Biochem 218 - 2010
Lecture 12

Clustering and Functional Analysis of Coordinately Regulated Genes

Gavin Sherlock

sherlock@genome.stanford.edu

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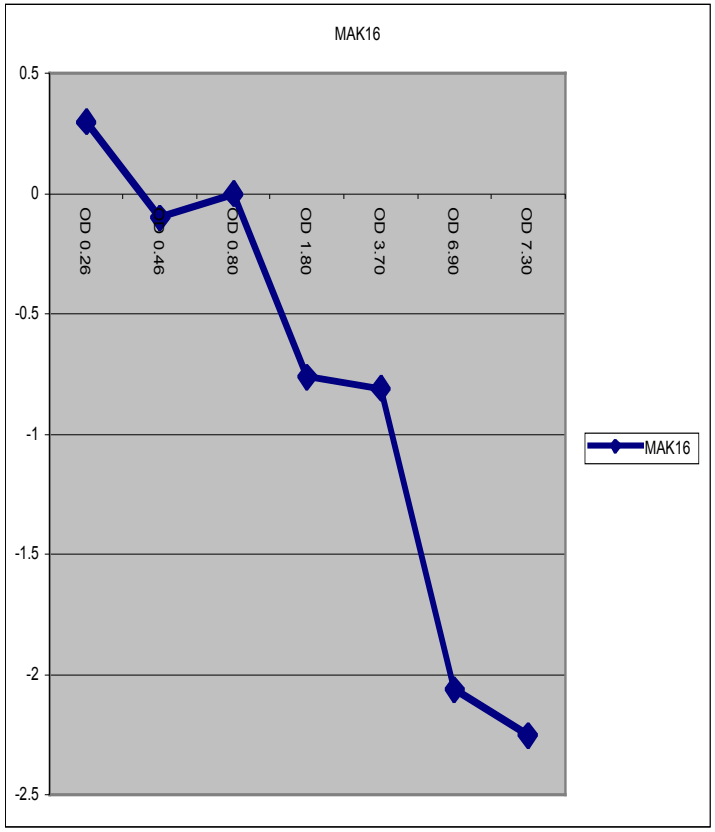
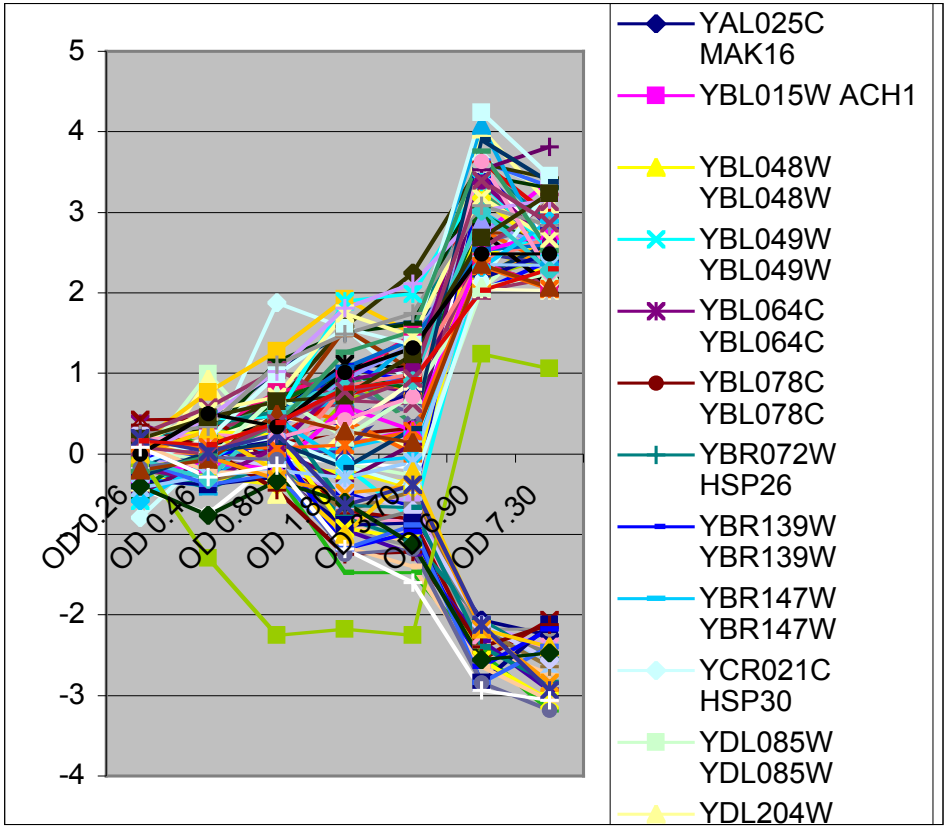
Clustering Coordinately Regulated Genes

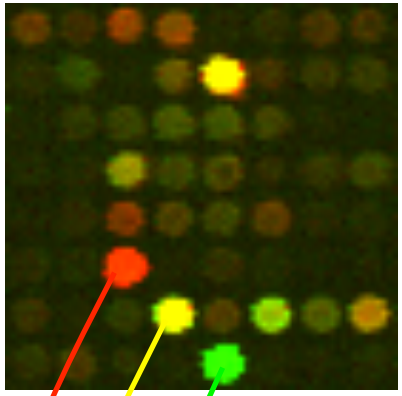
- What are the goals of typical expression experiments?
- How can we determine if two genes are ‘coexpressed’?
- What can we infer when we decide that two genes are coexpressed?

Visualizing Data

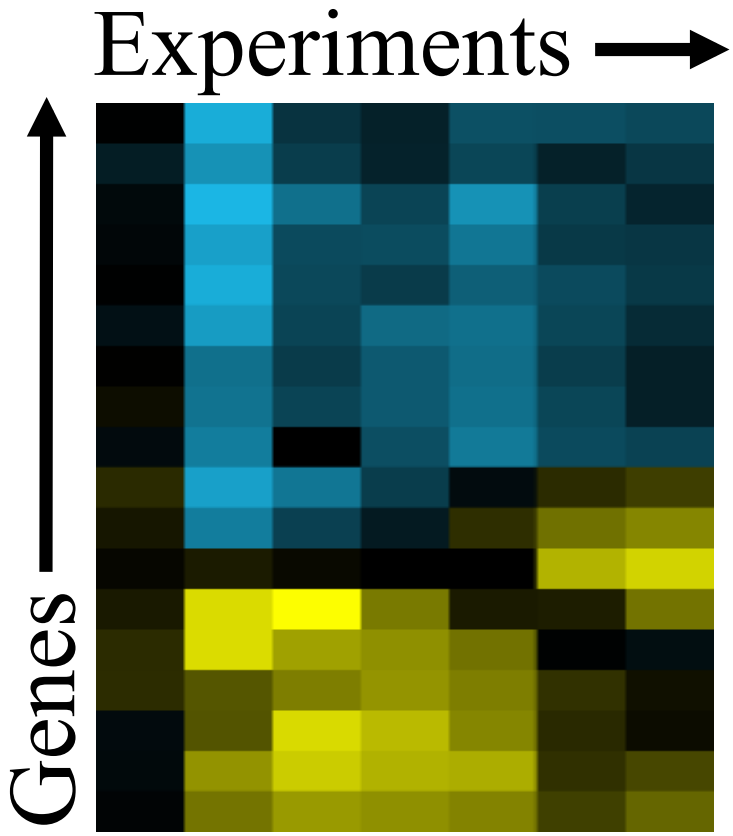




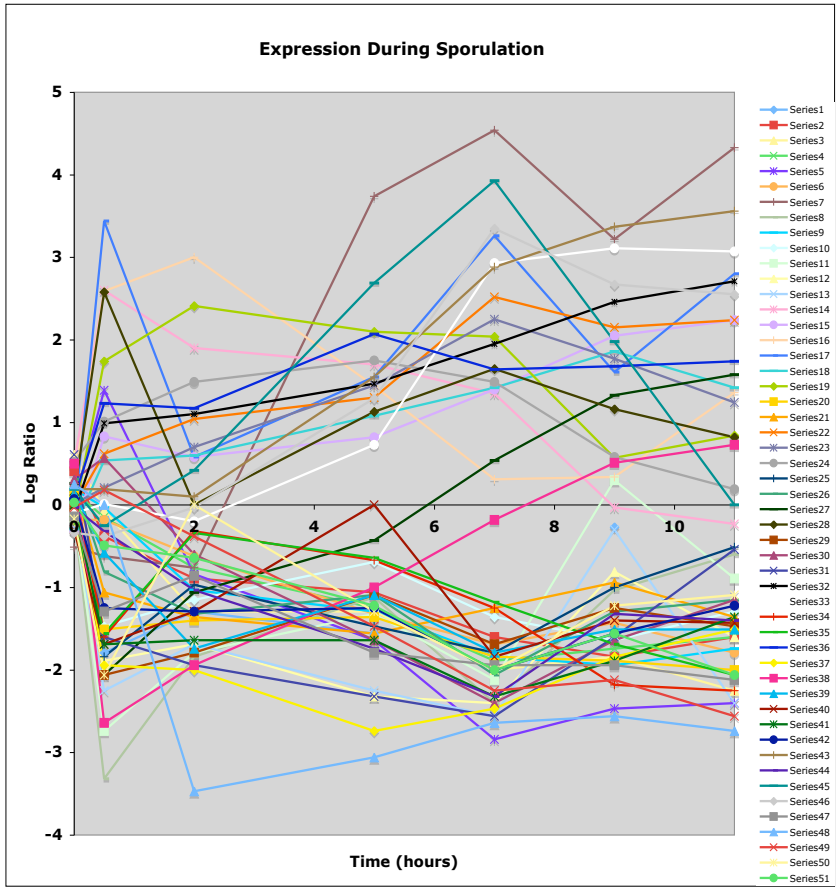
Extracting Data

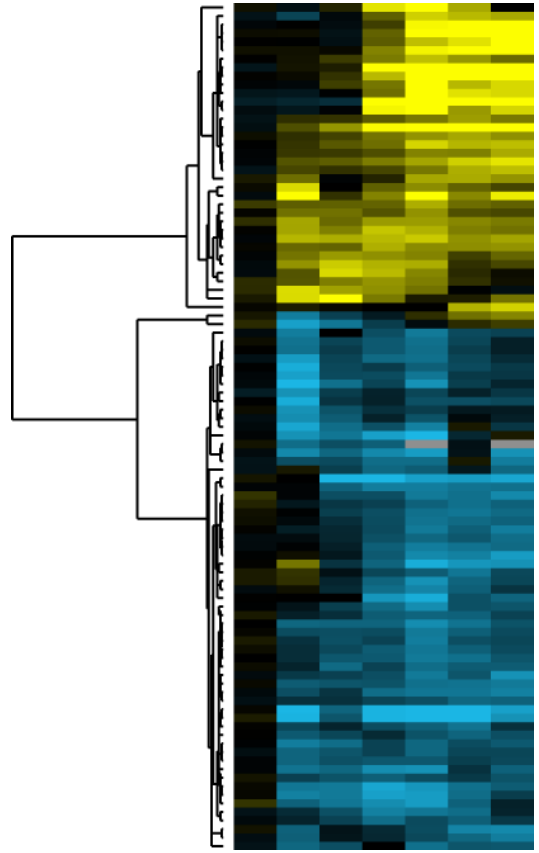


Cy3	Cy5	$\frac{Cy5}{Cy3}$	$\log_2\left(\frac{Cy5}{Cy3}\right)$
200	10000	50.00	5.64
4800	4800	1.00	0.00
9000	300	0.03	-4.91



Visualizing Data (cont.)



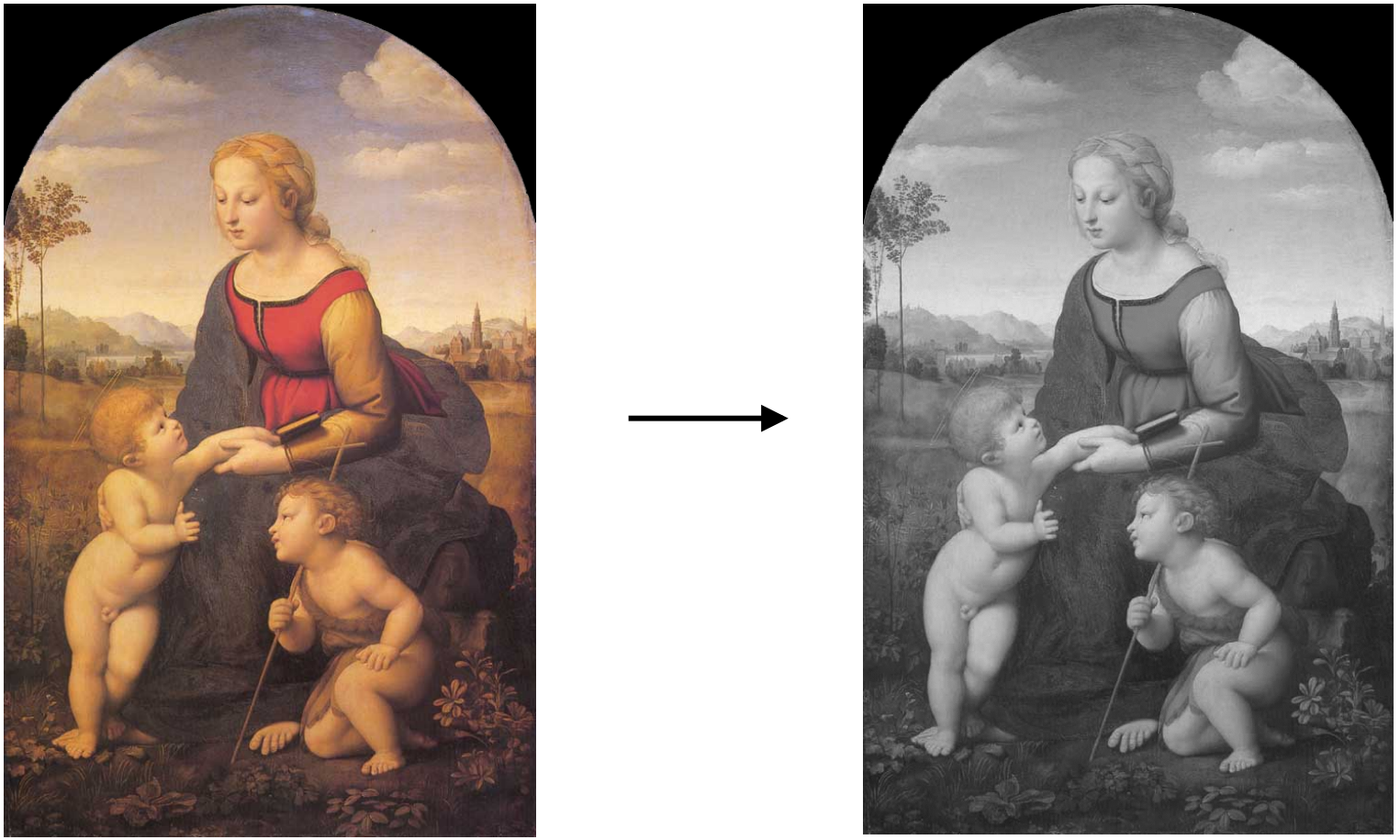


Organizing Data

In microarray studies, we often use clustering algorithms to help us identify patterns in complex data.

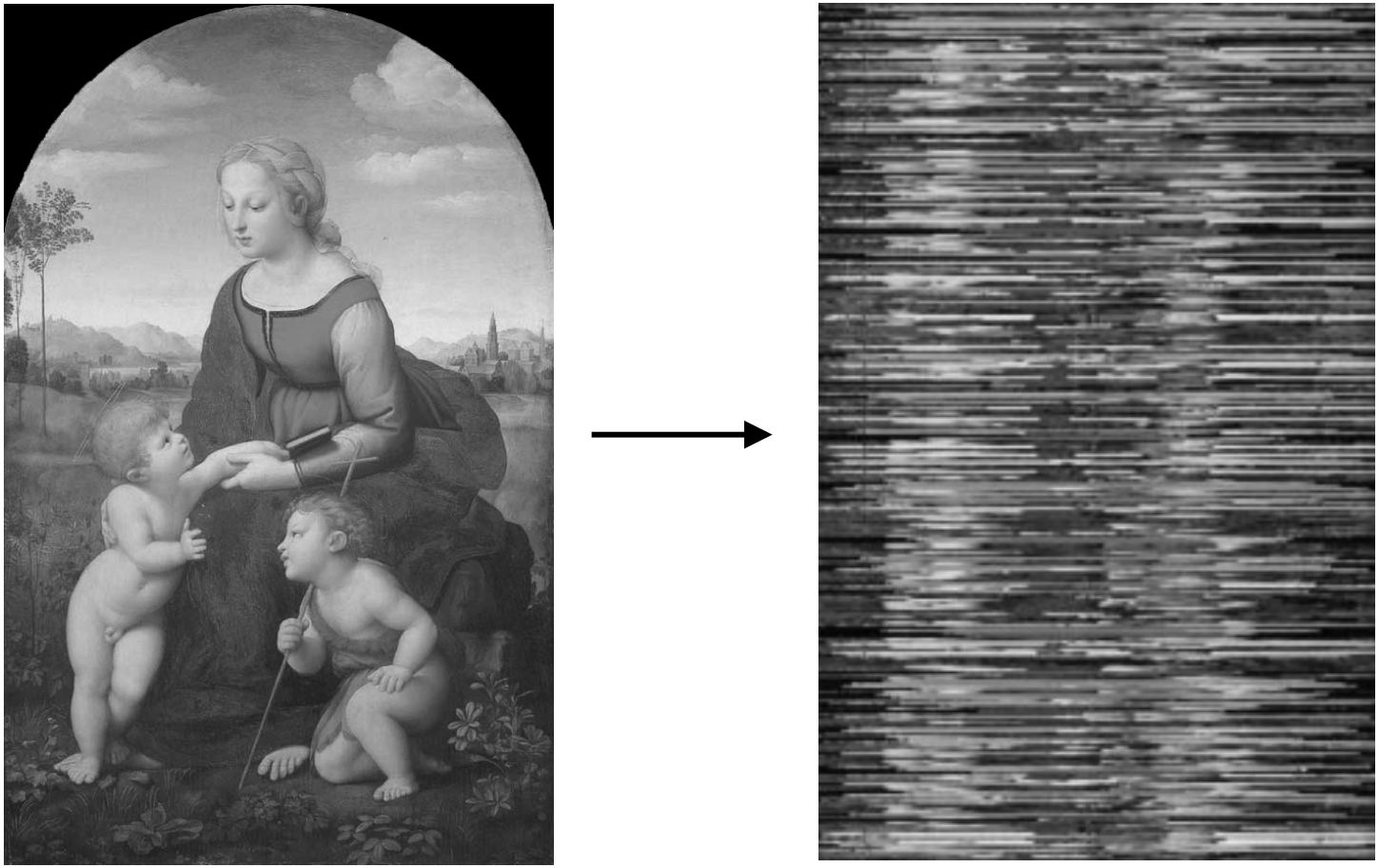
For example, we can randomize the data used to represent this painting and see if clustering will help us visualize the pattern.

Clustering algorithms



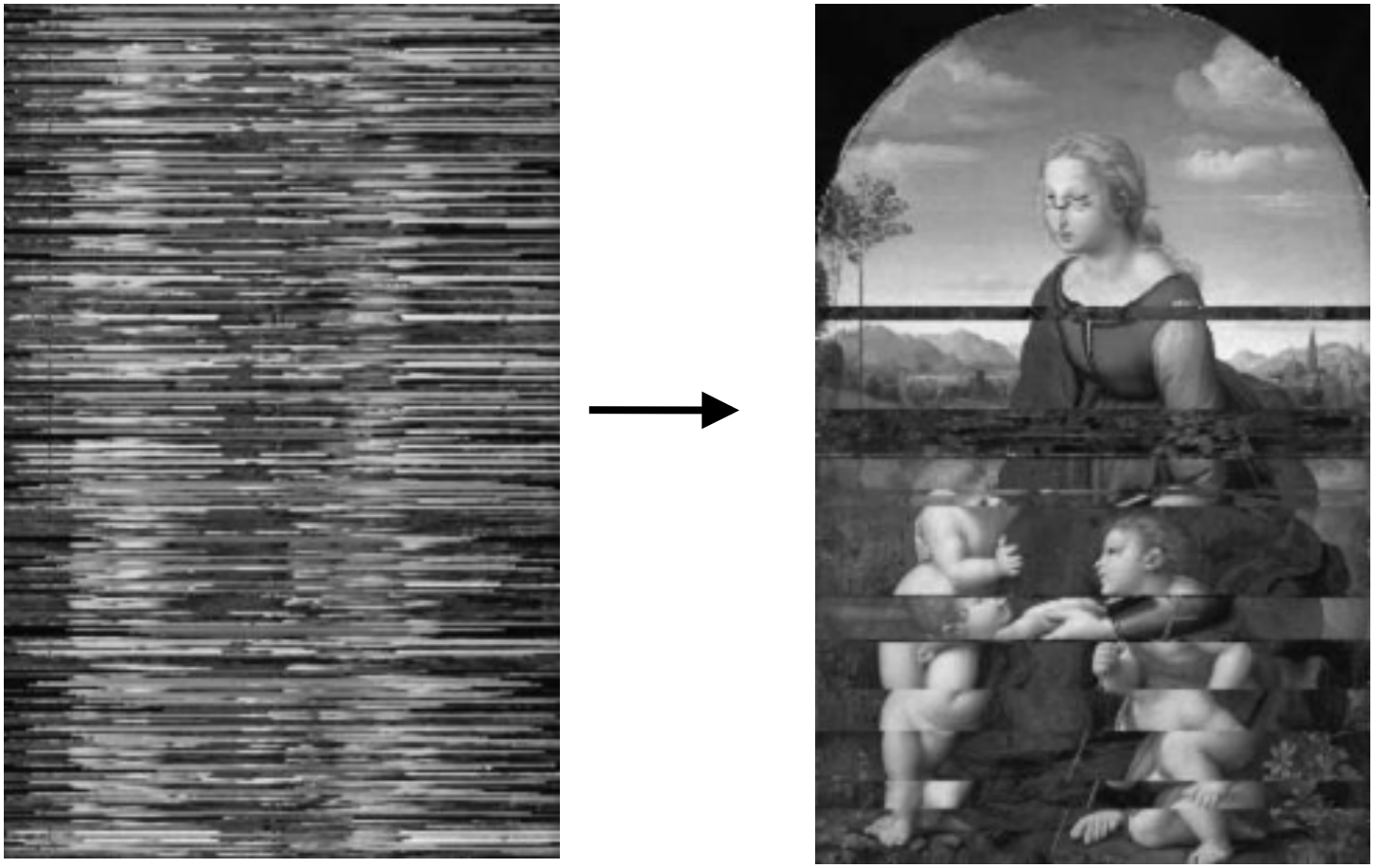
First, we represent the painting in black and white.

Clustering algorithms



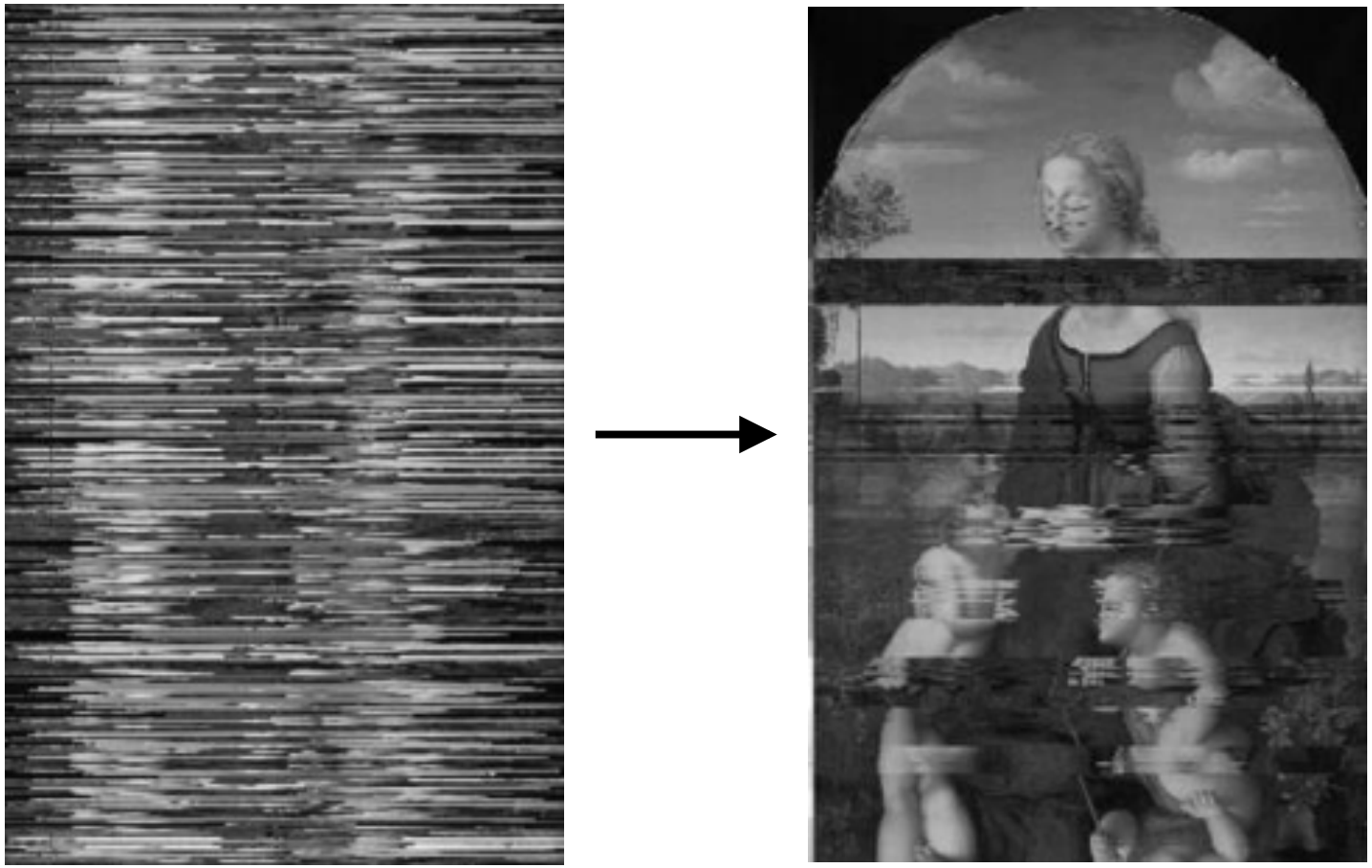
The painting is “sliced” into rows which are then randomized.

Clustering algorithms



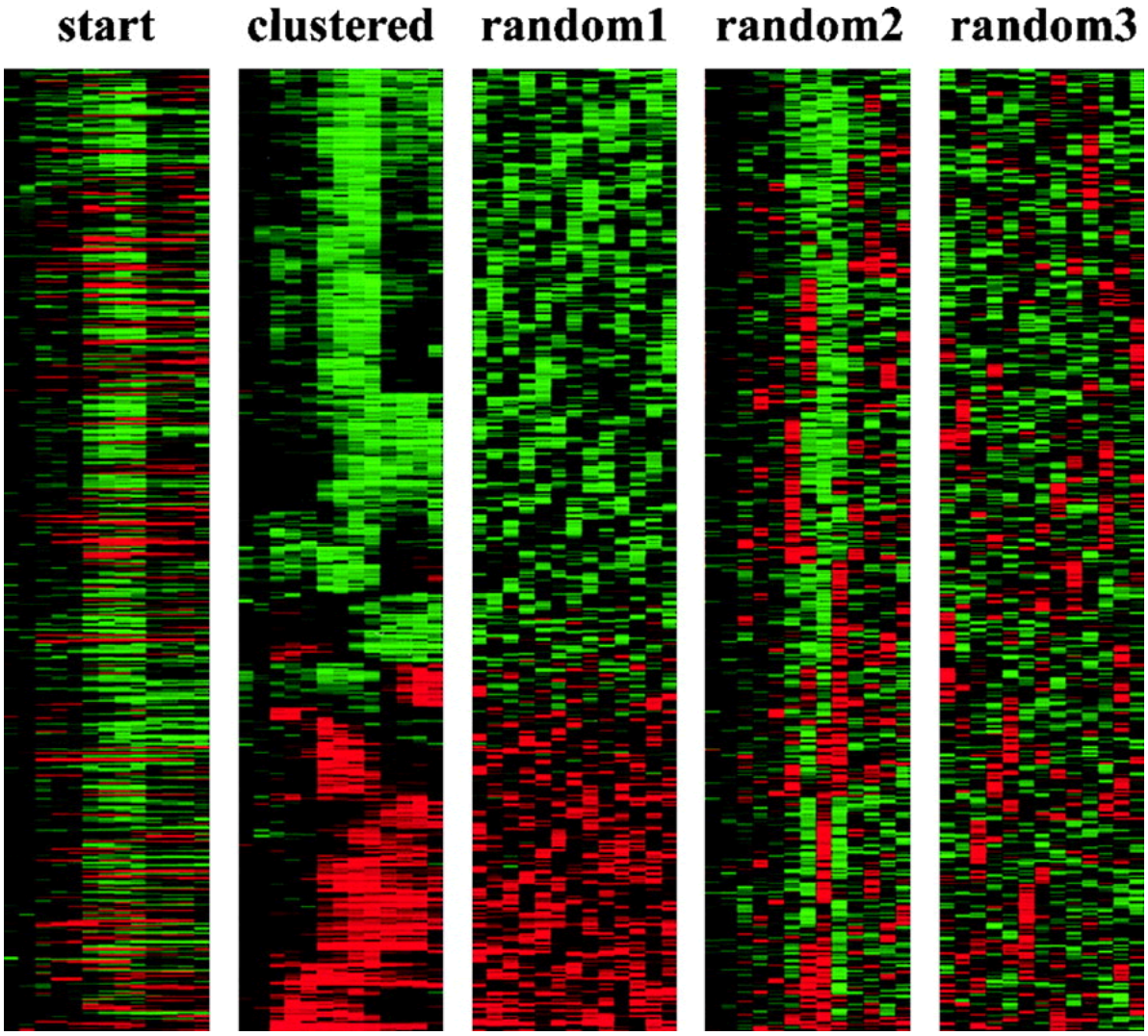
Rows ordered by hierarchical clustering with nodes flipped to optimize ordering

Clustering algorithms



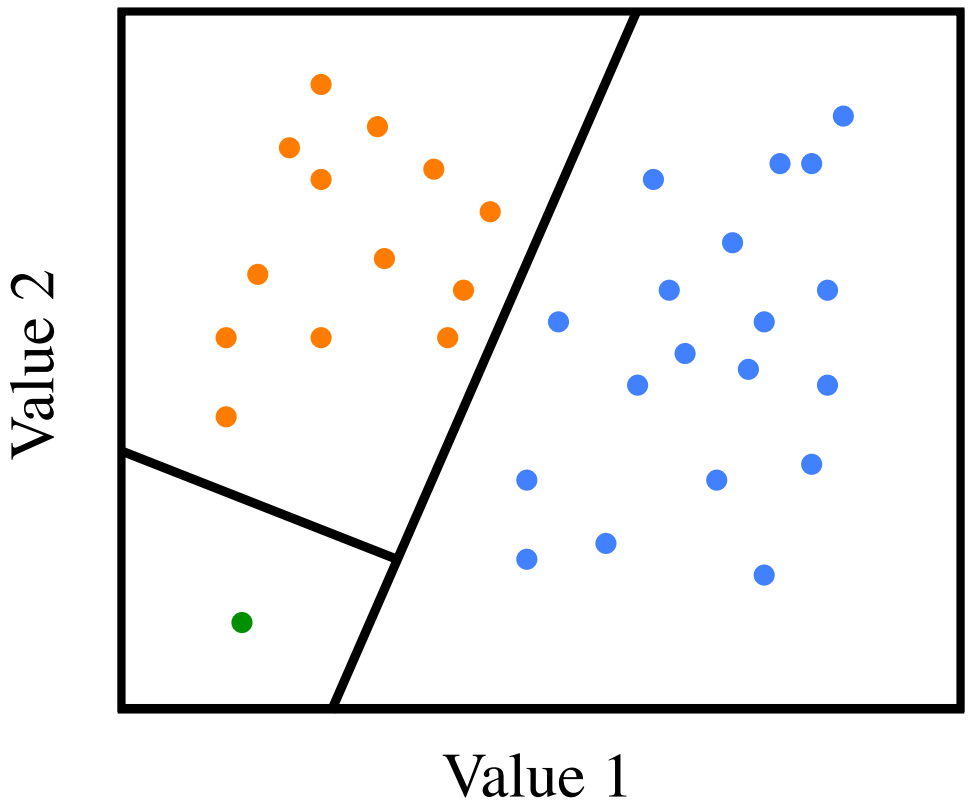
Rows ordered by using a Self-Organizing Map (SOM)

Random vs. Biological Data



From *Eisen MB, et al, PNAS 1998 95(25):14863-8*

Goal of Clustering



Types of Clustering

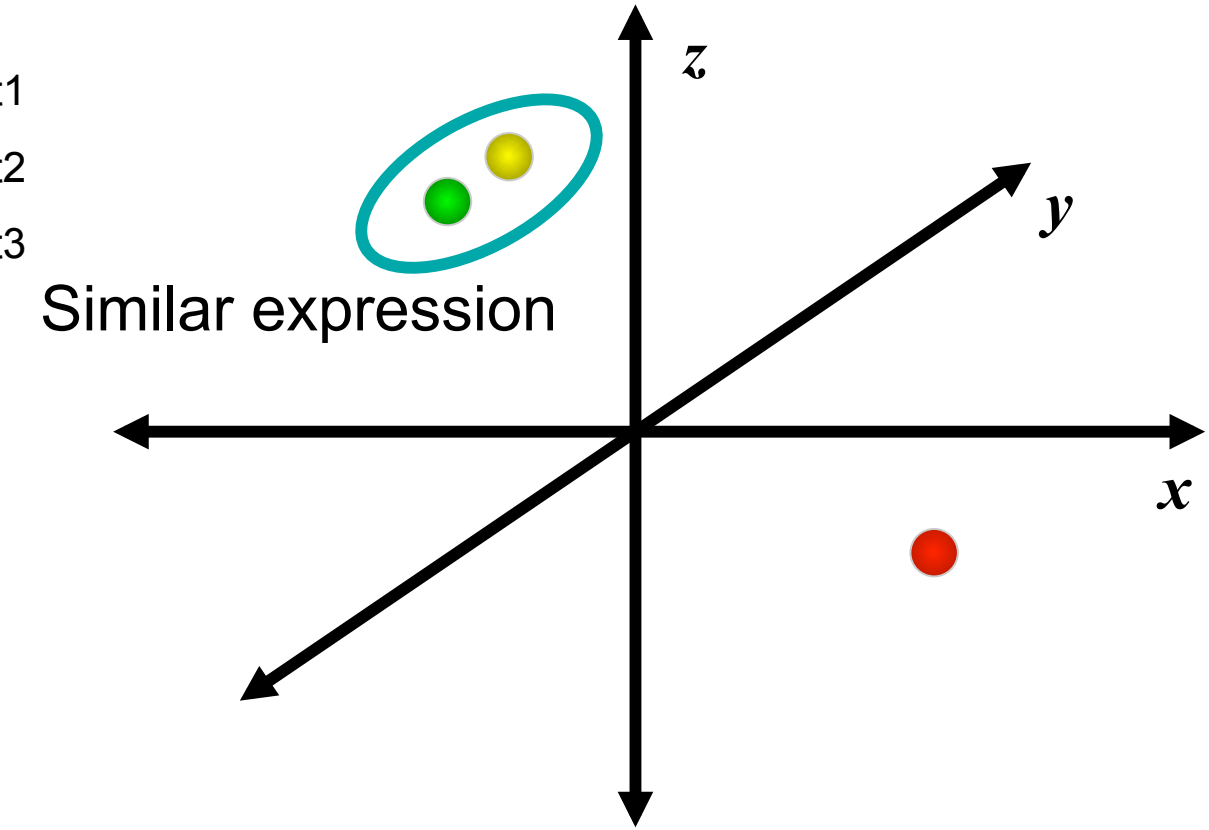
- Agglomerative
  - Bottom up approach
  - Different variants of hierarchical clustering
  - This is the typical clustering you see
- Partitioning / Divisive
  - Top down approach
  - K-means Clustering
  - Self-Organizing Maps
- All require the ability to compare expression patterns to each other.

How do we compare expression profiles?

- Treat expression data for a gene as a multidimensional vector.
- Use a distance/correlation metric to compare the vectors.

Expression Vectors

- Crucial concept for understanding clustering
- Each gene is represented by a vector where coordinates are its values - log(ratio) - in each experiment
  - $x = \log(\text{ratio})_{\text{expt1}}$
  - $y = \log(\text{ratio})_{\text{expt2}}$
  - $z = \log(\text{ratio})_{\text{expt3}}$
  - etc.



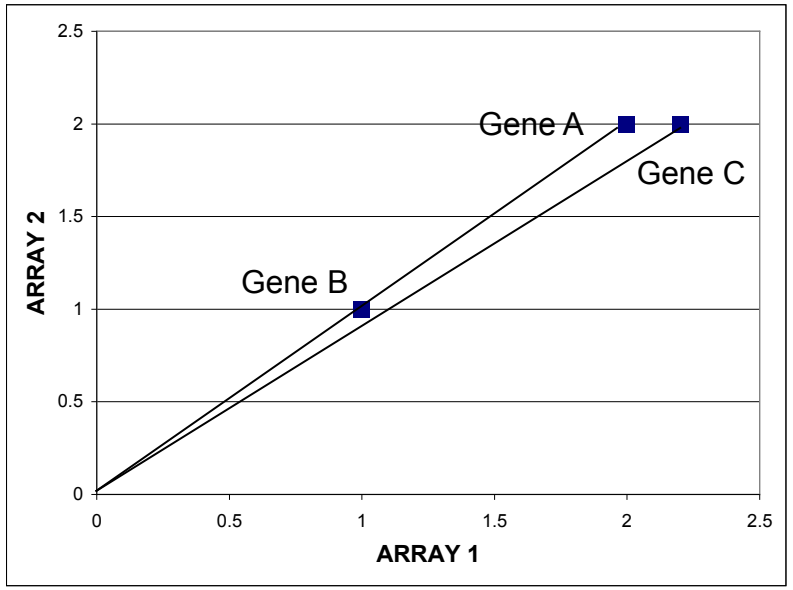
Distance metrics

- Distances or correlations are measured “between” expression vectors
- Many different ways to measure distance:
  - Euclidean distance
  - Pearson correlation coefficient(s)
  - Spearman’s Rank Correlation
  - Manhattan distance
  - Mutual information
  - Kendall’s Tau
  - etc.
- Each has different properties and can reveal different features of the data

Euclidean distance

- Euclidean distance metrics detect similar vectors by identifying those that are closest in space. In this example, Gene A and C are closest.

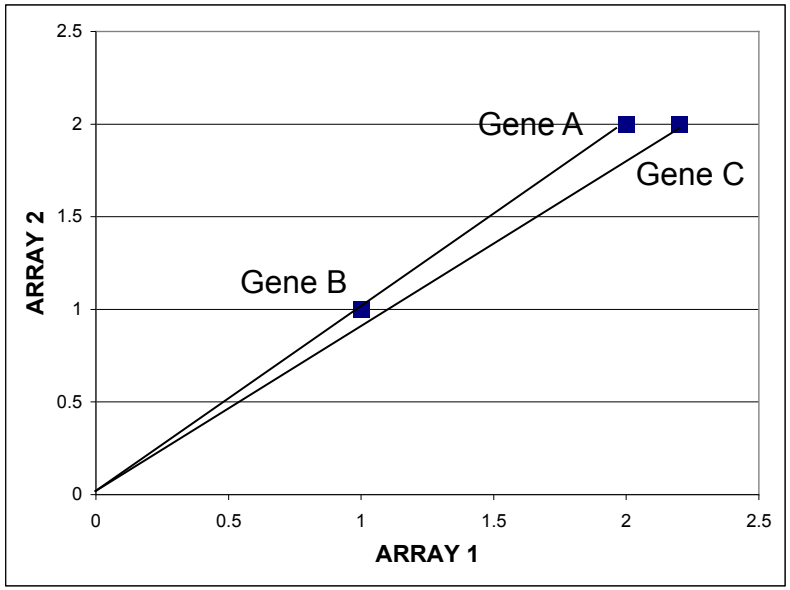
	A	B	C
1	NAME	ARRAY1	ARRAY2
2	GENE A	2	2
3	GENE B	1	1
4	GENE C	2.2	2



Pearson correlation

- The Pearson correlation disregards the magnitude of the vectors but instead compares their directions. In this example, Gene A and Gene B have the same slope, so would be most similar to each other.

	A	B	C
1	NAME	ARRAY1	ARRAY2
2	GENE A	2	2
3	GENE B	1	1
4	GENE C	2.2	2

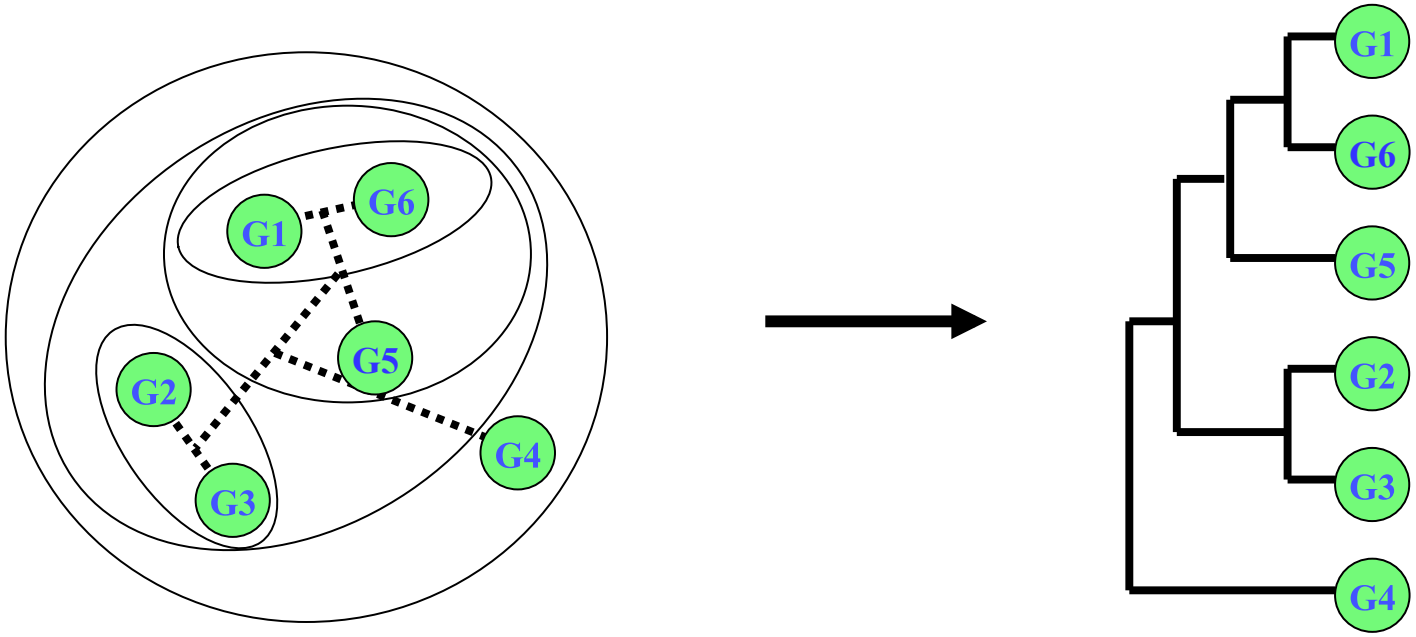


Agglomerative Hierarchical Clustering

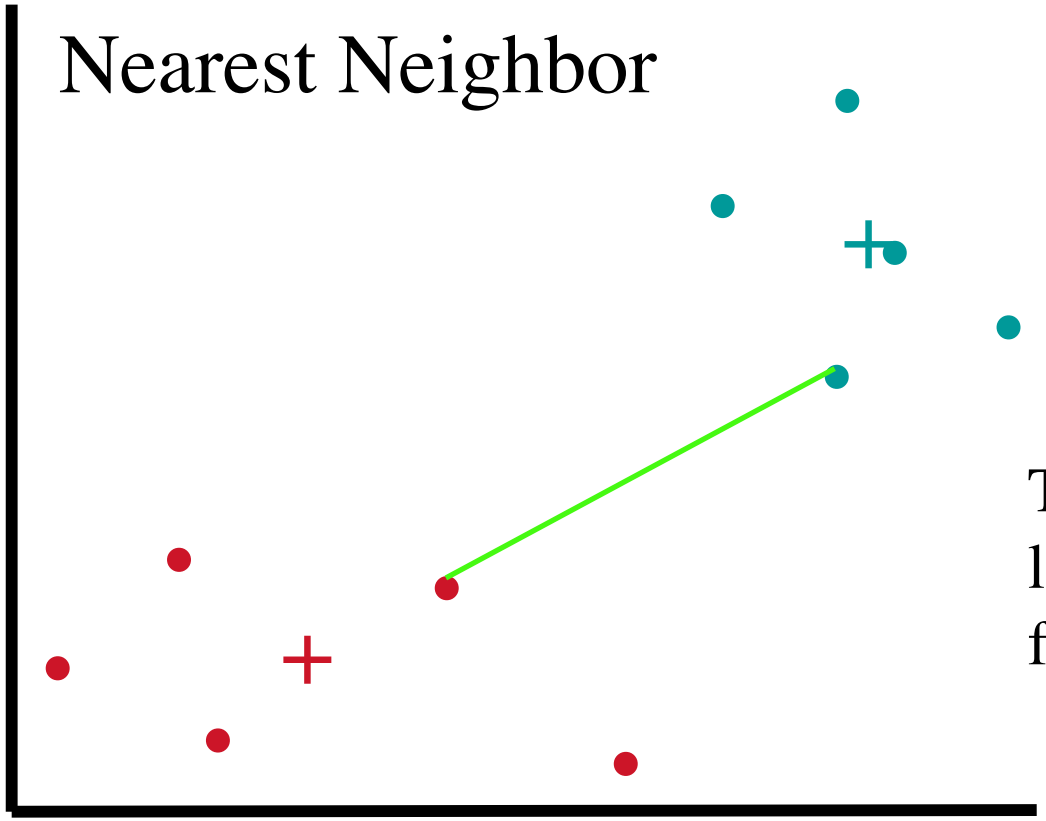
1. Compare all expression patterns to each other.
2. Join patterns that are the most similar out of all patterns.
3. Compare all joined and unjoined patterns.
4. Go to step 2, and repeat until all patterns are joined.

Need a rule to decide how to compare clusters to each other

Visualization of Hierarchical Clustering

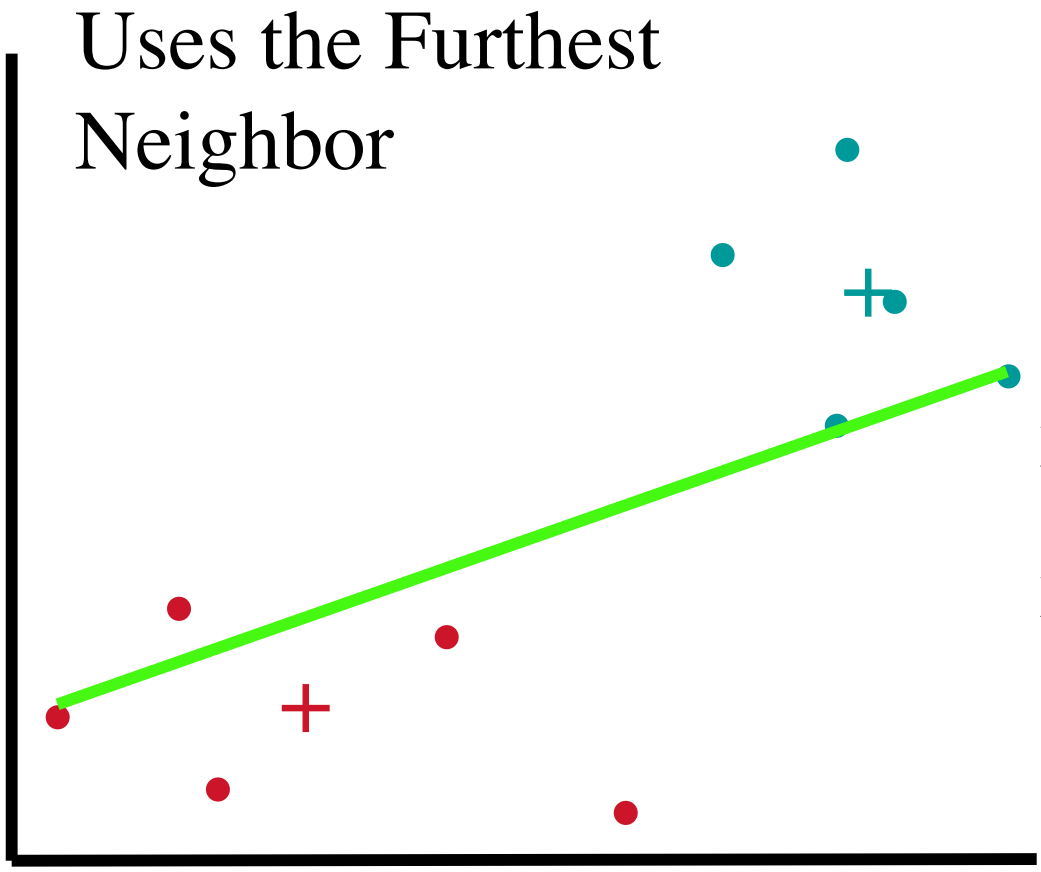


Single linkage Clustering



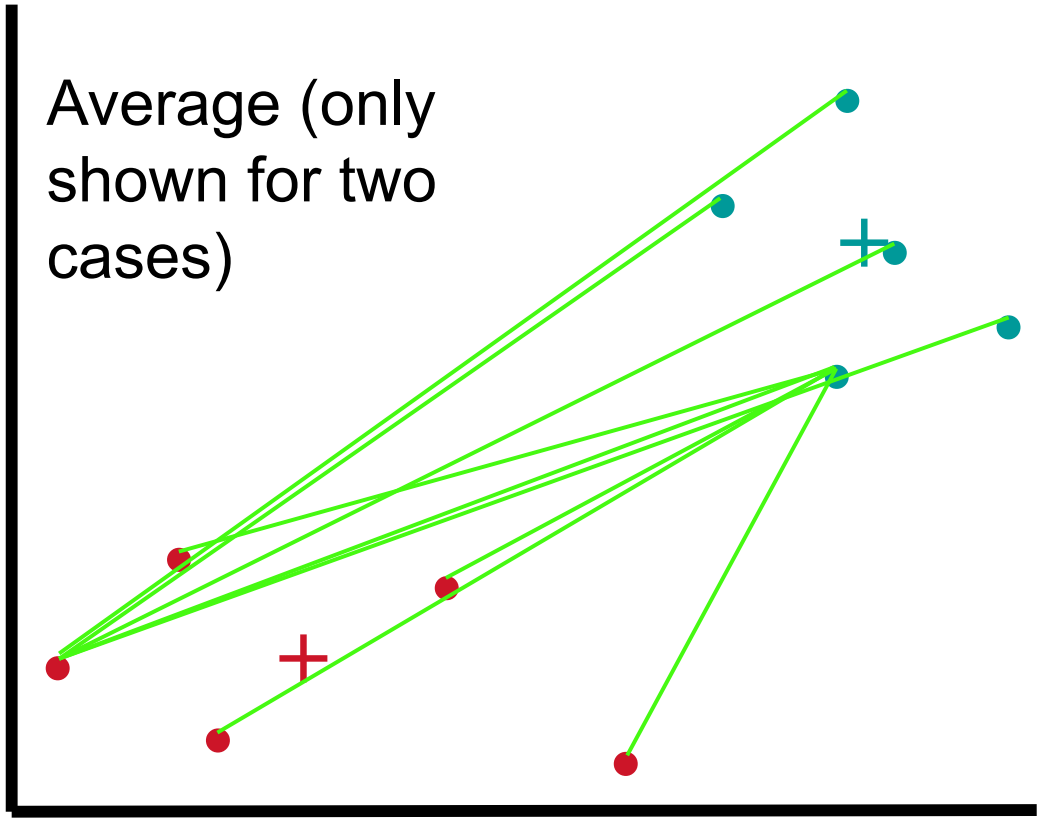
This method produces long chains which form straggly clusters.

Complete Linkage Clustering



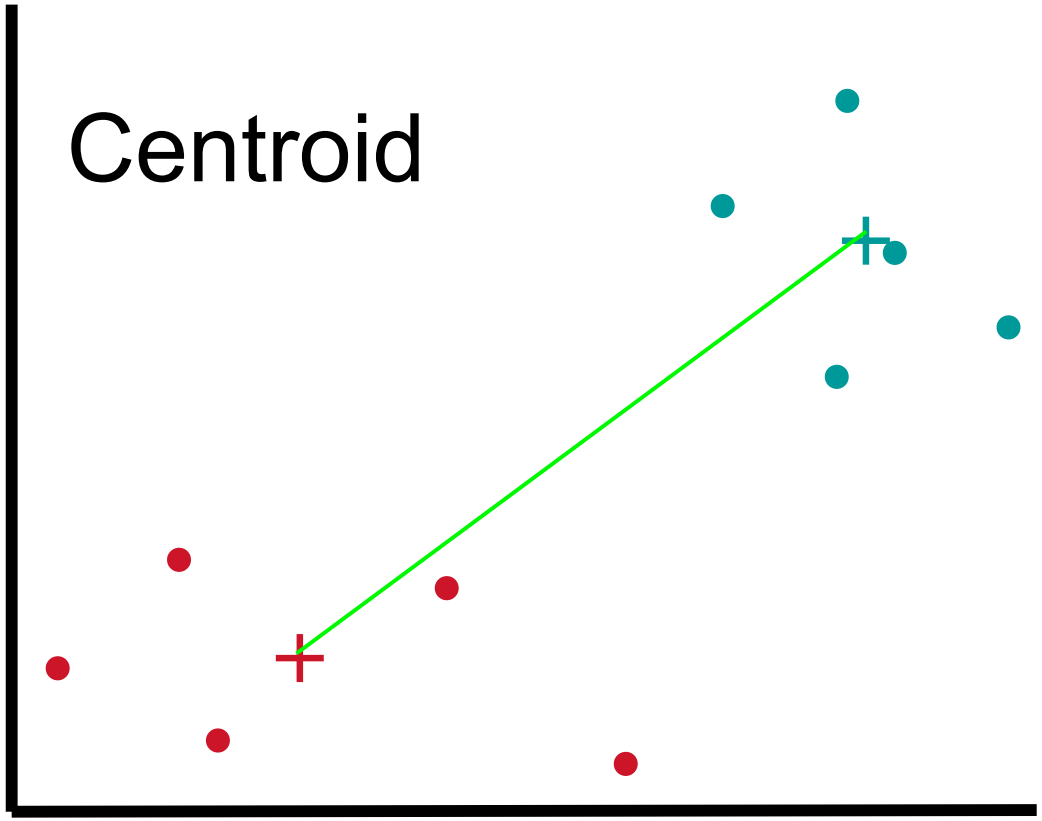
This method tends to produce very tight clusters of similar patterns

Average Linkage Clustering



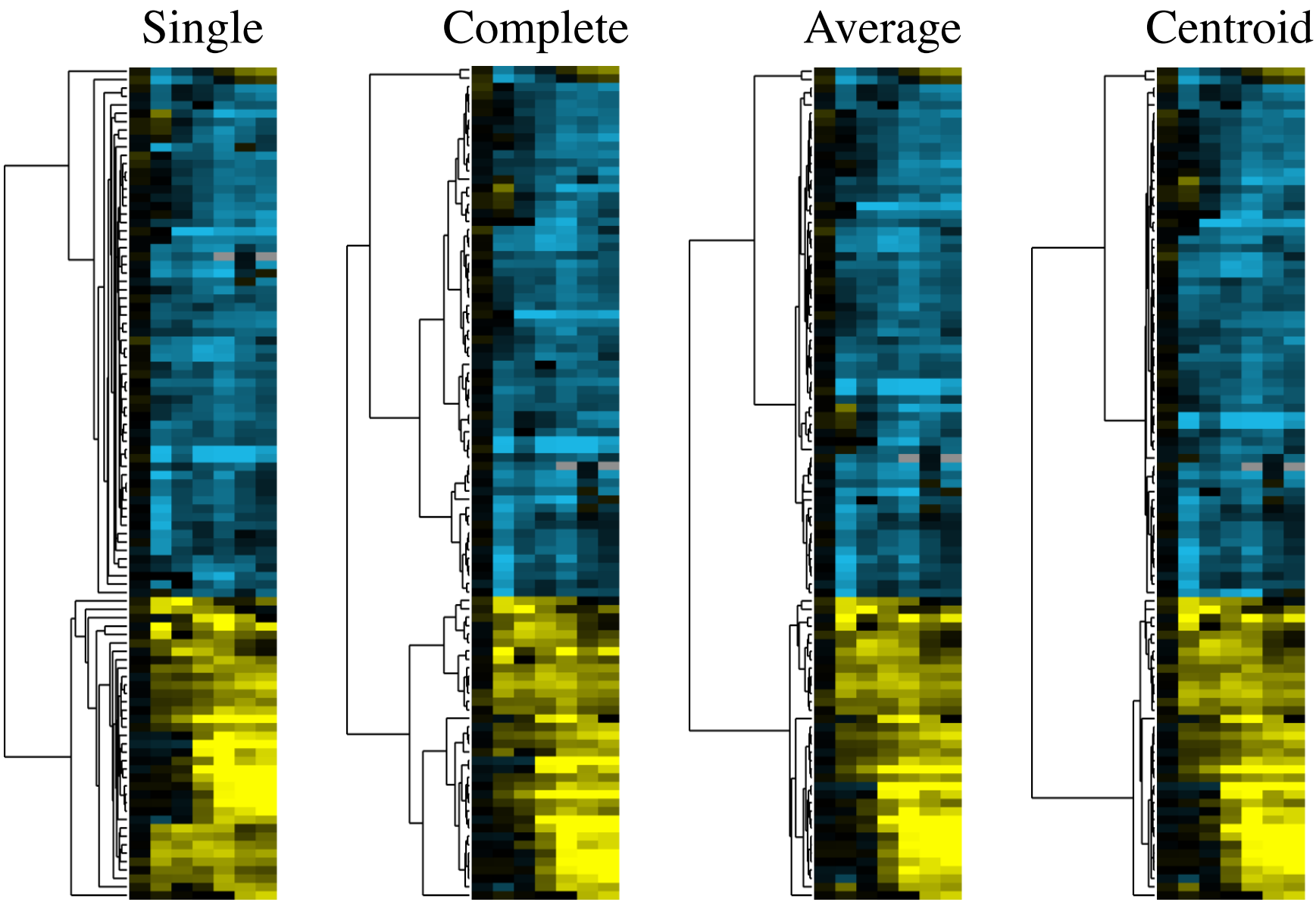
The red and blue '+' signs mark the centroids of the two clusters.

Centroid Linkage Clustering



The red and blue ‘+’ signs mark the centroids of the two clusters.

And we get a cluster:

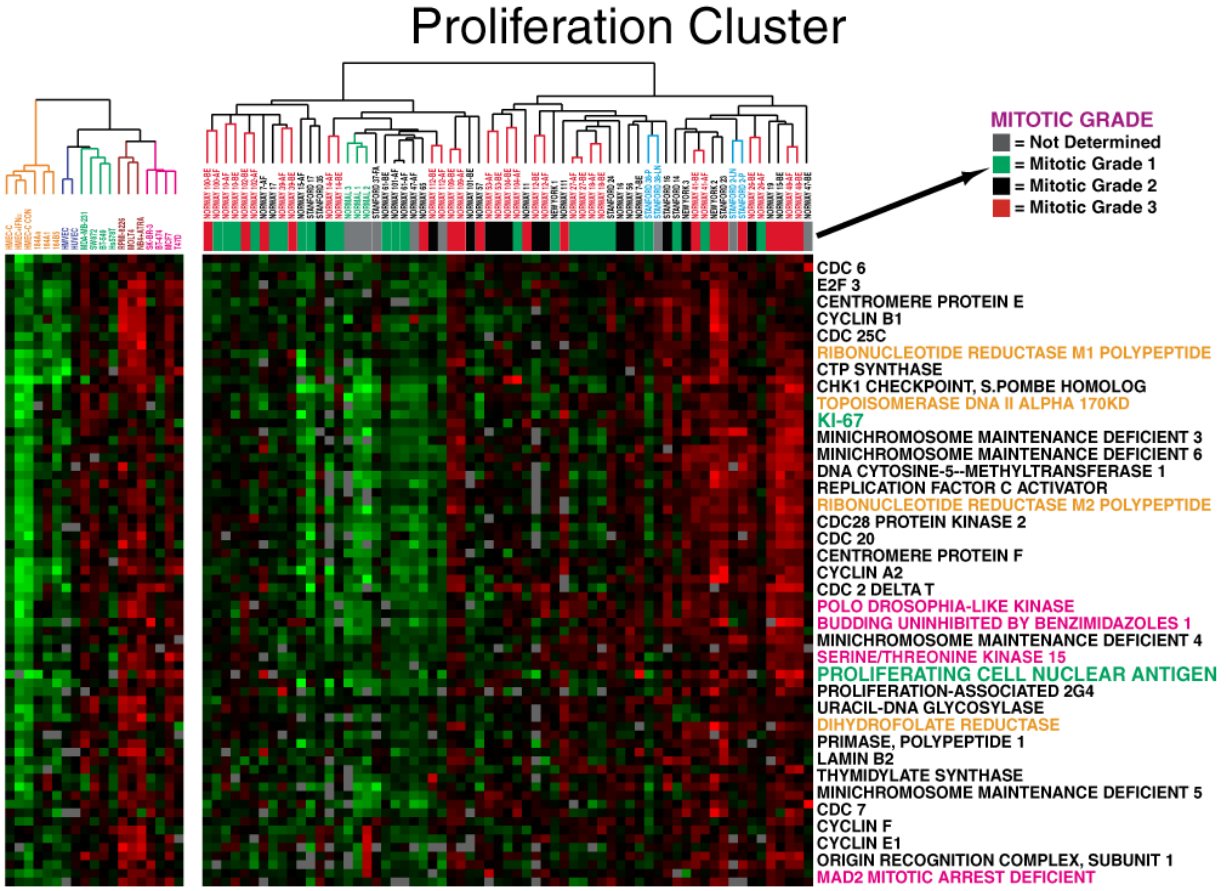


Two-way clustering

- Just as gene patterns are clustered, array patterns can be clustered.
- All the data points for an array can be used to construct a vector for that array and the vectors of multiple arrays can be compared.

Two-way Clustering

Two-way clustering can help show which samples are most similar, as well as which genes.



Agglomerative Hierachical Clustering

Advantages:

- Simple
- Easy to implement
- Easy to visualize

Disadvantages:

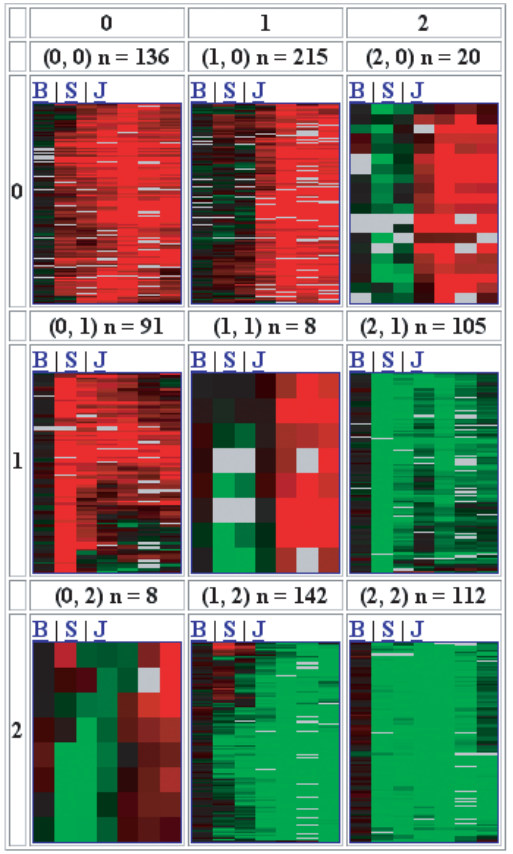
- Can lead to artifacts
- Discarding of subtleties in 2-way clustering

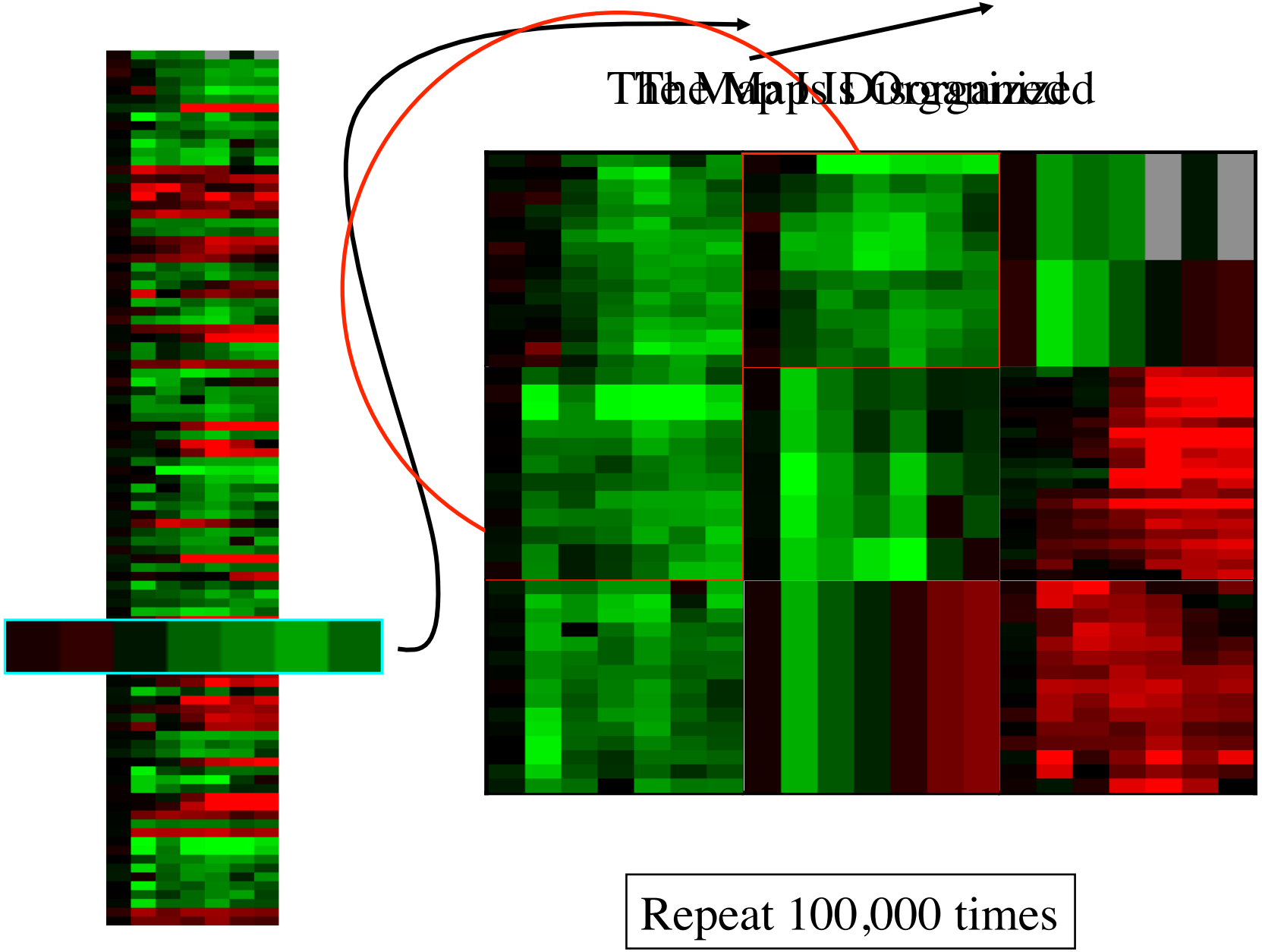
Partitioning Methods

- Split data up into smaller, more homogenous sets
- Should avoid artifacts associated with incorrectly joining dissimilar vectors
- Can cluster each partition independently of others, by genes and arrays
- Self-Organizing Maps and k-means clustering are two possible partitioning methods

Self Organizing Maps

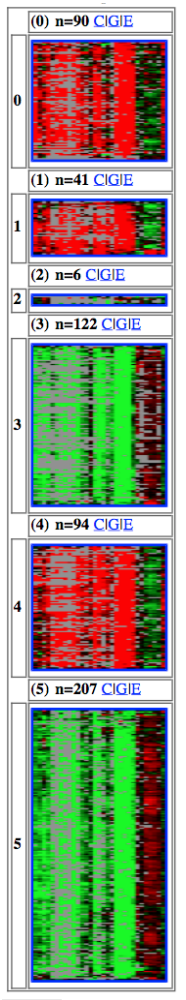
- Create a ‘Map’ of ‘n’ partitions, that is modeled on the expression data, where each partition in the map has an associated vector.
- Genes’ expression vectors are assigned to the partition with the most similar associated vector.
- Neighboring partitions are more similar to each other than they are to distant partitions.




The Map is Organized
Repeat 100,000 times

K-means Clustering

- Split data into ‘n’ partitions, each with an associated vector.
- Assign genes to partitions, and recalculate the vector associated with each partition as the centroid of its associated genes.
- Repeat until solution converges, or for a fixed number of iterations.

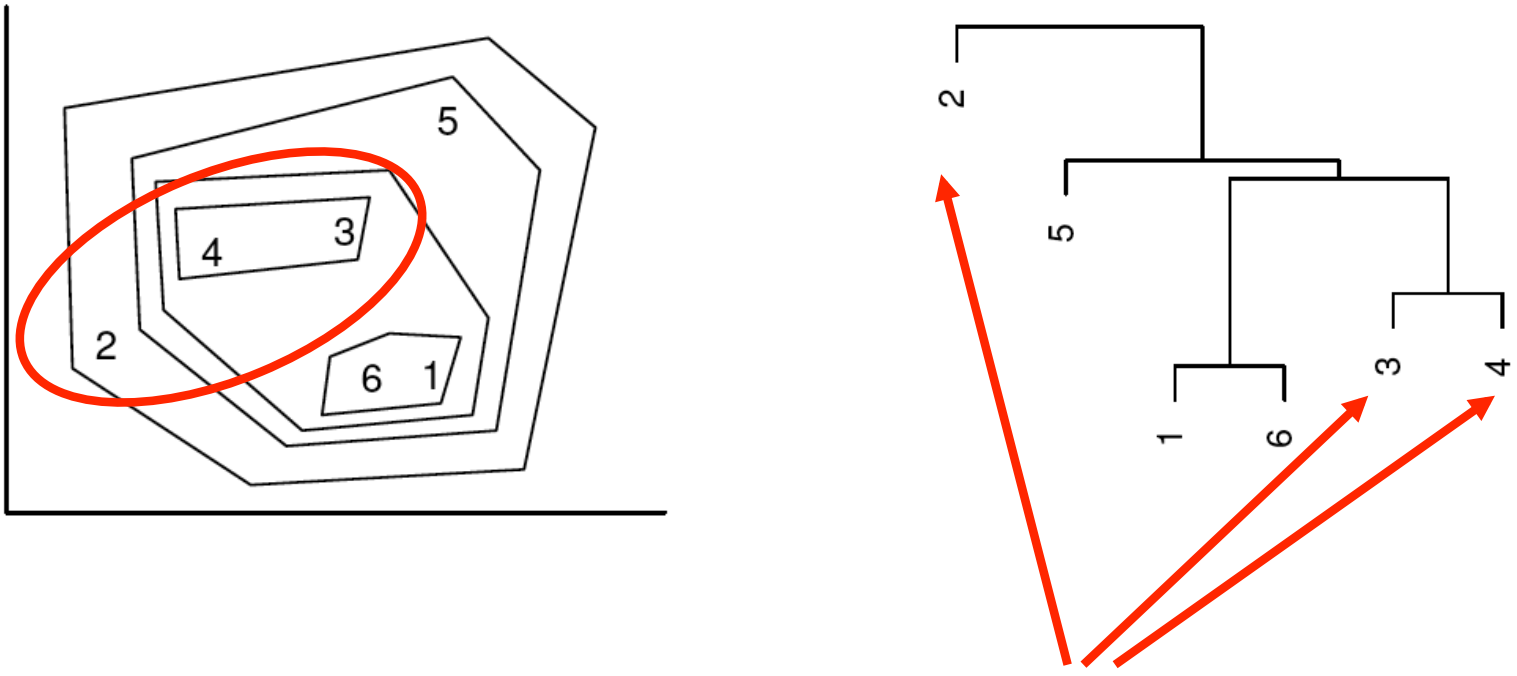


Divisive Hierarchical Clustering

- Iteratively use k-means clustering, with k set to 2.
- Successively divide data into smaller and smaller subsets.
- Allows you to build a tree describing how the data were successively split, similarly to agglomerative hierarchical clustering.

Agglomerative vs. Divisive

Agglomerative:

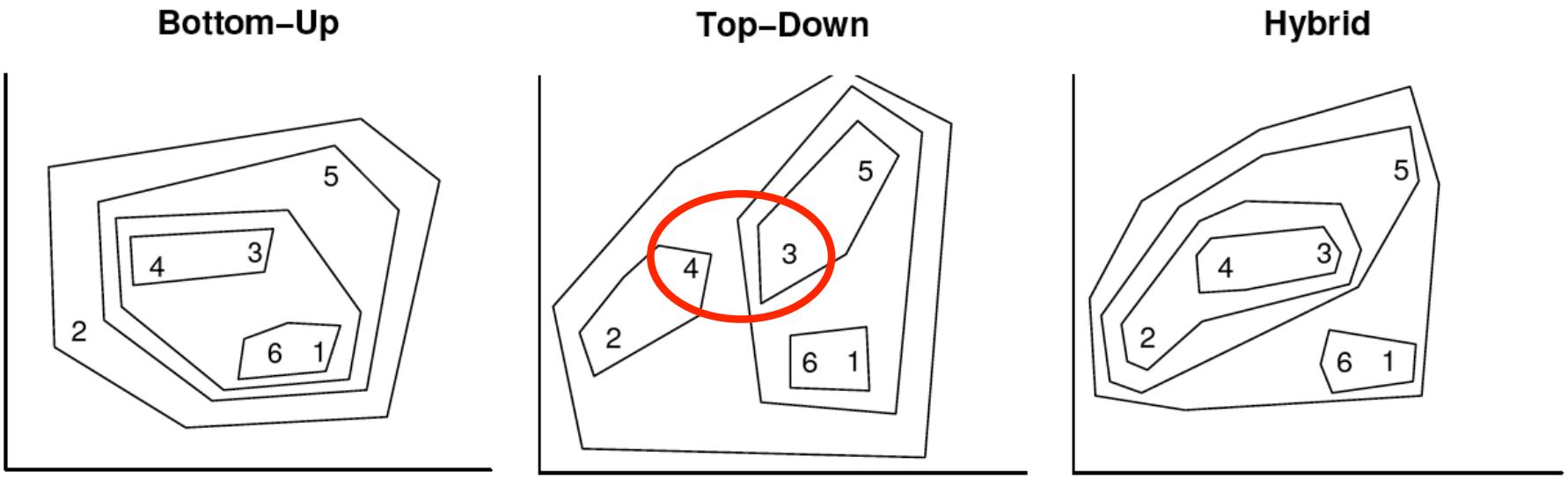


Chipman and Tibshirani, 2006

Agglomerative vs. Divisive

Agglomerative

Divisive



Chipman and Tibshirani, 2006

Summary For Clustering

- Many different methods exist for finding groups and patterns in data (including some I haven't mentioned).
- Many different parameters can be used in those methods.
- Caution should be exercised in interpreting the results.

Comparing Different Clustering Methods

Which technique is right?

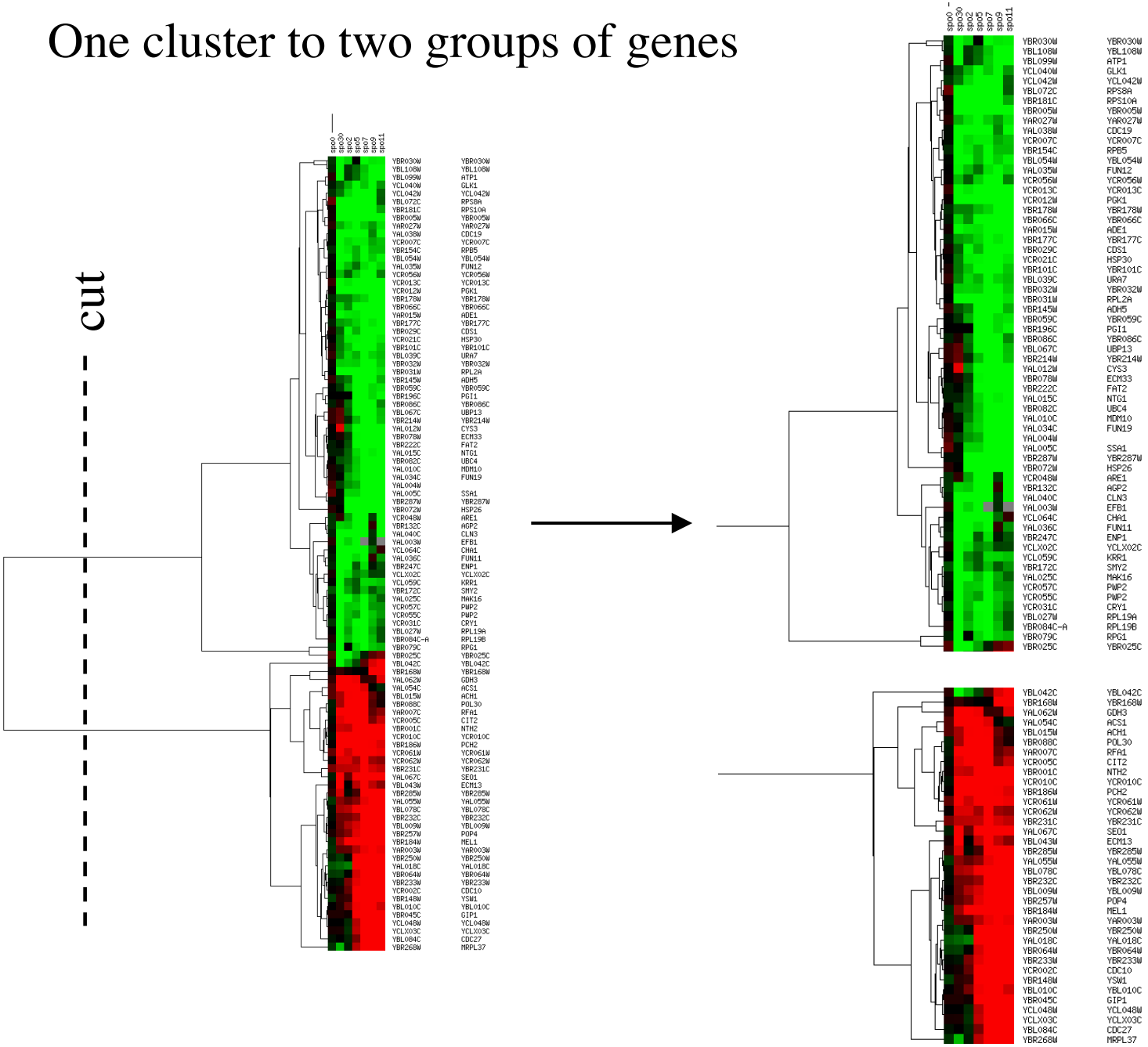
- Hierarchical clustering?
  - Single, Average, Complete, Centroid linkage, etc.?
- Self Organizing Maps
- K-means clustering
- Other algorithms?

What is a ‘cluster’?

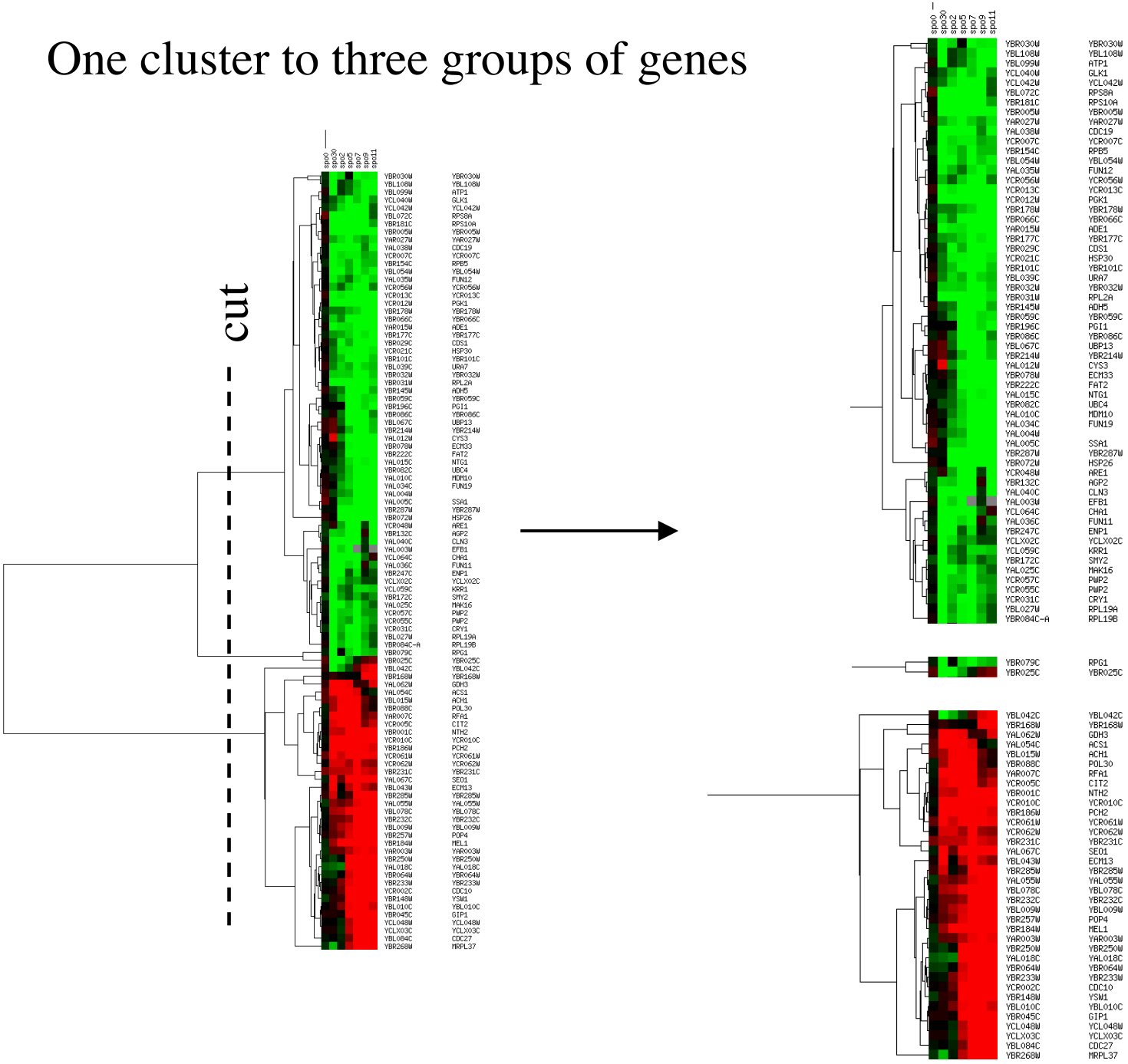
- And how do we know if it’s any good, or if one technique for producing clusters is better than another?

- Rather than think simply of clustering, think of all these methods as capable of producing groups of genes:

One cluster to two groups of genes



One cluster to three groups of genes



Now what?

- Try many methods, and demand they each produce the same number of groups of genes.
- Is there a metric that says which did best for a given number of groups?
- Can we come up with a metric for the best number of groups?

What do we think that co-expression means?

- Our general assumption is guilt by association:

  i.e. genes with similar expression patterns are more likely to participate in the same biological process.
- Therefore, we can exploit the Gene Ontology to assess our clusters:

How do we measure how 'good' the annotation is?

- Use a score that measures how coherent the level of annotation is compared to what would be expected from random clusters.
  - see Gibbons and Roth (2002). *Genome Research* **12**, 1574-1581.
  - Developed system, such that the higher the score, the better the annotation fit the clustering.

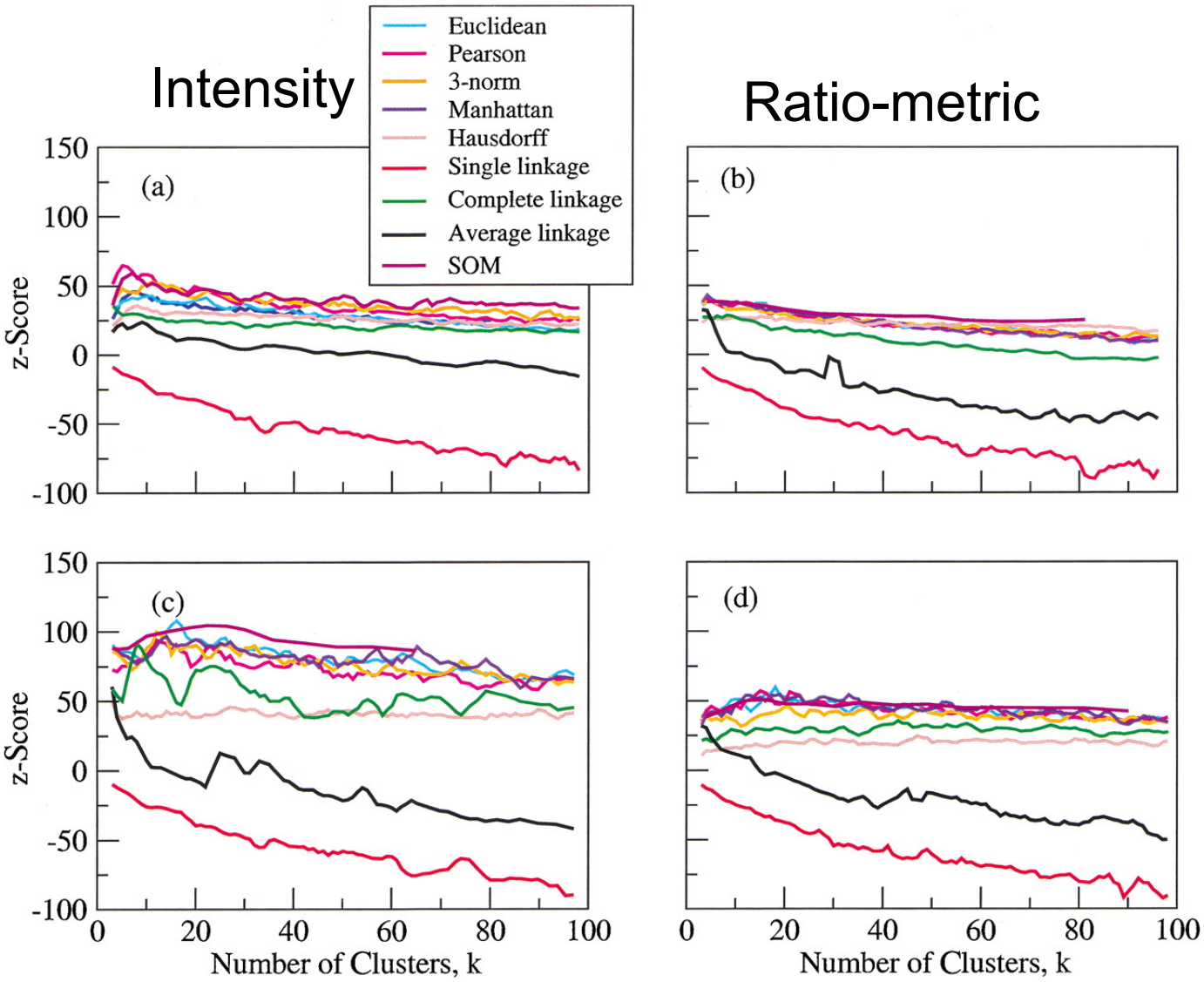


Figure 2. Four data sets clustered using *k*-means, hierarchical, and self-organized map algorithms. The horizontal axis shows the number of clusters desired, and the vertical axis shows *z*-scores. Data sets are (*a*) Cho, (*b*) CJRR, (*c*) Gasch, and (*d*) Spellman.

Gibbons F. D., Roth F. P. *Genome Res*. 2002;**12**:1574-1581

Characterization of clusters

- Now we have groups of genes that best fit their annotation, find the best annotation(s) that fits those groups.
- Calculate P-values for each GO term's association to a cluster, and choose those that are most significant.

Using the Gene Ontology to assess clusters

- Many microarray analyses result in a list of interesting genes
- Typically biologists can make up a story about any random list
- So, look at all GO annotations for the genes in a list, and see if the number of annotations for any GO node is significant

The Categories of GO

(The Gene Ontology)

- *Biological Process* = goal or objective (Why)

(e.g. DNA replication, Cell Cycle Control, Cell adhesion)

- *Molecular Function* = elemental activity/task (What)

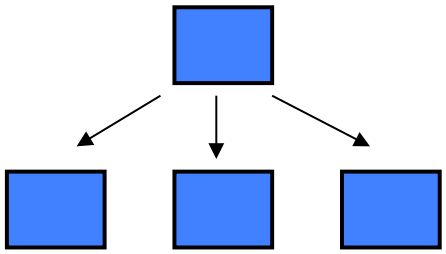
(e.g. Transcription factor, polymerase, protein kinase)

- *Cellular Component* = location or complex (Where)

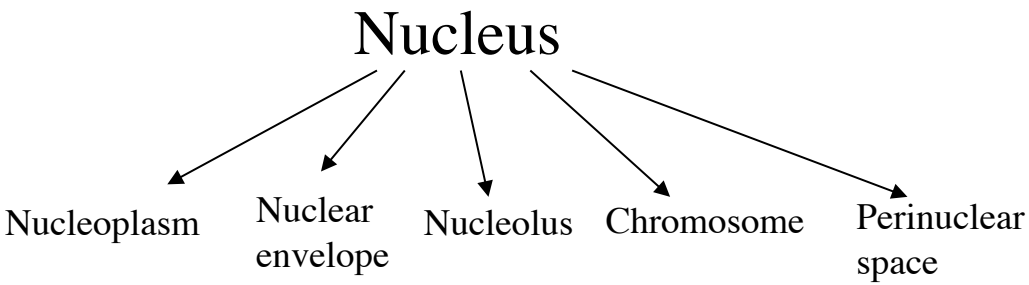
(e.g. pre-replication complex, kinetochore, membrane)

Each Category is a structured, controlled vocabulary

Parent-Child Relationships



A child is a subset of a parent's elements



The cell component term *Nucleus* has 5 children

Determining P-values for GO annotation for a list of genes

We can calculate the probability of having x of n genes having an annotation to a GO node, given that in the genome, M of N genes have that annotation, using the ***hypergeometric distribution***, as:

$$p = \frac{\binom{M}{x}\binom{N-M}{n-x}}{\binom{N}{n}}$$

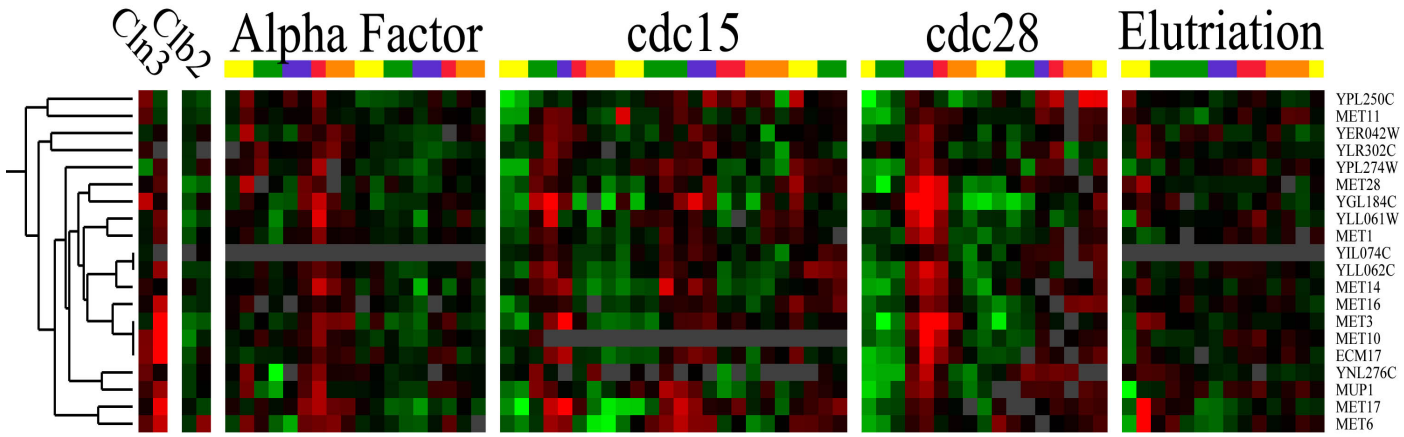
Determining GO significance

To calculate a P-value, we calculate the probability of having ***at least*** x of n annotations:

$$\text{P-value} = 1 - \sum_{i=0}^{x-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{i}}$$

Then do multiple hypothesis correction on the p-values

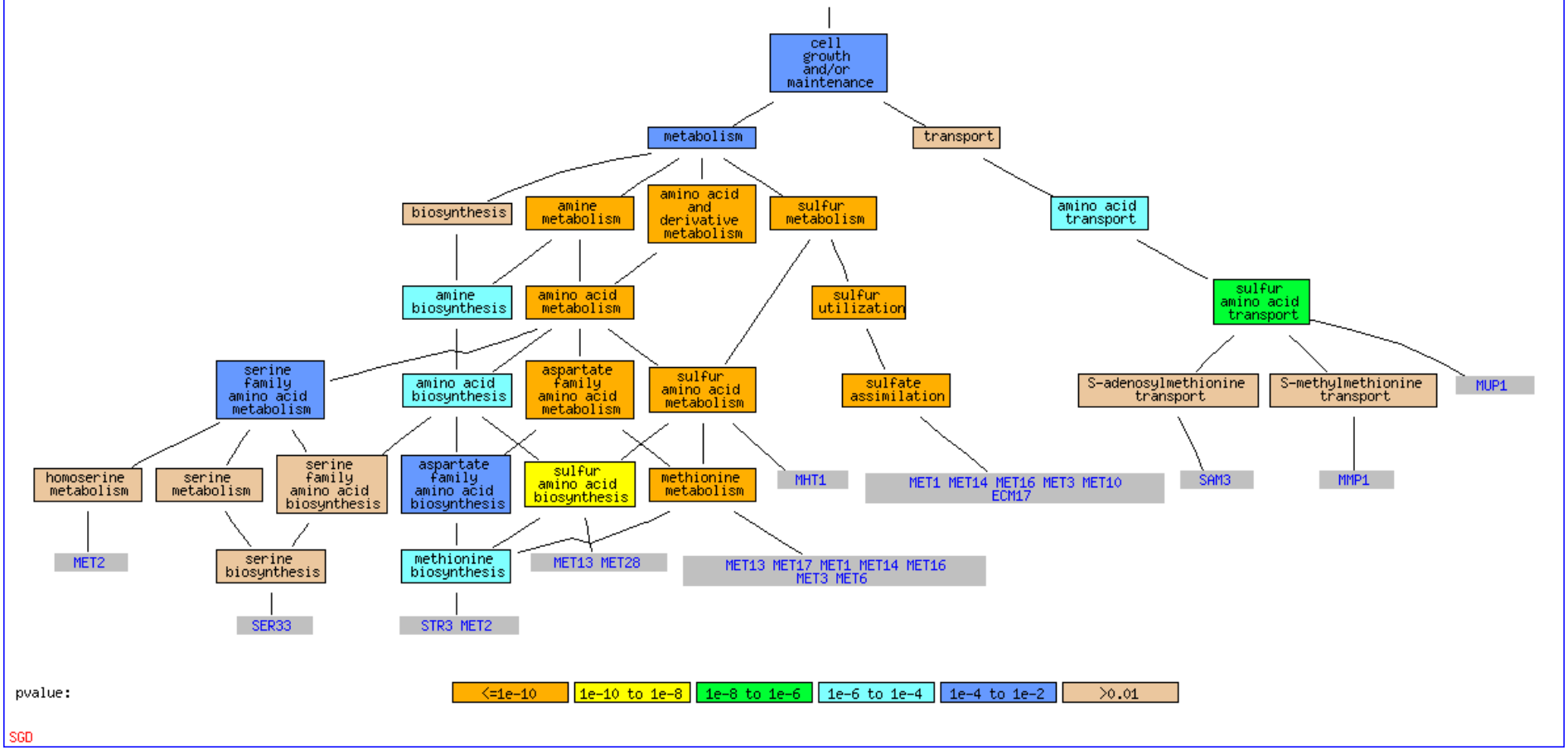
Methionine Cluster



YPL250C
MET11
YER042W
YLR302C
YPL274W
MET28
YGL184C
YLL061W
MET1
YIL074C
YLL062C
MET14
MET16
MET3
MET10
ECM17
YNL276C
MUP1
MET17
MET6

GO Annotations

- sulfur metabolic process : 2.43e-19 (12/18 vs 66/6608)
- methionine metabolic process : 1.40e-14 (10/18 vs 24/6608)



Recommended reading : Clustering

- **Eisen MB, Spellman PT, Brown PO, Botstein D.** (1998). Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci U S A* **95**(25):14863-8.
- **Tamayo P, Slonim D, Mesirov J, Zhu Q, Kitareewan S, Dmitrovsky E, Lander ES, Golub TR** (1999). Interpreting patterns of gene expression with self-organizing maps: methods and application to hematopoietic differentiation. *Proc Natl Acad Sci USA* **96** (6):2907.
- **Tavazoie S, Hughes JD, Campbell MJ, Cho RJ, Church GM** (1999). Systematic determination of genetic network architecture. *Nat Genet*. **22**(3):281-5.
- **Tusher VG, Tibshirani R, Chu G** (2001). Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci USA* **98**(9):5116-21
- **Slonim DK.** (2002). From patterns to pathways: gene expression data analysis comes of age. *Nat Genet*. **32** Suppl:502-8.
- **McShane LM, Radmacher MD, Freidlin B, Yu R, Li MC, Simon R.** (2002). Methods for assessing reproducibility of clustering patterns observed in analyses of microarray data. *Bioinformatics* **18**(11):1462-9.
- **Bryan J** (2004). Problems in gene clustering based on gene expression data. *Journal of Multivariate Analysis* **90**, 44–66.
- **D'haeseleer P** (2005). How does gene expression clustering work? *Nat Biotechnol*. **23** (12):1499-501.
- **Chipman H and Tibshirani R** (2006). Hybrid Hierarchical Clustering with Applications to Microarray Data. *Biostatistics*, **7**(2):286-301.

Recommended reading for Cluster Validation / Analysis

- **Yeung KY, Haynor DR, Ruzzo WL.** (2001). Validating clustering for gene expression data. *Bioinformatics* **17**, 309-318.
- **Gibbons FD, Roth FP.** (2002). Judging the quality of gene expression-based clustering methods using gene annotation. *Genome Res*. **12**(10):1574-81.
- **Slonim DK.** (2002). From patterns to pathways: gene expression data analysis comes of age. *Nat Genet*. **32** Suppl:502-8.
- **McShane LM, Radmacher MD, Freidlin B, Yu R, Li MC, Simon R.** (2002). Methods for assessing reproducibility of clustering patterns observed in analyses of microarray data. *Bioinformatics* **18**(11):1462-9.
- **Zhou X, Kao MC, Wong WH.** (2002). Transitive functional annotation by shortest-path analysis of gene expression data. *Proc Natl Acad Sci U S A*. **99**(20):12783-8.
- **Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, Groop LC.** (2003). PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet*. **34**, 267-73.
- **Breitling R, Amtmann A, Herzyk P** (2004). Iterative Group Analysis (iGA): a simple tool to enhance sensitivity and facilitate interpretation of microarray experiments. *BMC Bioinformatics* **5**(1):34.
- **Boyle EI, Weng S, Gollub J, Jin H, Botstein D, Cherry JM, Sherlock G** (2004). GO::TermFinder--open source software for accessing Gene Ontology information and finding significantly enriched Gene Ontology terms associated with a list of genes. *Bioinformatics*. **20**(18):3710-5.
- **Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP** (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A*. **102**, 15545-50.
- **Handl J, Knowles J, Kell DB** (2005). Computational cluster validation in post-genomic data analysis. *Bioinformatics*. **21**(15):3201-12.