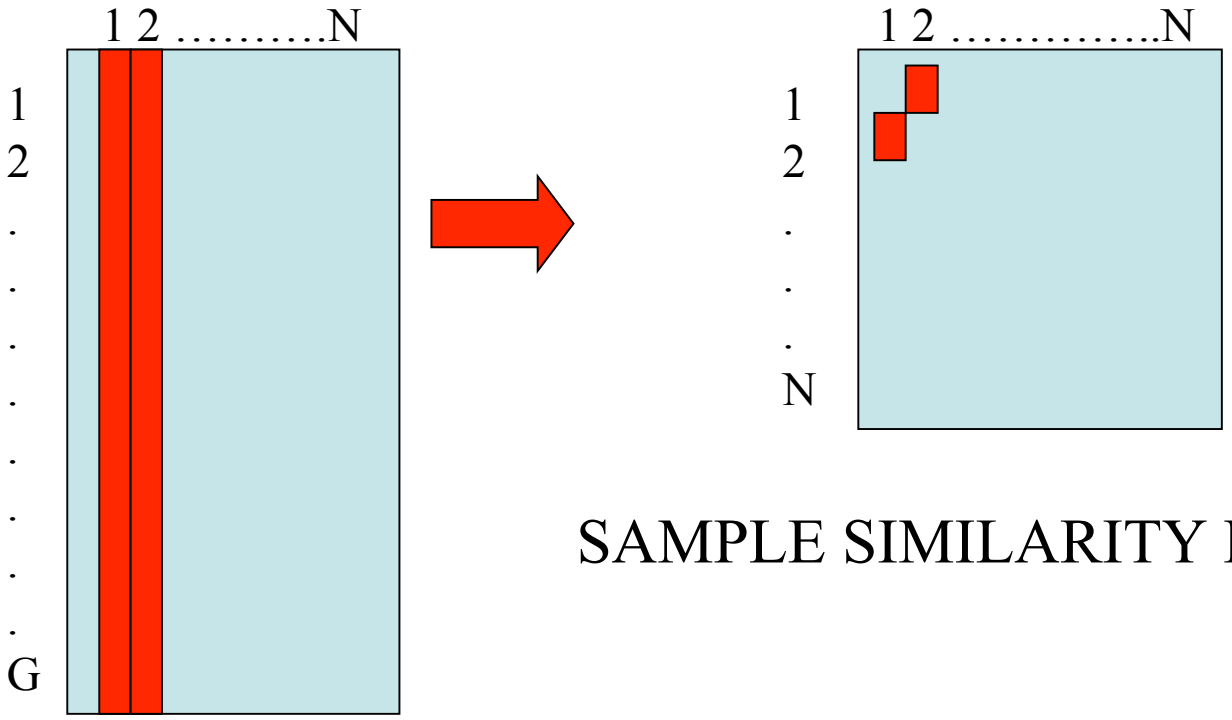
The similarity/distance matrices



DATA MATRIX

GENE SIMILARITY MATRIX

The similarity/distance matrices



SAMPLE SIMILARITY MATRIX

DATA MATRIX

Limitation of Clustering

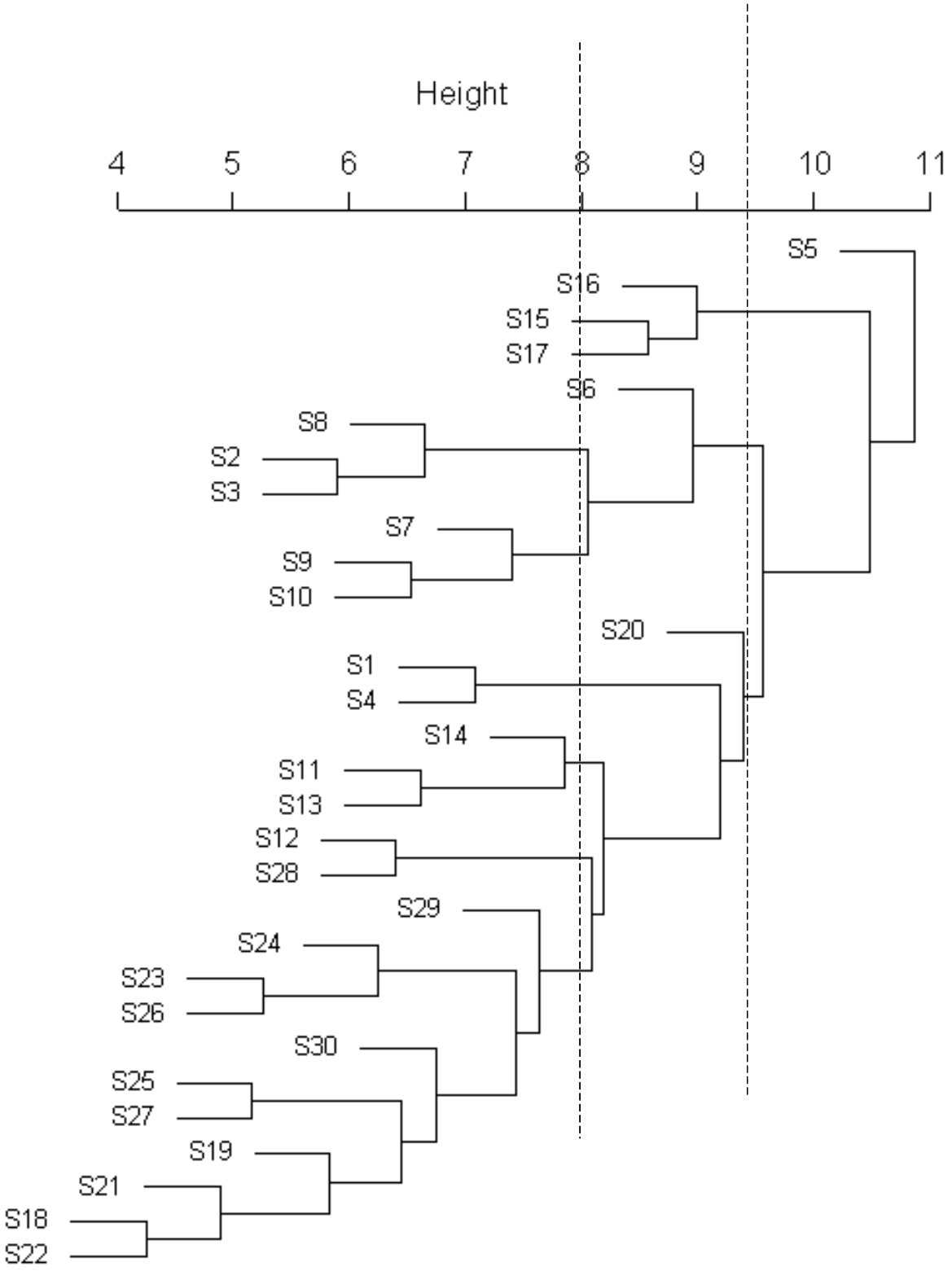
- The clustering structure can ONLY be as good as the distance/similarity matrix
- Generally, not enough thought and time is spent on choosing and estimating the distance/similarity matrix.
- “Garbage in ➔ Garbage out”

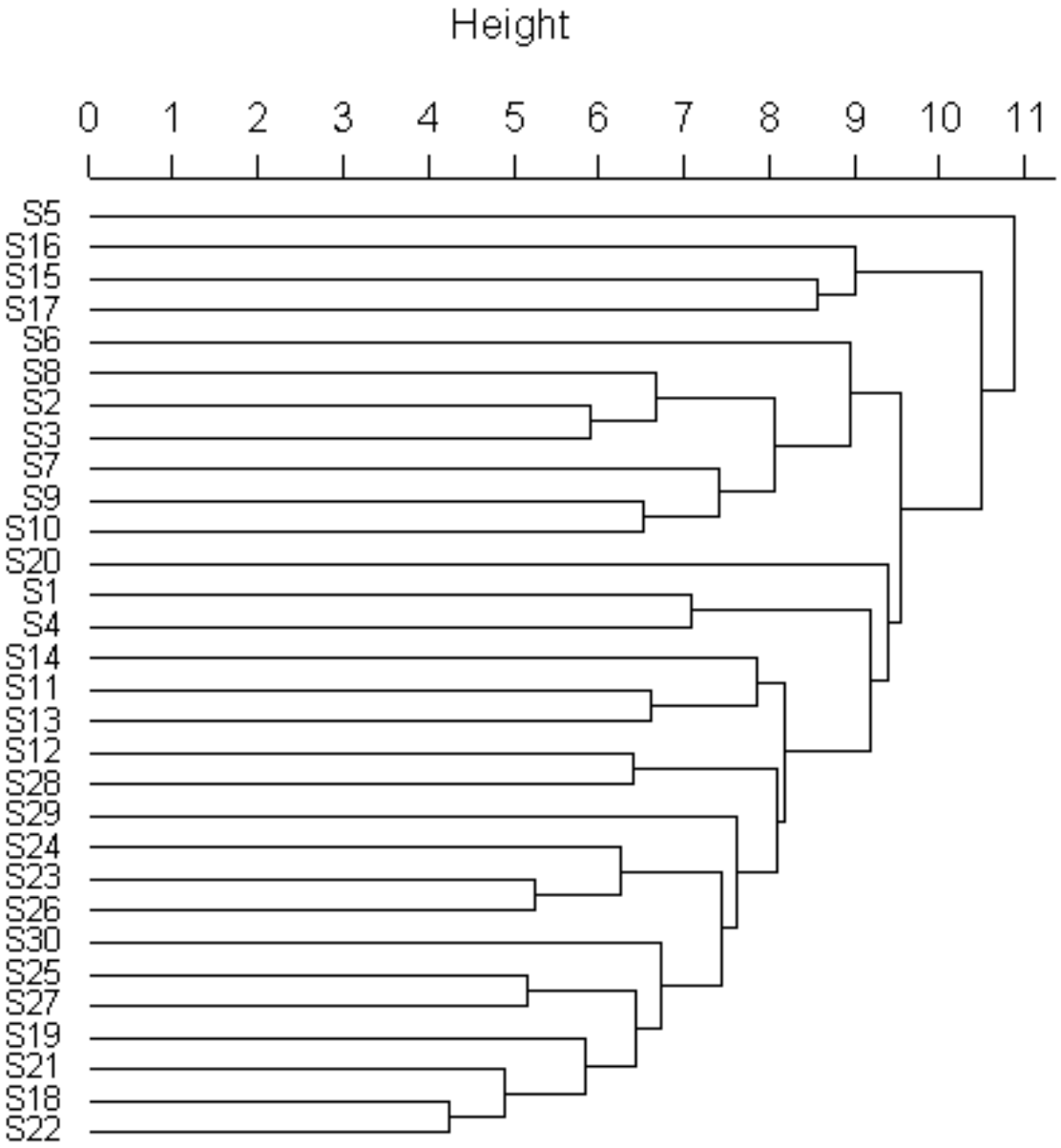
Two commonly seen clustering approaches in gene expression data analysis

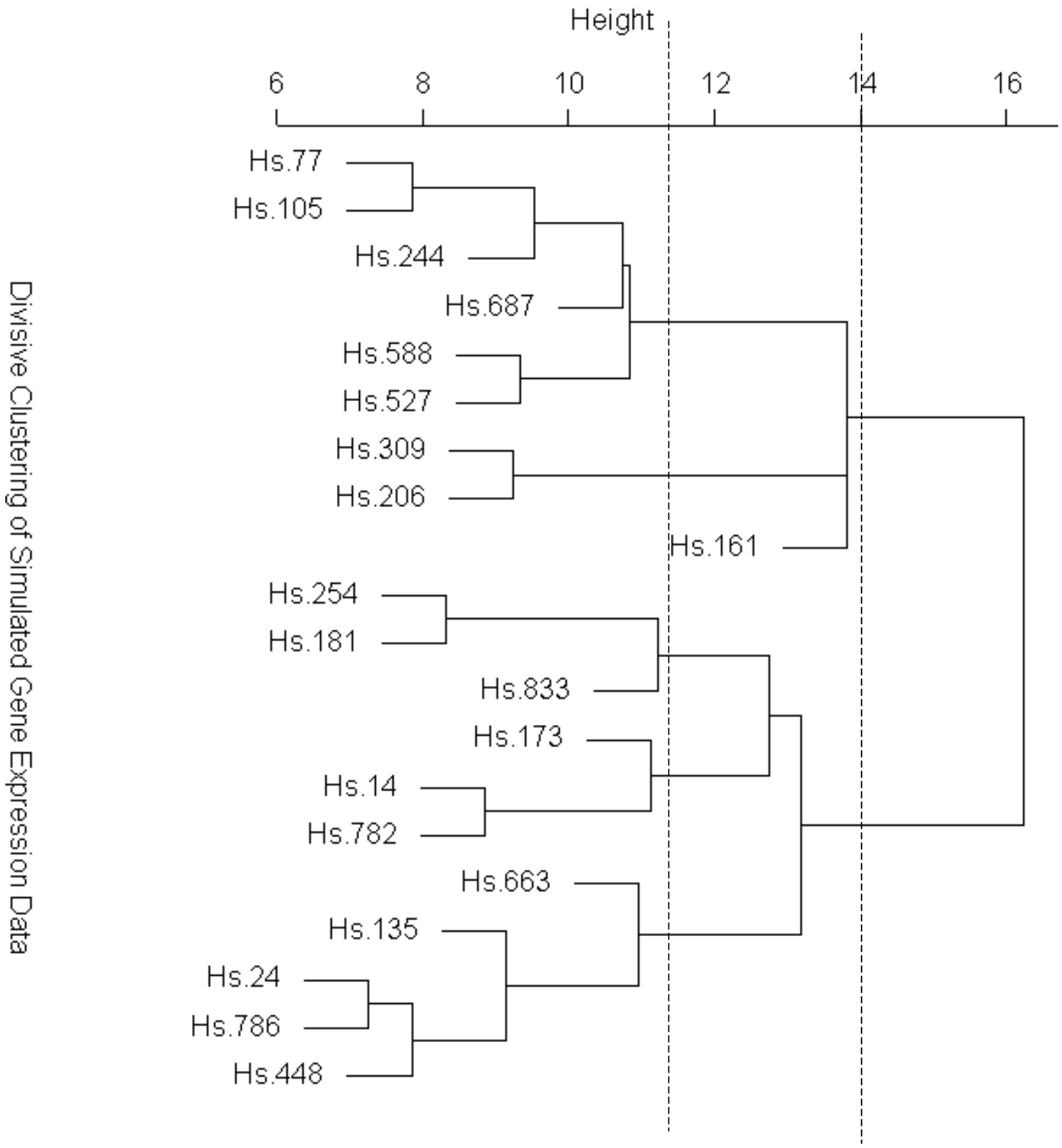
- Hierarchical clustering
  - Dendrogram (red-green picture)
  - Allows us to cluster both genes and samples in one picture and see whole dataset "organized"
- K-means/K-medoids
  - Partitioning method
  - Requires user to define K = # of clusters a priori
  - No picture to (over)interpret

Hierarchical Clustering

- **The most overused statistical method in gene expression analysis**
- Gives us pretty red-green picture with patterns
- But, pretty picture tends to be pretty unstable.
- Many different ways to perform hierarchical clustering
- Tend to be sensitive to small changes in the data
- Provided with clusters of every size: where to "cut" the dendrogram is user-determined


Height
4
5
6
7
8
9
10
11
S5
S16
S15
S17
S6
S8
S2
S3
S7
S9
S10
S20
S1
S4
S14
S11
S13
S12
S28
S29
S24
S23
S26
S30
S25
S27
S19
S21
S18
S22


Height
0
1
2
3
4
5
6
7
8
9
10
11
S5
S16
S15
S17
S6
S8
S2
S3
S7
S9
S10
S20
S1
S4
S14
S11
S13
S12
S28
S29
S24
S23
S26
S30
S25
S27
S19
S21
S18
S22


Height
6
8
10
12
14
16
Hs.77
Hs.105
Hs.244
Hs.687
Hs.588
Hs.527
Hs.309
Hs.206
Hs.161
Hs.254
Hs.181
Hs.833
Hs.173
Hs.14
Hs.782
Hs.663
Hs.135
Hs.24
Hs.786
Hs.448
Divisive Clustering of Simulated Gene Expression Data

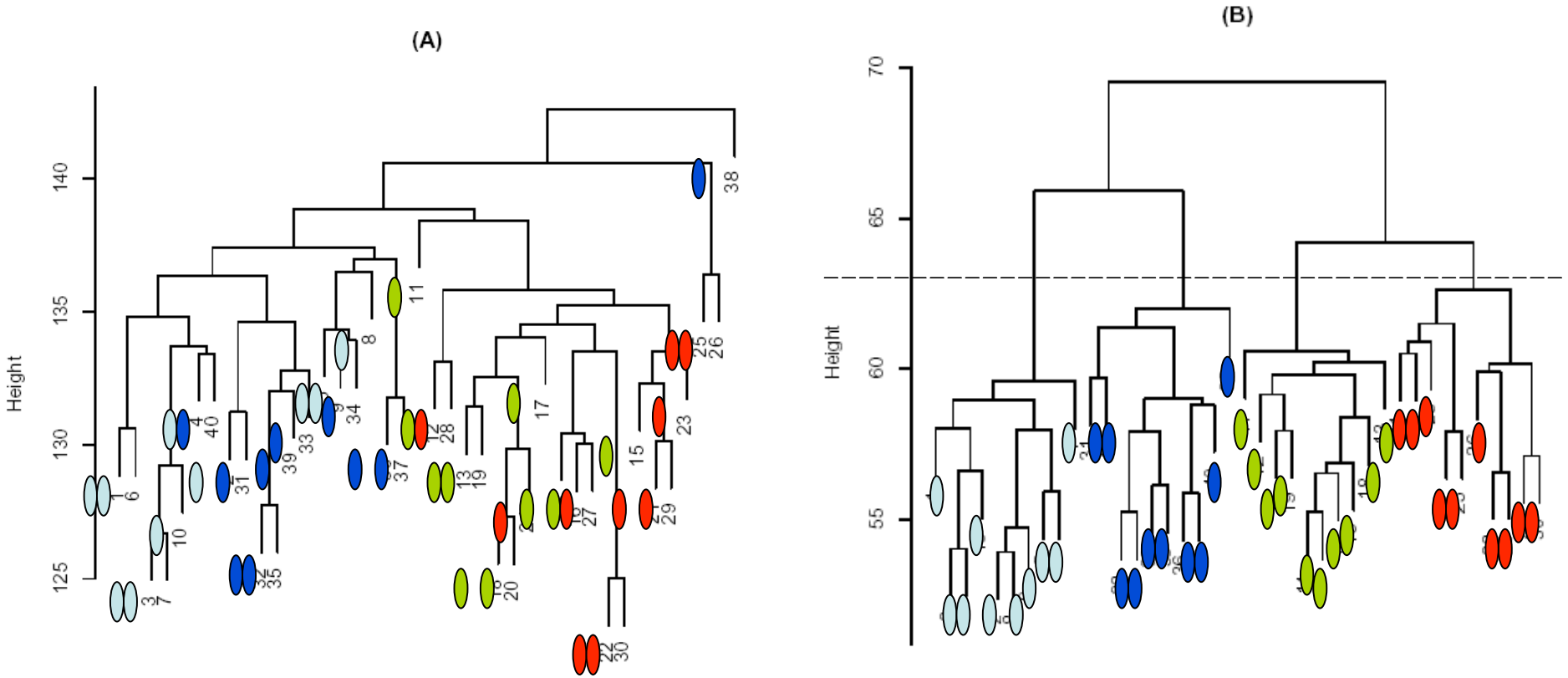
How to make a hierarchical clustering

1. Choose samples and genes to include in cluster analysis
2. Choose similarity/distance metric
3. Choose clustering direction (top-down or bottom-up)
4. Choose linkage method (if bottom-up)
5. Calculate dendrogram
6. Choose height/number of clusters for interpretation
7. Assess cluster fit and stability
8. Interpret resulting cluster structure

1. Choose samples and genes to include

- Important step!
- Do you want housekeeping genes included?
- What to do about replicates from the same individual/tumor?
- Genes that contribute noise will affect your results.
- Including all genes: dendrogram can’t all be seen at the same time.
- Perhaps screen the genes?

Simulated Data with 4 clusters: 1-10, 11-20, 21-30, 31-40



A: 450 relevant genes plus
450 “noise” genes.

B: 450 relevant genes.

2. Choose similarity/distance matrix

- Think hard about this step!
- Remember: garbage in → garbage out
- The metric that you pick should be a valid measure of the distance/similarity of genes.
- Examples:
  - Applying correlation to highly skewed data will provide misleading results.
  - Applying Euclidean distance to data measured on categorical scale will be invalid.
- Not just “wrong”, but which makes most sense

Some correlations to choose from

- Pearson Correlation:

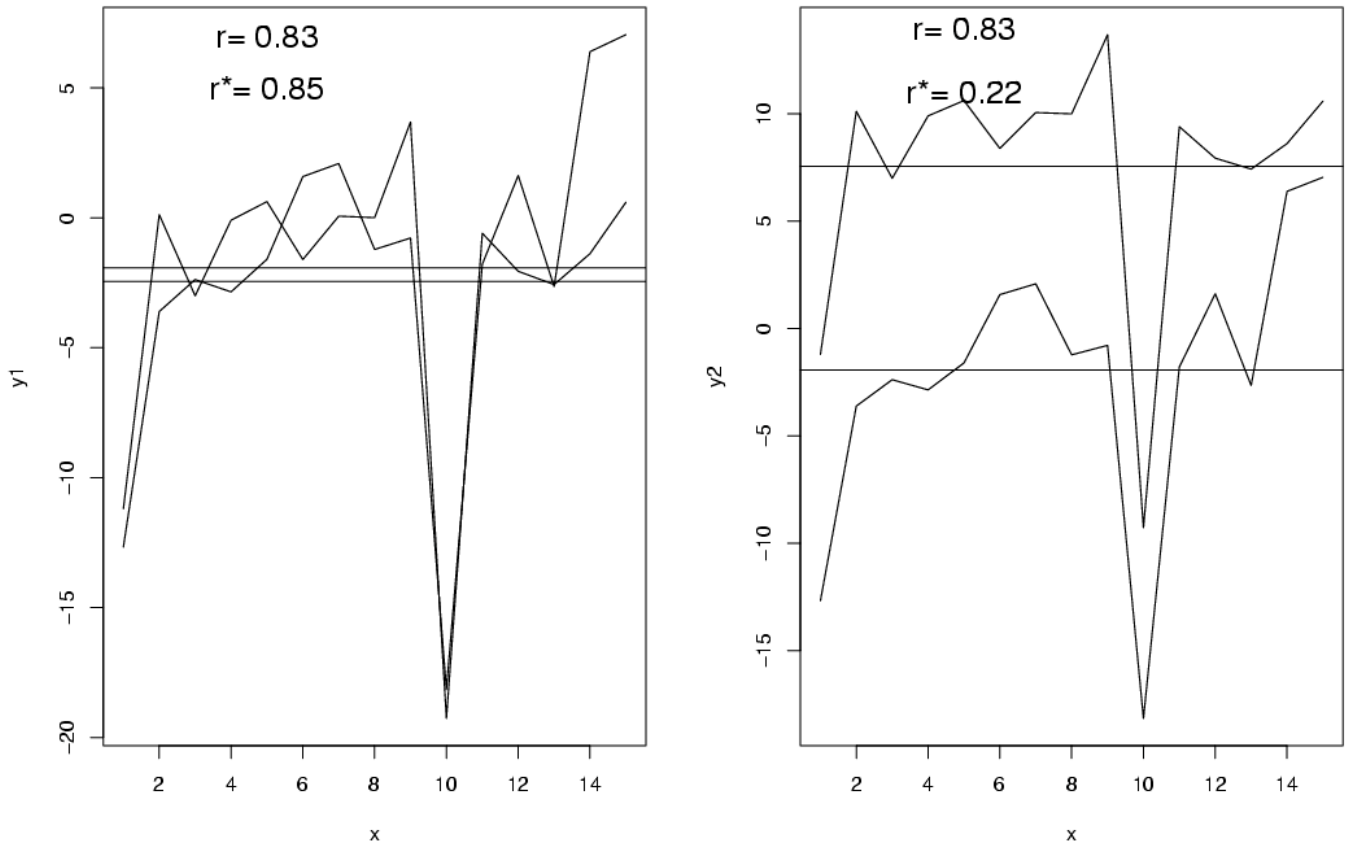
$$s(x_1, x_2) = \frac{\sum_{k=1}^{K} (x_{1k} - \bar{x}_1)(x_{2k} - \bar{x}_2)}{\sqrt{\sum_{k=1}^{K} (x_{1k} - \bar{x}_1)^2 \sum_{k=1}^{K} (x_{2k} - \bar{x}_2)^2}}$$

- Uncentered Correlation:

$$s(x_1, x_2) = \frac{\sum_{k=1}^{K} x_{1k} x_{2k}}{\sqrt{\sum_{k=1}^{K} x_{1k}^{\ 2} \sum_{k=1}^{K} x_{2k}^{\ 2}}}$$

- Absolute Value of Correlation:

$$s(x_1, x_2) = \left| \frac{\sum_{k=1}^{K} (x_{1k} - \bar{x}_1)(x_{2k} - \bar{x}_2)}{\sqrt{\sum_{k=1}^{K} (x_{1k} - \bar{x}_1)^2 \sum_{k=1}^{K} (x_{2k} - \bar{x}_2)^2}} \right|$$



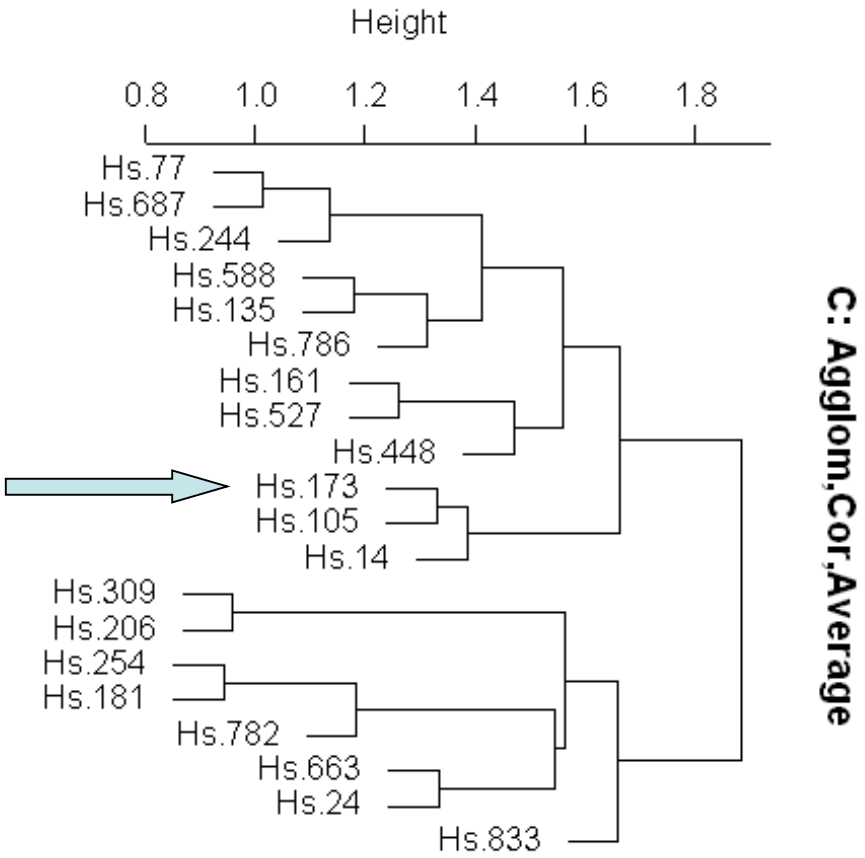
The difference is that, if you have two vectors X and Y with identical shape, but which are offset relative to each other by a fixed value, they will have a standard Pearson correlation (centered correlation) of 1 but will not have an uncentered correlation of 1.

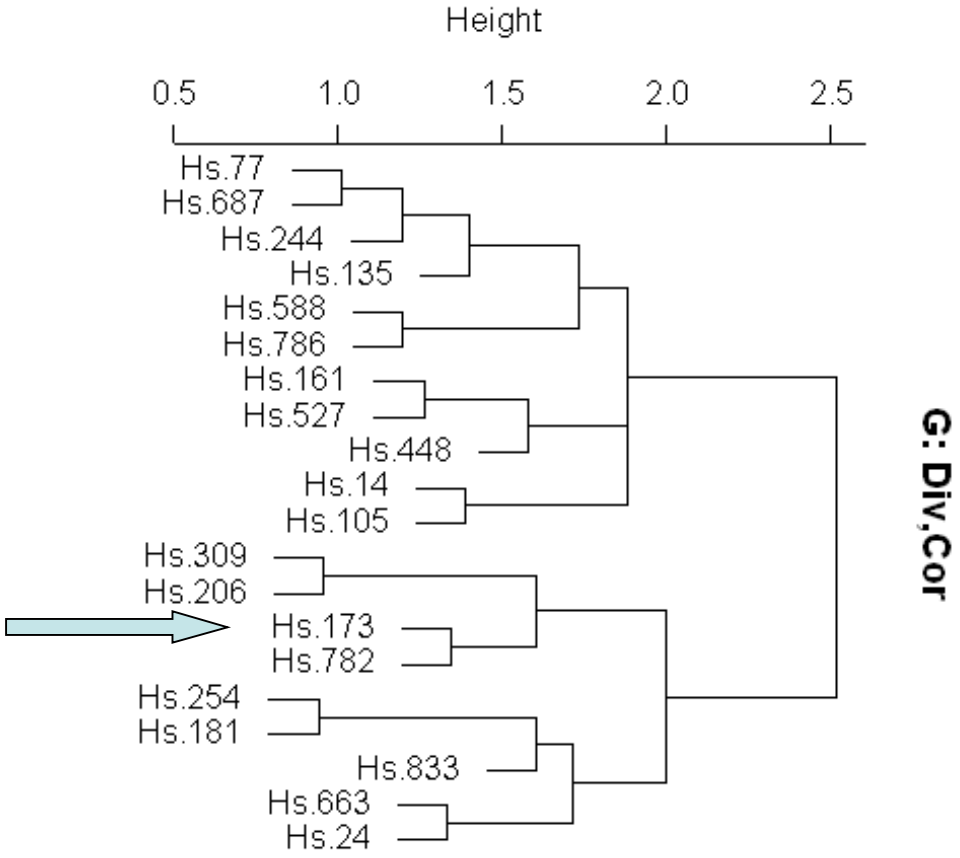
3. Choose clustering direction (top-down or bottom-up)

- Agglomerative clustering (bottom-up)
  - Starts with as each gene in its own cluster
  - Joins the two most similar clusters
  - Then, joins next two most similar clusters
  - Continues until all genes are in one cluster
- Divisive clustering (top-down)
  - Starts with all genes in one cluster
  - Choose split so that genes in the two clusters are most similar (maximize “distance” between clusters)
  - Find next split in same manner
  - Continue until all genes are in single gene clusters

Which to use?

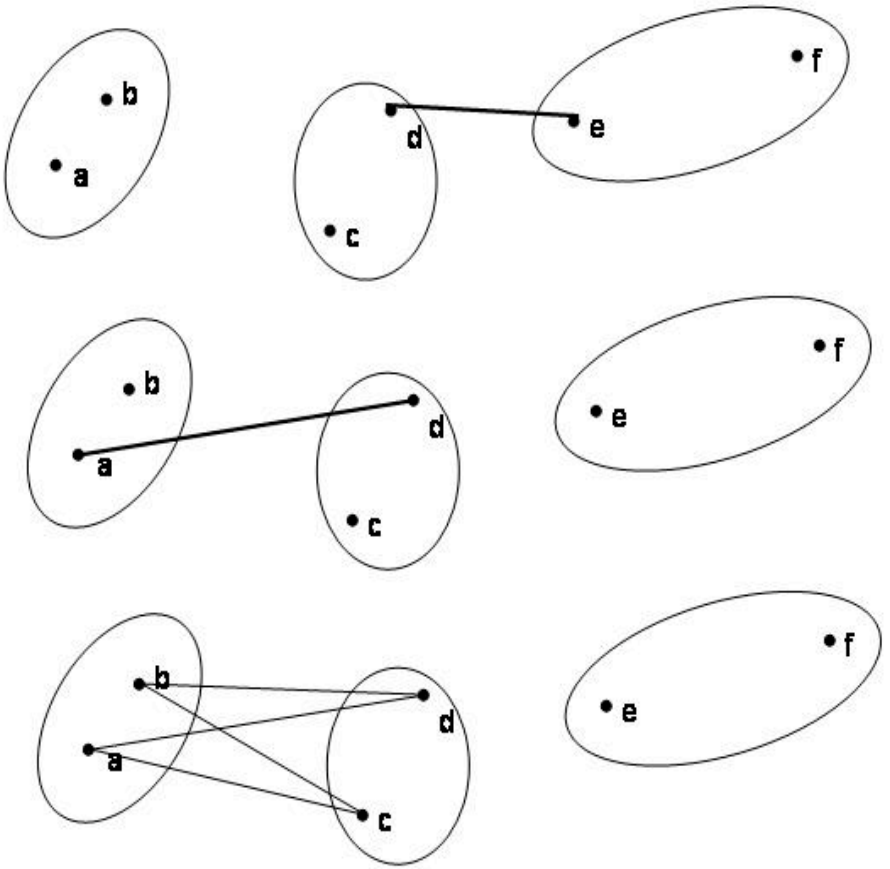
- Both are **only** 'step-wise' optimal: at each step the optimal split or merge is performed
- This does not imply that the final cluster structure is optimal!
- Agglomerative/Bottom-Up
  - Computationally simpler, and more available.
  - More "precision" at bottom of tree
  - When looking for small clusters and/or many clusters, use agglomerative
- Divisive/Top-Down
  - More "precision" at top of tree.
  - When looking for large and/or few clusters, use divisive
- **In gene expression applications, divisive makes more sense**.
- Results ARE sensitive to choice!


Height
0.8
1.0
1.2
1.4
1.6
1.8
Hs.77
Hs.687
Hs.244
Hs.588
Hs.135
Hs.786
Hs.161
Hs.527
Hs.448
Hs.173
Hs.105
Hs.14
Hs.309
Hs.206
Hs.254
Hs.181
Hs.782
Hs.663
Hs.24
Hs.833
C: Agglom,Cor,Average


Height
0.5
1.0
1.5
2.0
2.5
Hs.77
Hs.687
Hs.244
Hs.135
Hs.588
Hs.786
Hs.161
Hs.527
Hs.448
Hs.14
Hs.105
Hs.309
Hs.206
Hs.173
Hs.782
Hs.254
Hs.181
Hs.833
Hs.663
Hs.24
G: Div,Cor

4. Choose linkage method (if bottom-up)

- Single Linkage: join clusters whose distance between closest genes is smallest (elliptical)
- Complete Linkage: join clusters whose distance between furthest genes is smallest (spherical)
- Average Linkage: join clusters whose average distance is the smallest.



5. Calculate dendrogram
6. Choose height/number of clusters for interpretation

- In gene expression, we don't see "rule-based" approach to choosing cutoff very often.
- Tend to look for what makes a good story.
- There are more rigorous methods. (more later)
- "Homogeneity" and "Separation" of clusters can be considered. (Chen et al. Statistica Sinica, 2002)
- Other methods for assessing cluster fit can help determine a reasonable way to "cut" your tree.

7. Assess cluster fit and stability

- One approach is to try different approaches and see how tree differs.
  - Use average instead of complete linkage
  - Use divisive instead of agglomerative
  - Use Euclidean distance instead of correlation
- More later on assessing cluster structure

K-means and K-medoids

- Partitioning Method
- Don’t get pretty picture
- MUST choose number of clusters K a priori
- More of a “black box” because output is most commonly looked at purely as assignments
- Each object (gene or sample) gets assigned to a cluster
- Begin with initial partition
- Iterate so that objects within clusters are most similar

K-means (continued)

- Euclidean distance most often used
- Spherical clusters.
- Can be hard to choose or figure out K.
- Not unique solution: clustering can depend on initial partition
- No pretty figure to (over)interpret

How to make a K-means clustering

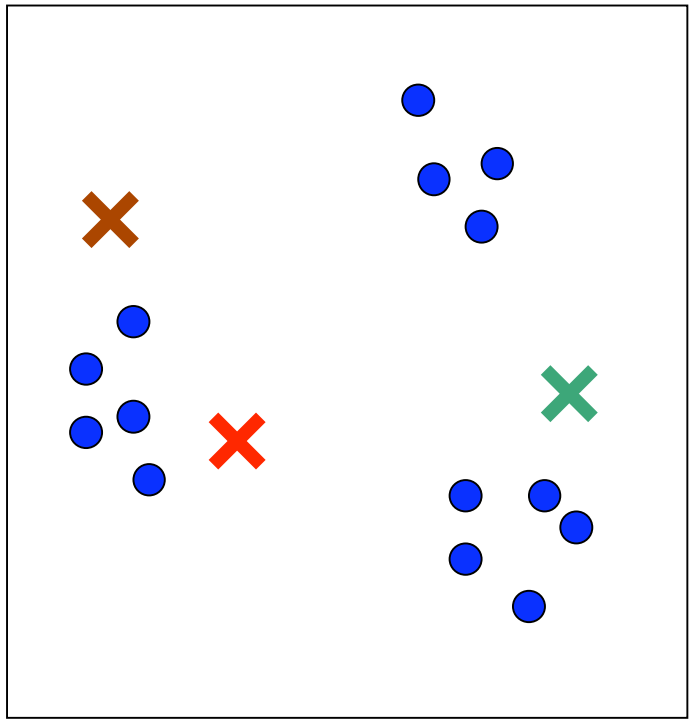
1. Choose samples and genes to include in cluster analysis
2. Choose similarity/distance metric (generally Euclidean)
3. Choose K.
4. Perform cluster analysis.
5. Assess cluster fit and stability
6. Interpret resulting cluster structure

K-means Algorithm

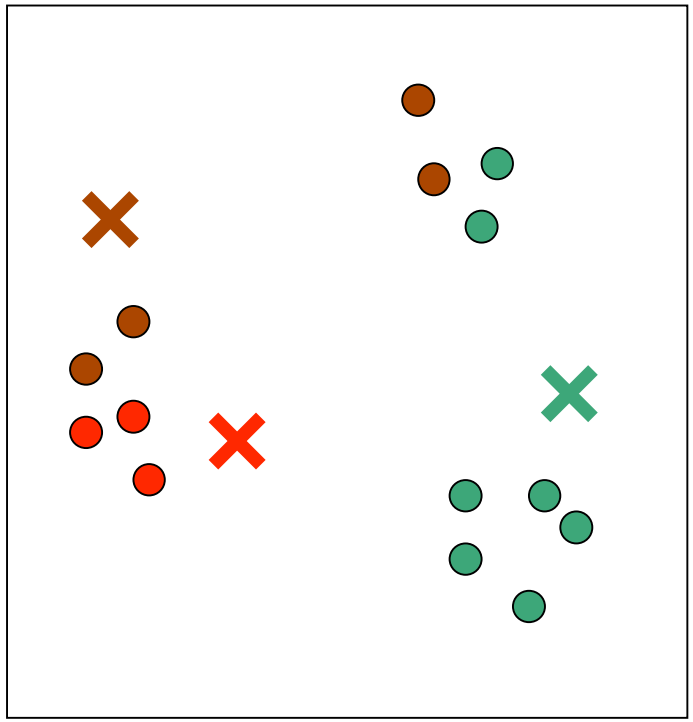
1. Choose K centroids at random
2. Make initial partition of objects into k clusters by assigning objects to closest centroid
3. Calculate the centroid (mean) of each of the k clusters.
4. a. For object i, calculate its distance to each of the centroids.

   b. Allocate object i to cluster with closest centroid.

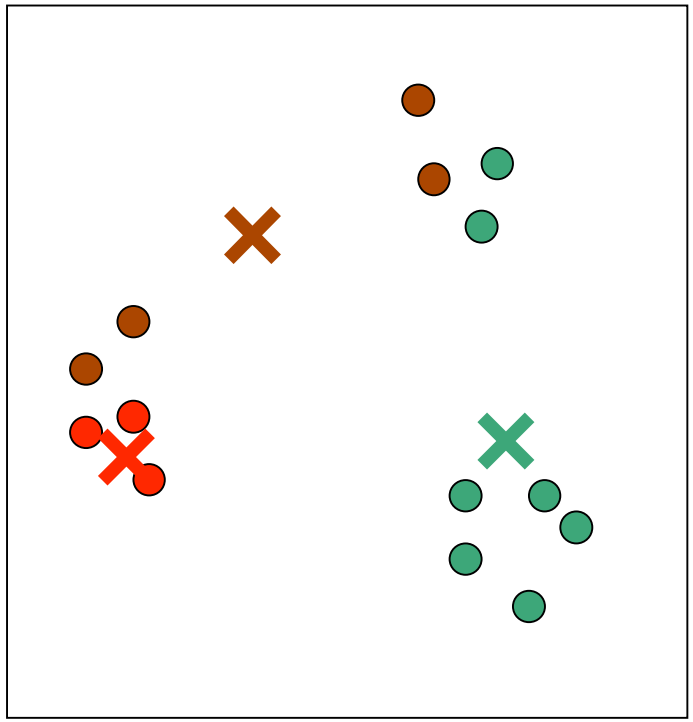
   c. If object was reallocated, recalculate centroids based on new clusters.
4. Repeat 3 for object i = 1,….N.
5. Repeat 3 and 4 until no reallocations occur.
6. Assess cluster structure for fit and stability



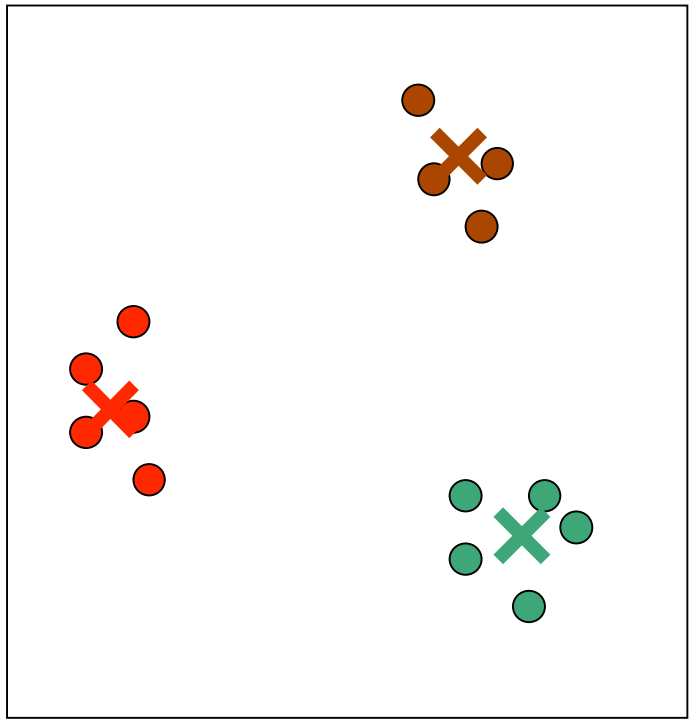
Iteration = 0



Iteration = 1



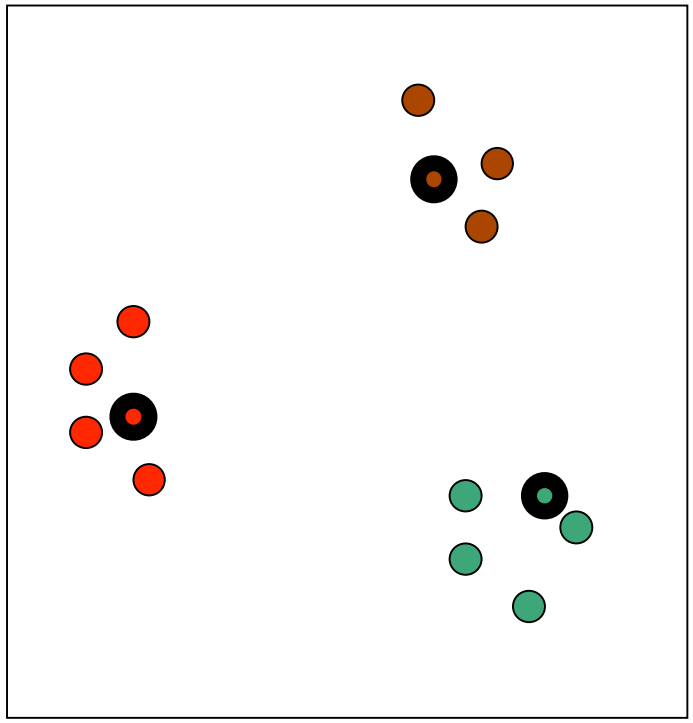
Iteration = 2



Iteration = 3

K-medoids

- A little different
- Centroid: The average of the samples within a cluster
- Medoid: The "representative object" within a cluster.
- Initializing requires choosing medoids at random.



7. Assess cluster fit and stability

- PART OF THE MISUNDERSTOOD!
- Most often ignored.
- Cluster structure is treated as reliable and precise
- BUT! Usually the structure is rather unstable, at least at the bottom.
- Can be VERY sensitive to noise and to outliers
- Homogeneity and Separation
- Cluster Silhouettes and Silhouette coefficient: how similar genes within a cluster are to genes in other clusters (composite separation and homogeneity) (more later with K-medoids) (Rousseeuw Journal of Computation and Applied Mathematics, 1987)

Assess cluster fit and stability (continued)

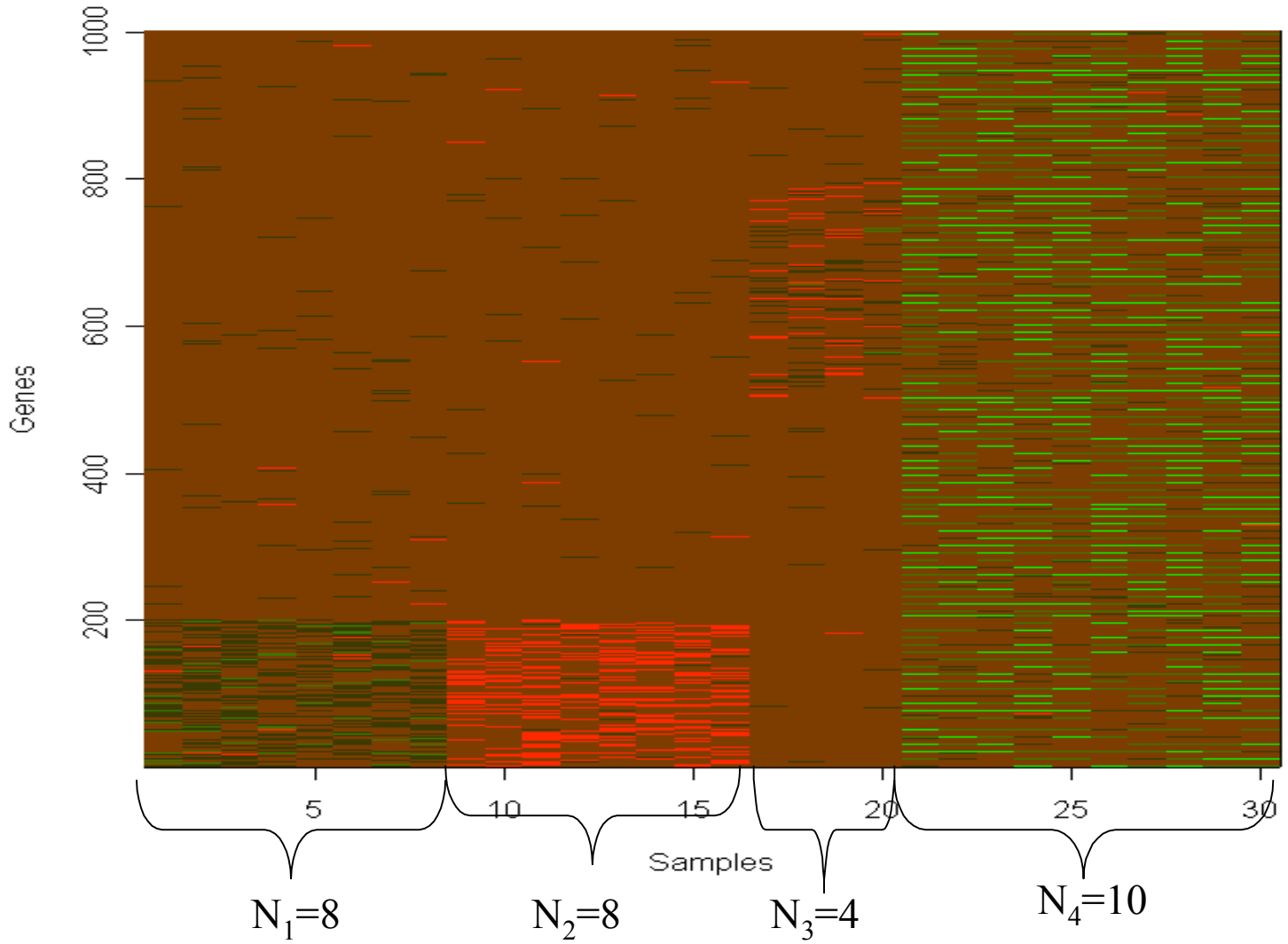
- WADP: Weighted Average Discrepant Pairs
  - Bittner et al. Nature, 2000
  - Fit cluster analysis using a dataset
  - Add random noise to the original dataset
  - Fit cluster analysis to the noise-added dataset
  - Repeat many times.
  - Compare the clusters across the noise-added datasets.
- Consensus Trees
  - Zhang and Zhao Functional and Integrative Genomics, 2000.
  - Use parametric bootstrap approach to sample new data using original dataset
  - Proceed similarly to WADP.
  - Look for nodes that are in a “majority” of the bootstrapped trees.
- More not mentioned…..

Careful though….

- Some validation approaches are more suited to some clustering approaches than others.
- Most of the methods require us to define number of clusters, even for hierarchical clustering.
  - Requires choosing a cut-point
  - If true structure is hierarchical, a cut tree won't appear as good as it might truly be.

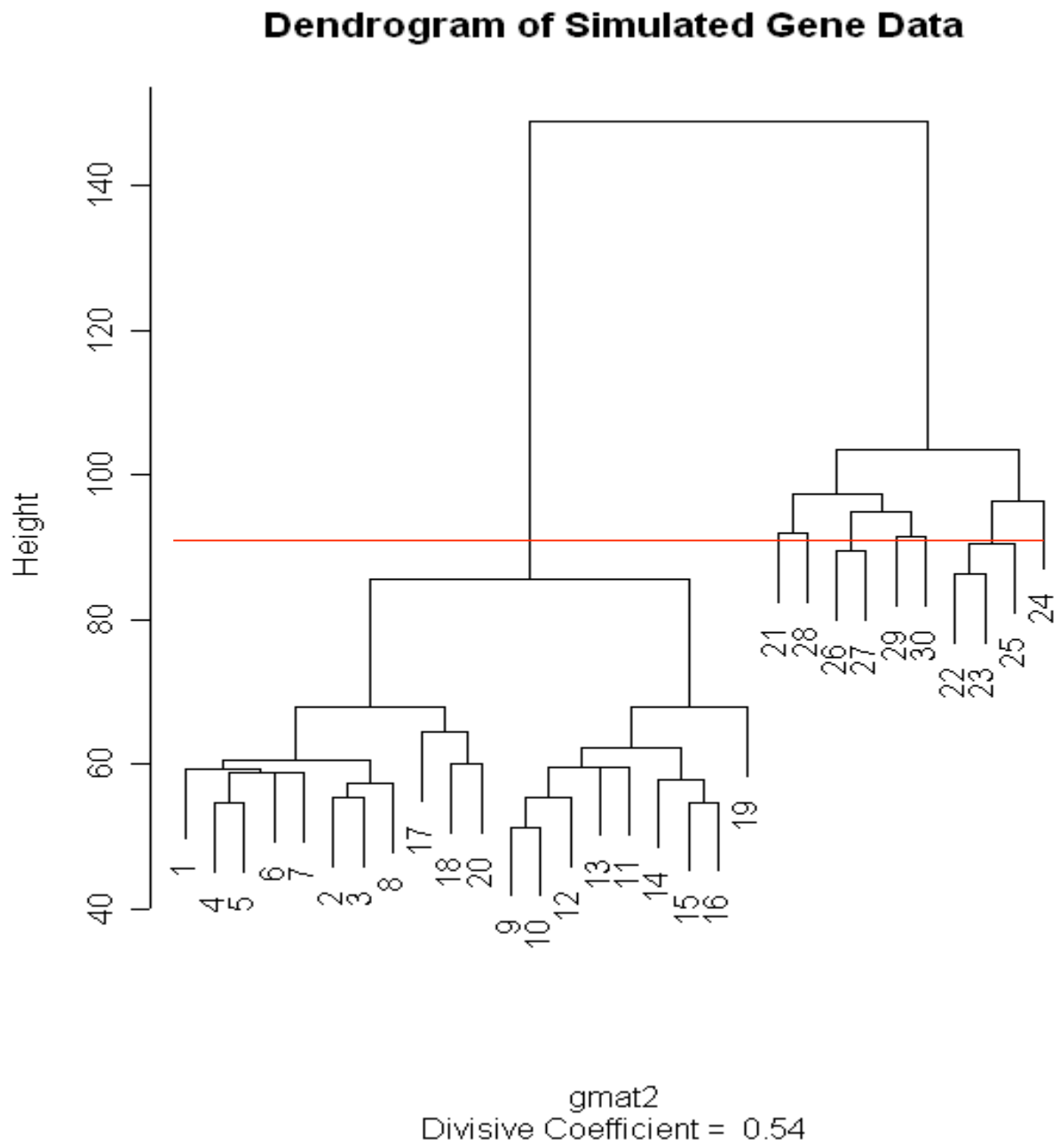
Example with Simulated Gene Expression Data

Four groups of samples: determined by k-means type assumptions.



K-Means

True Class	cluster 1	2	3	4
1	8	0	0	0
2	0	8	0	0
3	3	1	0	0
4	0	0	7	3



Silhouettes

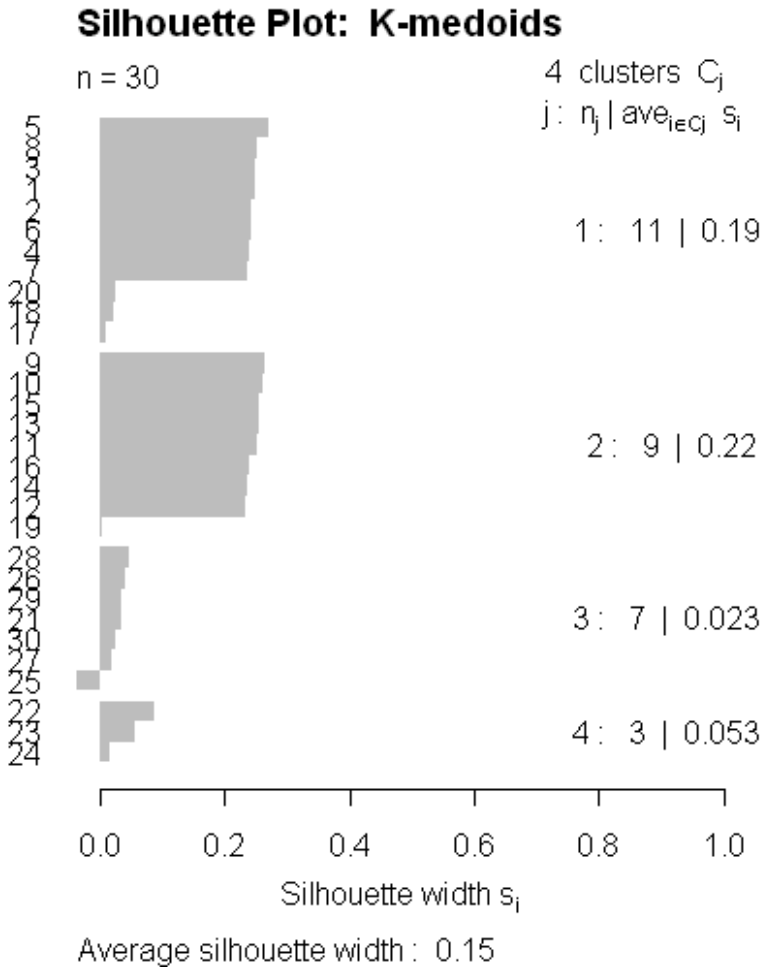
- Silhouette of gene *i* is defined as:

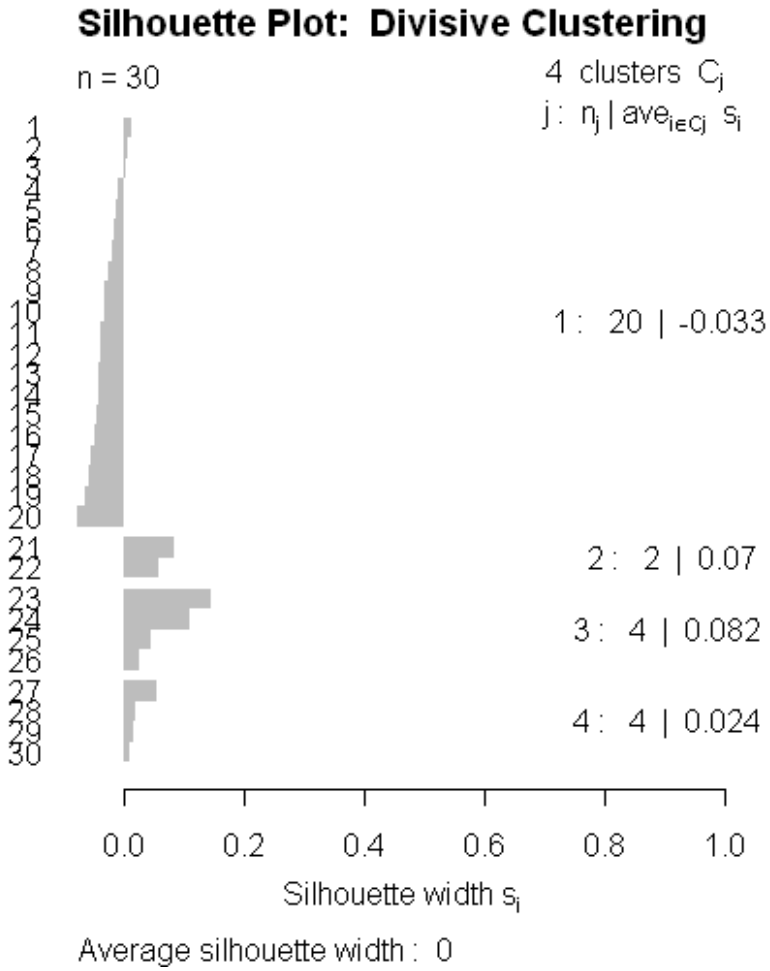
$$s(i) = \frac{b_i - a_i}{\max(a_i, b_i)}$$

- a_i = average distance of gene *i* to other genes in same cluster
- b_i = average distance of gene *i* to genes in its nearest neighbor cluster

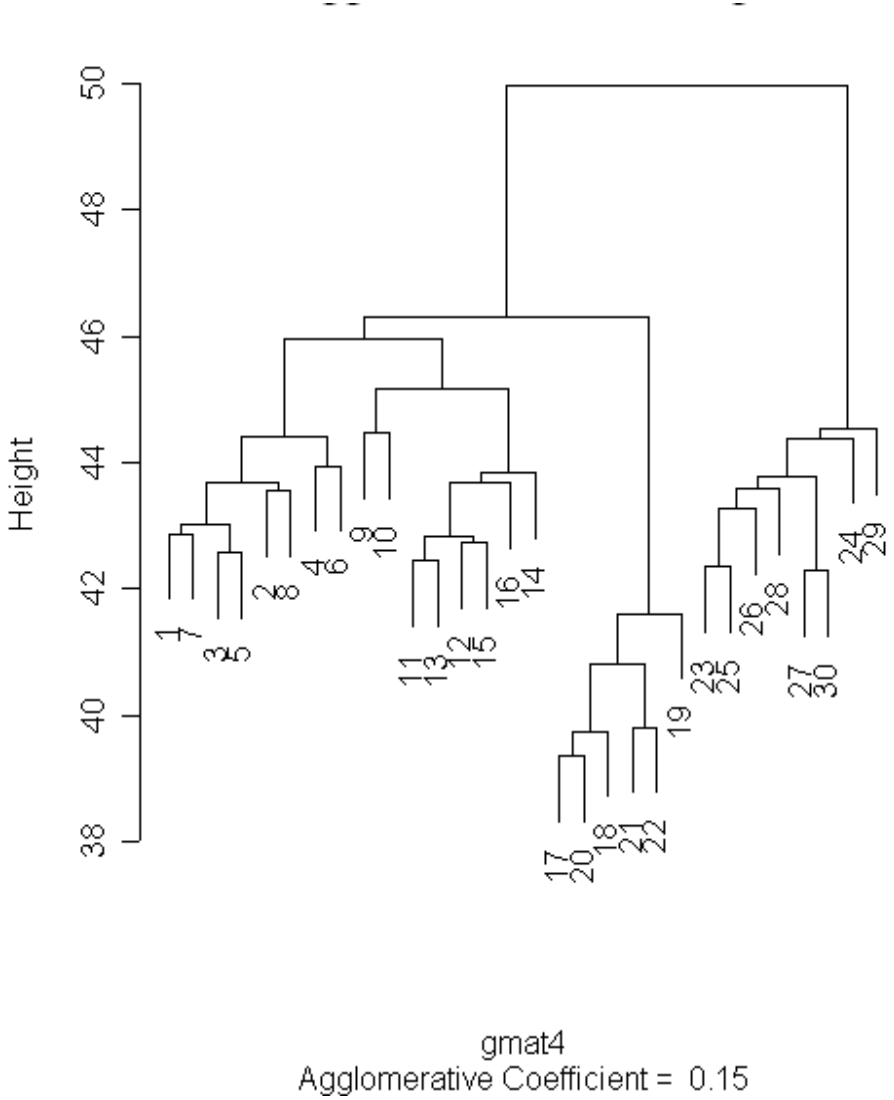
Silhouette Plots (Kaufman and Rousseeuw)

Assumes 4 classes



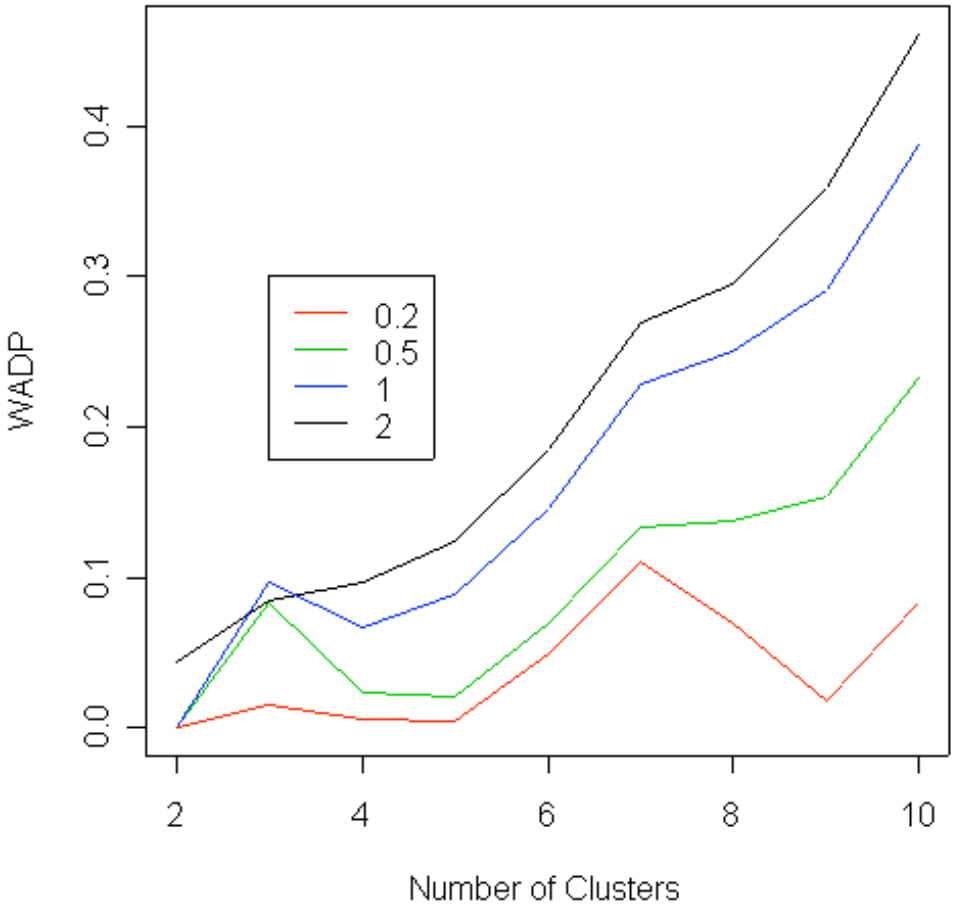


WADP:
Weighted Average Discrepancy Pairs



- Add perturbations to original data
- Calculate the number of paired samples that cluster together in the original cluster that didn't in the perturbed
- Repeat for every cutoff (i.e. for each k)
- Do iteratively
- Estimate for each k the proportion of discrepant pairs.

WADP



- Different levels of noise have been added
- By Bittner's recommendation, 1.0 is appropriate for our dataset
- But, not well-justified.
- External information would help determine level of noise for perturbation
- We look for largest k before WADP gets big.

Some Take-Home Points

- Clustering can be a useful exploratory tool
- Cluster results are very sensitive to noise in the data
- It is crucial to assess cluster structure to see how stable your result is
- Different clustering approaches can give quite different results
- For hierarchical clustering, interpretation is almost always subjective