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Ernest Fraenkel

Structure of the Unit

- Wet lab and computational lab focus on measuring and understanding response to a drug (etoposide) in cell culture
- In the computational labs, you will compare etoposide changes in 20.109 data and a published dataset.
- We will use concepts you have seen in 6.0002 to analyze these data.
- Computational assignments will give you the building blocks for your written report.
- The lab will be conducted in the programming environment called “R”.

Why R?

Lecture Schedule

Date	Topic
March 10th	Clustering and PCA
March 12th	Analyzing RNA-Seq
March 17th	Big Data for BE
March 19th	Transcriptional Regulation
SPRING BREAK	
March 31st	Molecular Networks
April 2nd	Single-cell Analysis

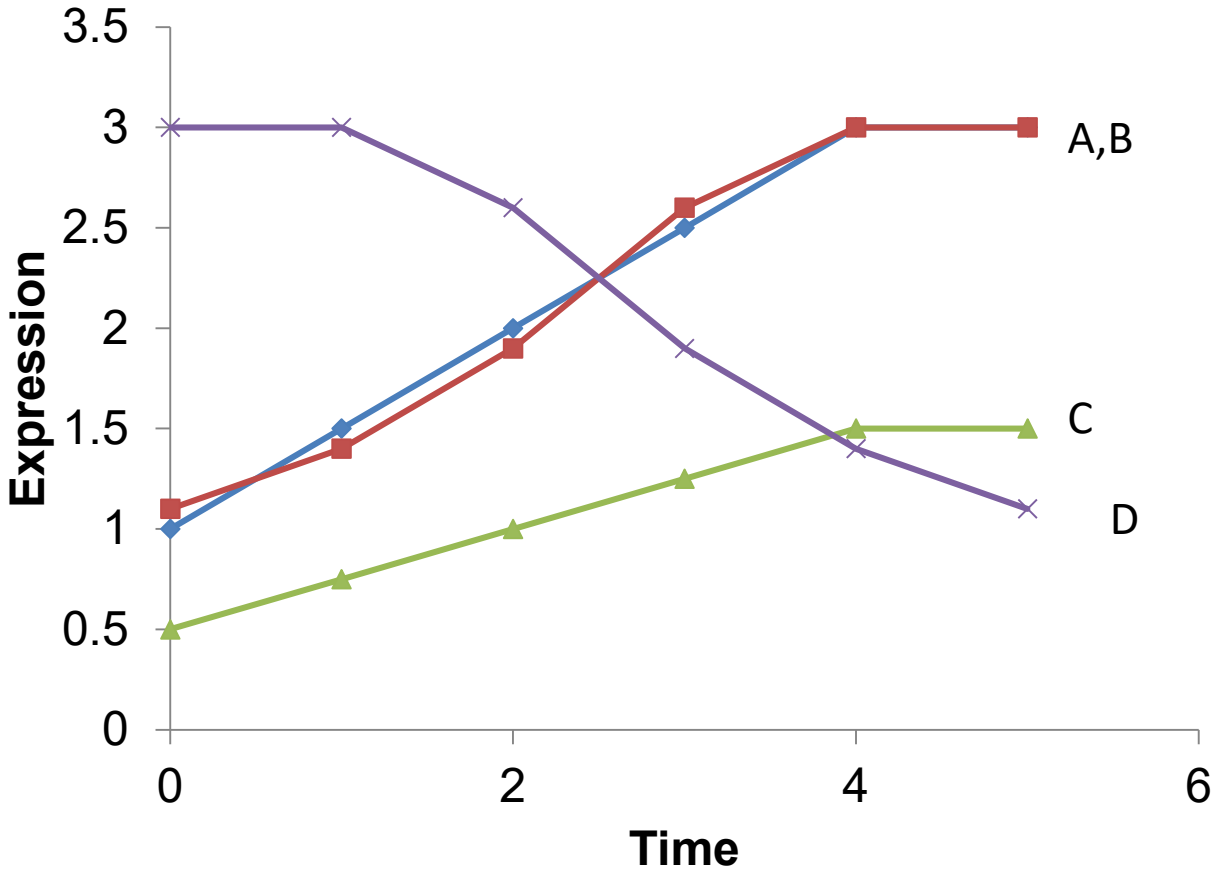
Write on Board Before Class:

Learning Objectives

- See the big picture of this unit
- Choose the right distance metric to compare the expression of two genes
- Describe why you would cluster expression by genes or experiments
- Manually cluster small vectors using hierarchical or k-means clustering
- Read a dendrogram
- Describe the results of Principal Component Analysis (PCA)

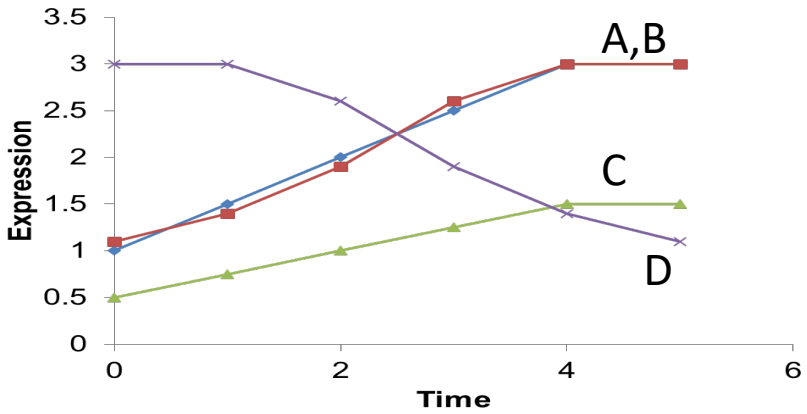
Comparing
the Expression of Genes

Draw on LEFT Board and keep



Comparing gene expression

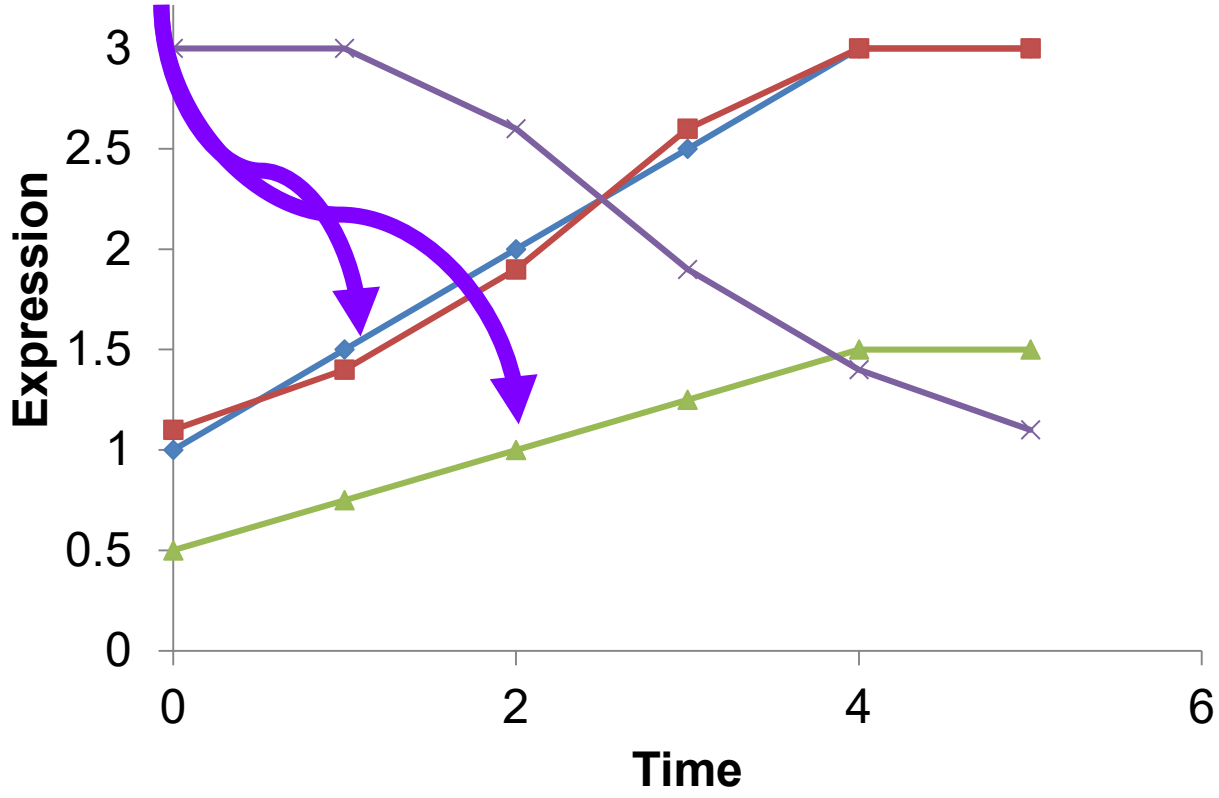
- Draw gene expression patterns on board
- Which of the genes on this plot are most similar?
- How do we quantify similarity of expression?
- Let's consider the simplest description first.
  - A and B are most similar.
  - Euclidean distance would describe this type of similarity.



Distance Metrics

Which other pairs of genes might be co-regulated?

Can we capture the similarity of these patterns?



Euclidean distance provides an intuitive description:

In our timecourse:

$X_A=(x_{A1}, x_{A2},\ldots x_{AN})$

$X_b=(x_{B1}, x_{B2},\ldots x_{BN})$

$$d(X_A, X_B) = \sqrt{\sum_{i=1}^{N}(x_{Ai} - x_{Bi})^2}$$

Pearson Correlation

- To understand Pearson Correlation, we need to define a Z-score
- Z_{Ai}= z-score of gene *A* in experiment i:

Z-score

$$Z_{Ai} = \frac{X_{Ai} - \bar{X}_A}{\sigma}$$

Standard deviation

$$\sigma = \sqrt{\frac{\sum(X_{Ai} - \bar{X}_A)^2}{N}}$$

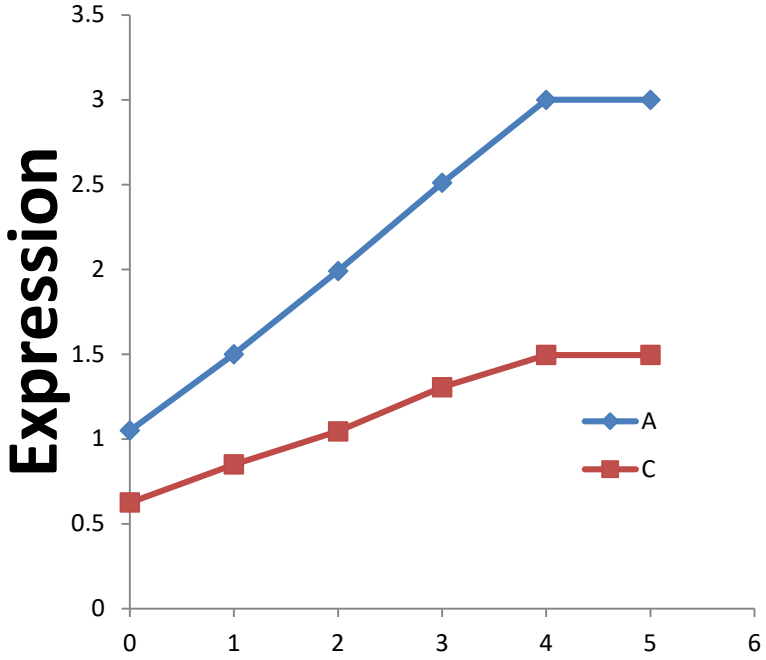
over all experiments

$$r_{A,B} = \frac{\sum_{i=1}^{N_{expt}} Z_{Ai} Z_{Bi}}{N}$$

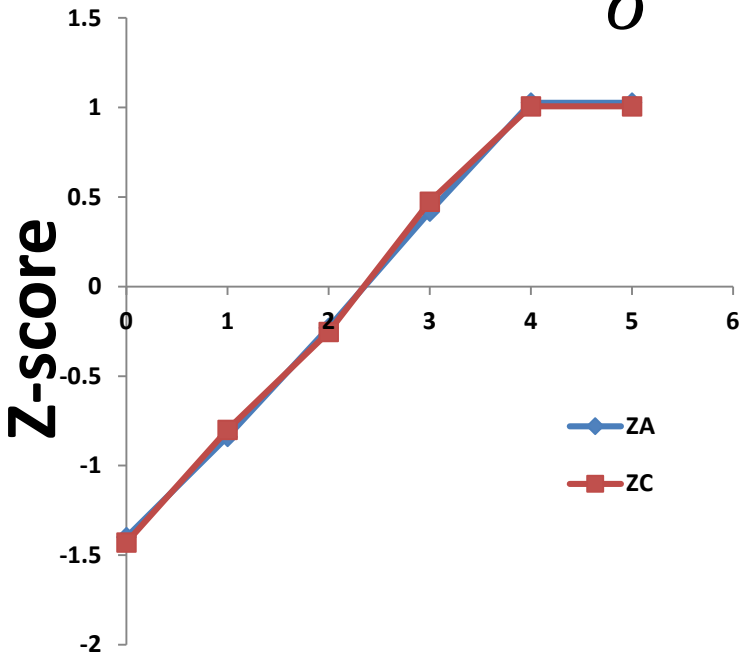
Pearson correlation

from +1 (perfect correlation) to -1 (anti-correlated)

Distance = $1-r_{A,B}$

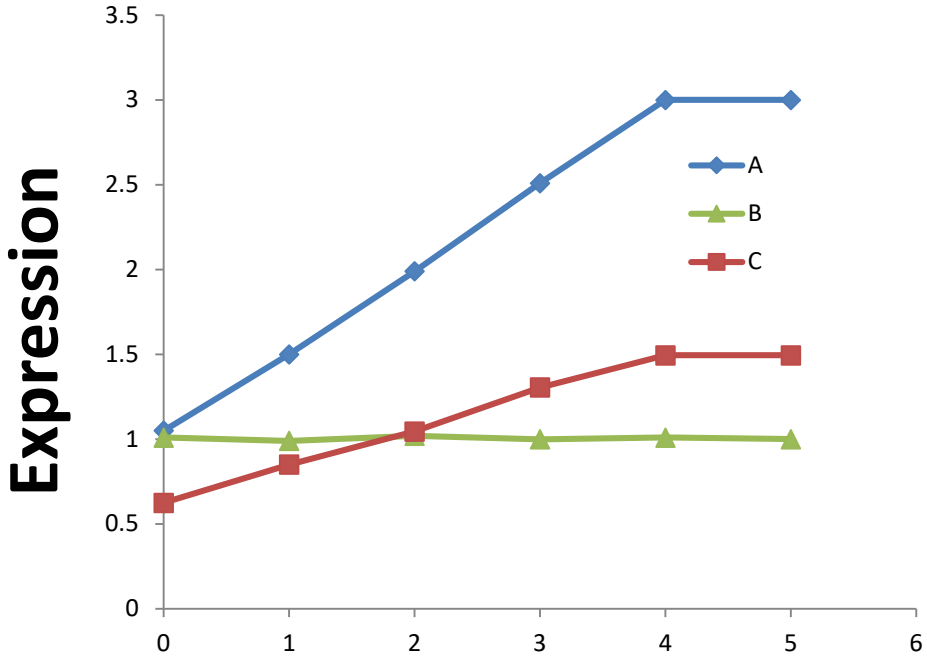


$$Z_{Ki} = \frac{X_{Ki} - \bar{X}_K}{\sigma}$$



$r_{A,C}$= 0.999

$$r_{A,B} = \frac{\sum_{i=1}^{N_{expt}} Z_{Ai} Z_{Bi}}{N}$$



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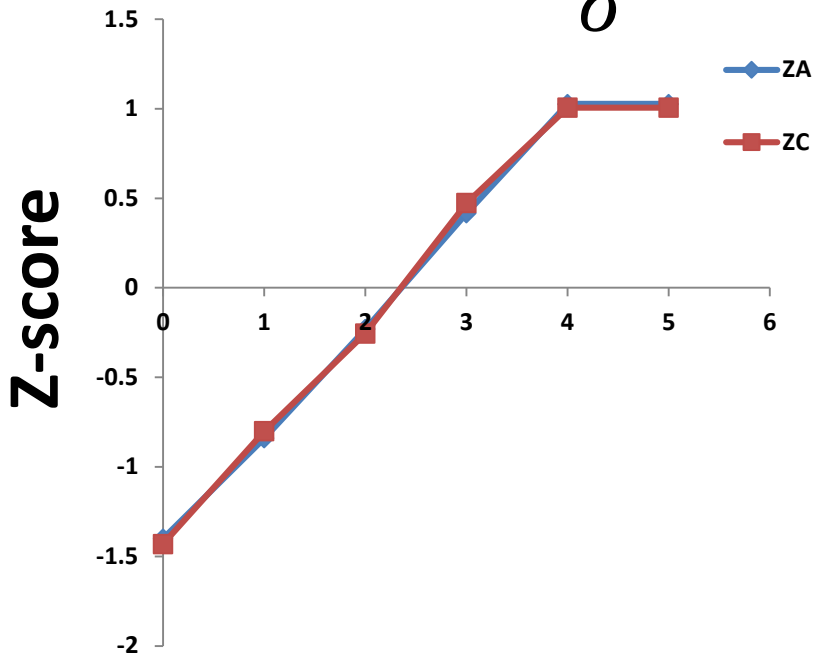
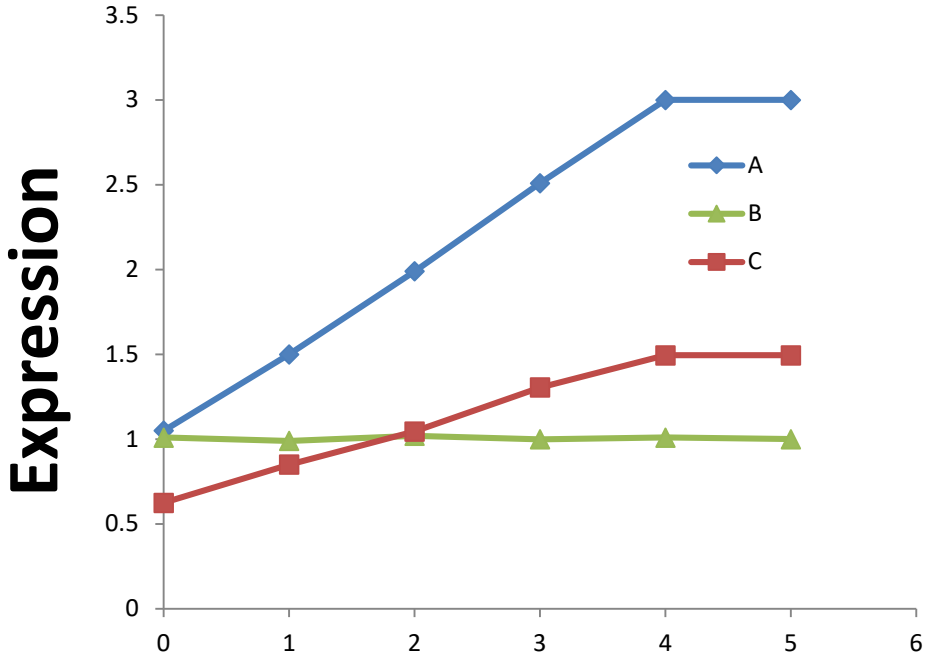


$$r_{A,B} = \frac{\sum_{i=1}^{N_{expt}} Z_{Ai} Z_{Bi}}{N}$$



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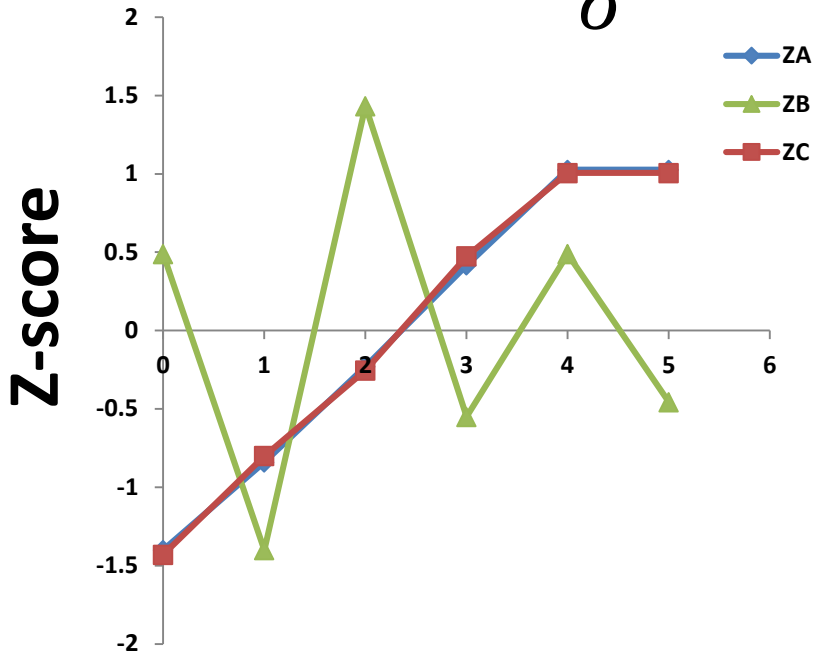


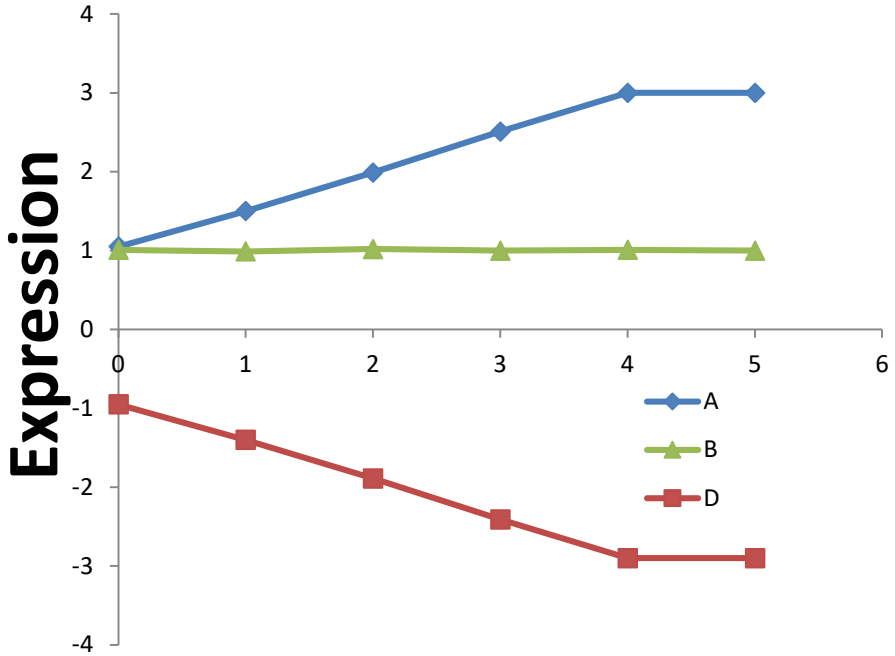
$r_{A,B}$= -0.01

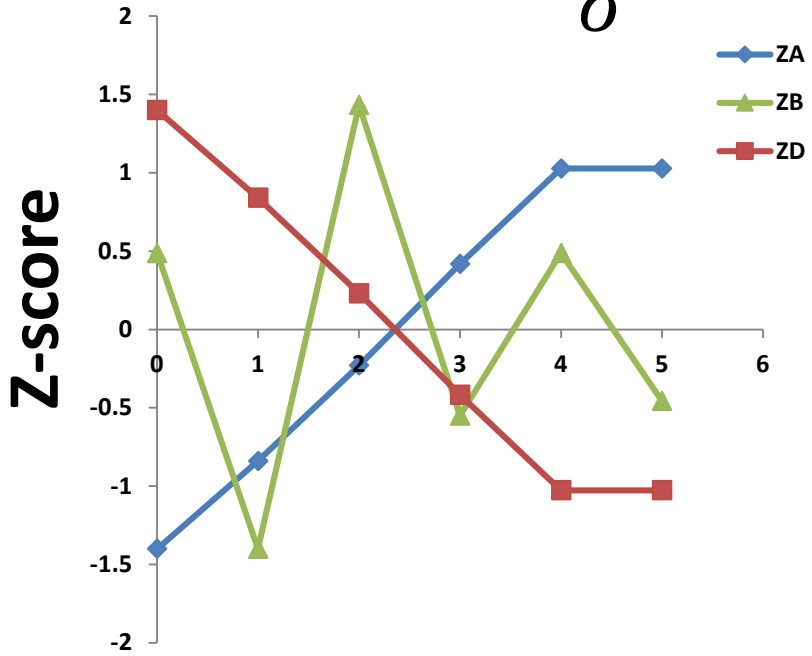
$r_{A,C}$= 0.999

$r_{B,C}$= -0.03

$$r_{A,B} = \frac{\sum_{i=1}^{N_{expt}} Z_{Ai} Z_{Bi}}{N}$$

$$Z_{Ki} = \frac{X_{Ki} - \bar{X}_K}{\sigma}$$





$r_{A,B}$= -0.01

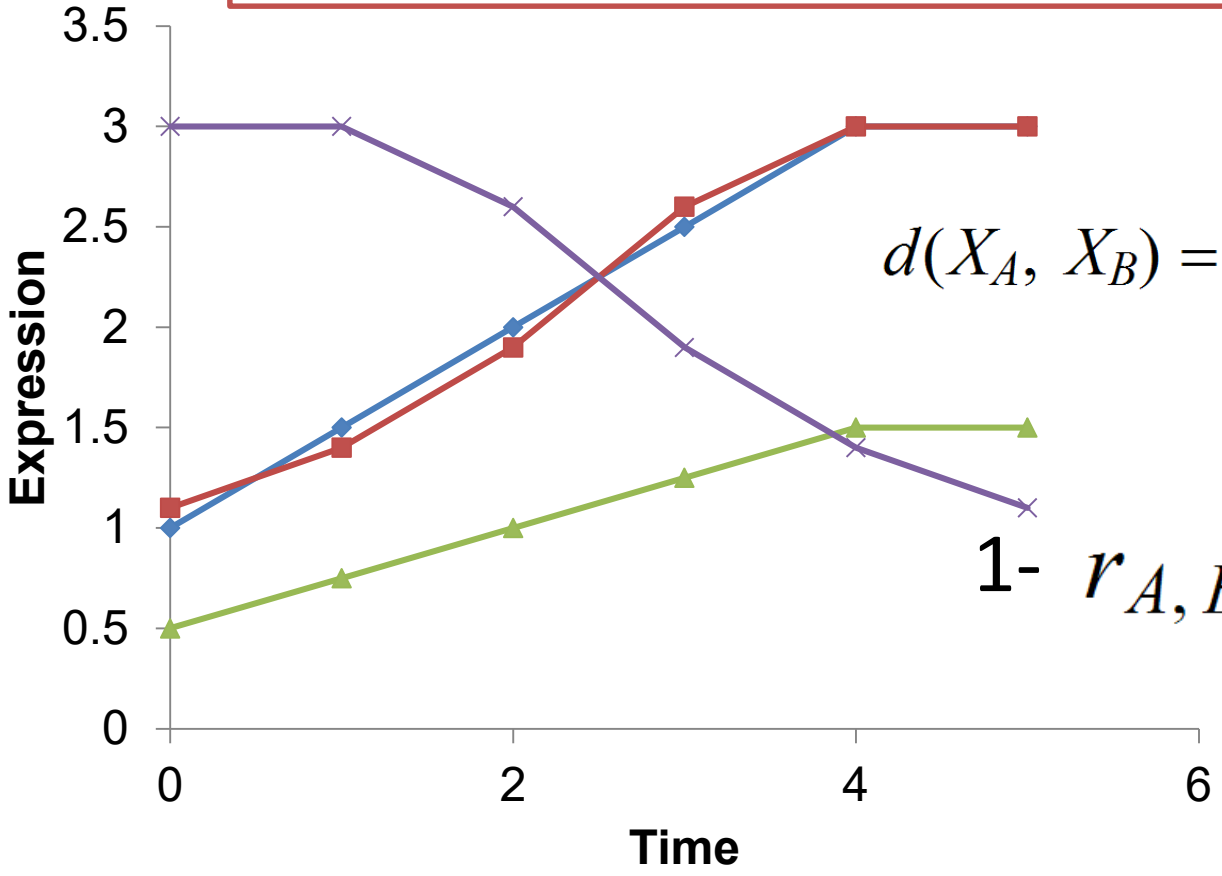
$r_{A,D}$= -1.0

$r_{B,D}$= 0.007

$$r_{A,B} = \frac{\sum_{k=1}^{N_{expt}} Z_{kA} Z_{kB}}{N}$$

Distance Metrics

Which would you use to find co-regulated genes?



$$d(X_A,\ X_B) = \sqrt{\sum_{k=1}^{N} (X_{A,k} - X_{B,k})^2}$$

$$1\text{-}\ r_{A,B} = 1\text{-}\frac{\sum Z_A Z_B}{N}$$

Write on Board: Learning Objectives

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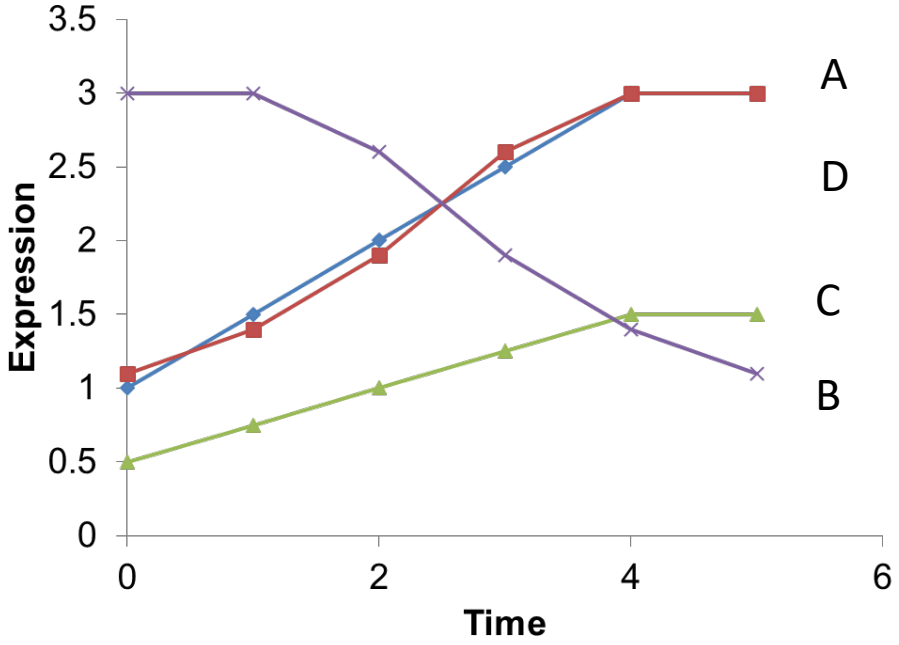
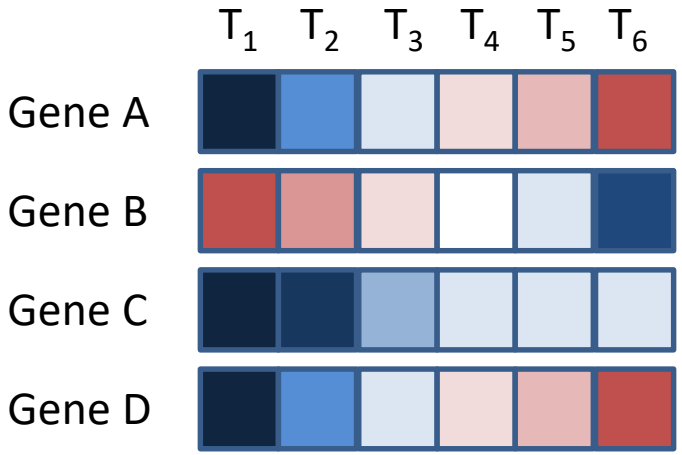
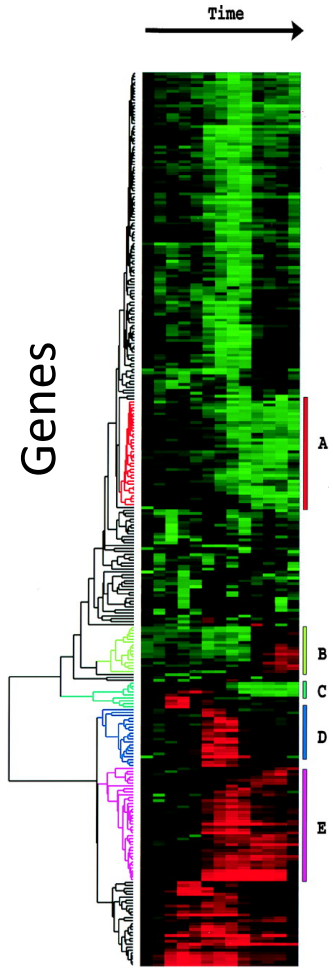
Many ways to plot expression



Heatmap



Many ways to plot expression



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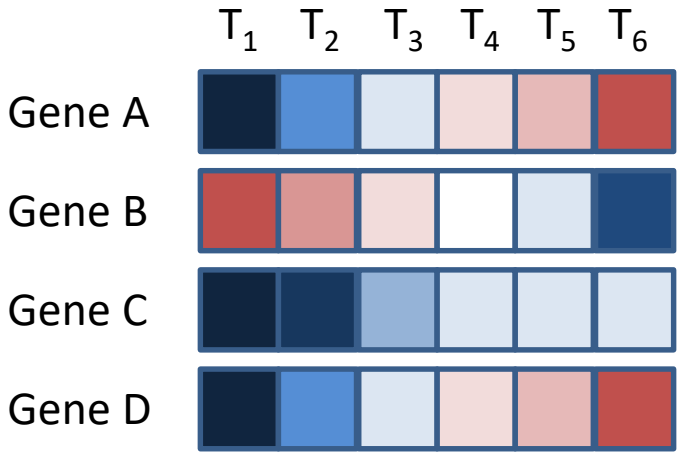
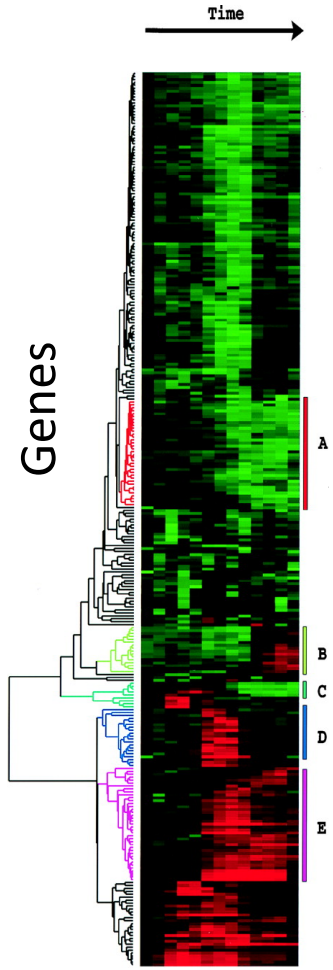
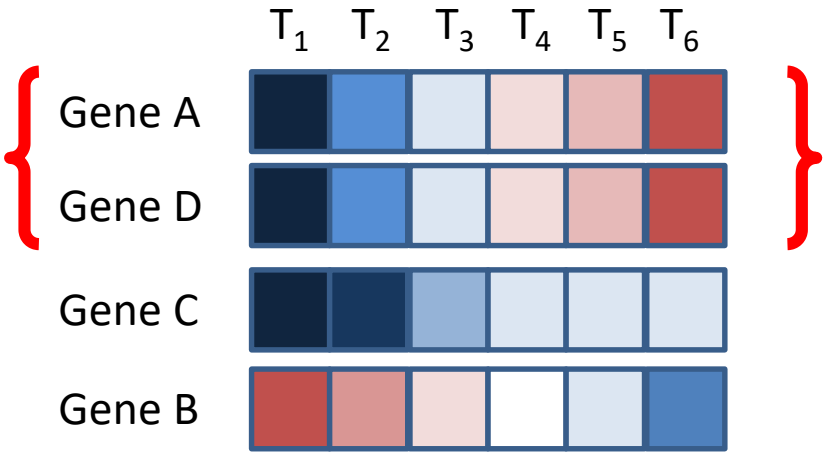
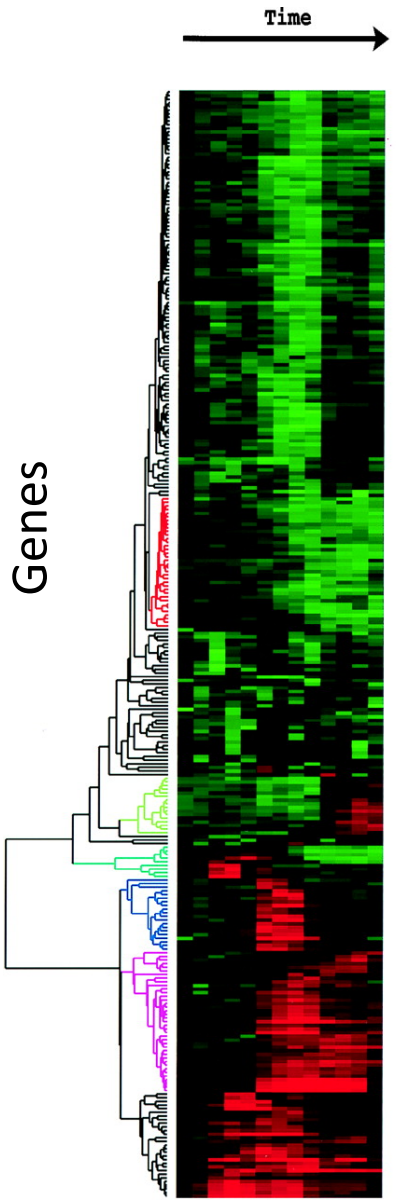


Clustering







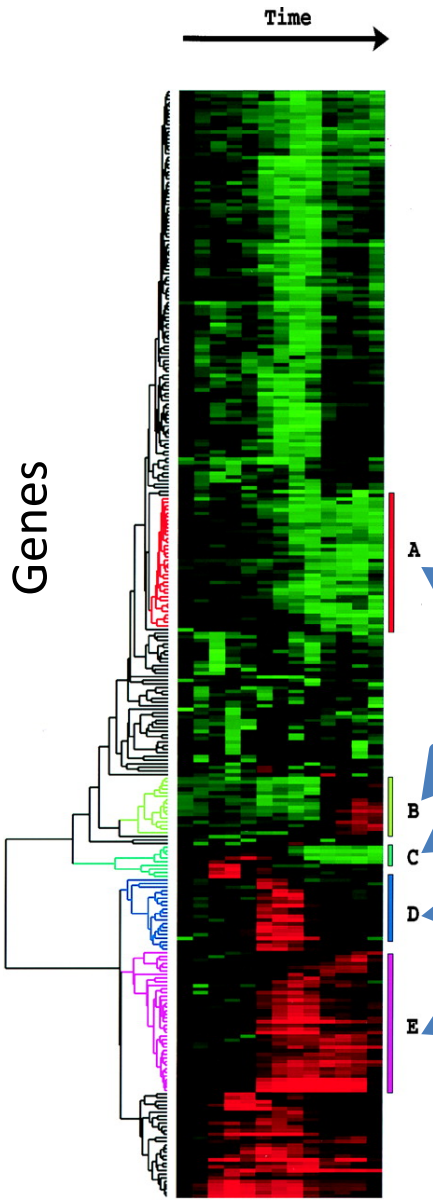
Clustering 8600 human genes based on time course of expression following serum stimulation of fibroblasts

Key: Black = little change Green = down Red = up

(relative to initial time point)

What can you learn from the clustering genes?

Iyer et al. *Science* 1999



Clustering 8600 human genes based on time course of expression following serum stimulation of fibroblasts

Key: Black = little change Green = down Red = up

(relative to initial time point)

Why might you cluster experiments?

(A) cholesterol biosynthesis

(B) the cell cycle

(C) the immediate-early response

(D) signaling and angiogenesis

(E) wound healing and tissue remodeling

Iyer et al. *Science* 1999

Why cluster?

- Cluster genes (rows)
  - Measure expression at multiple time-points, different conditions, etc.

Similar expression patterns may suggest similar functions of genes

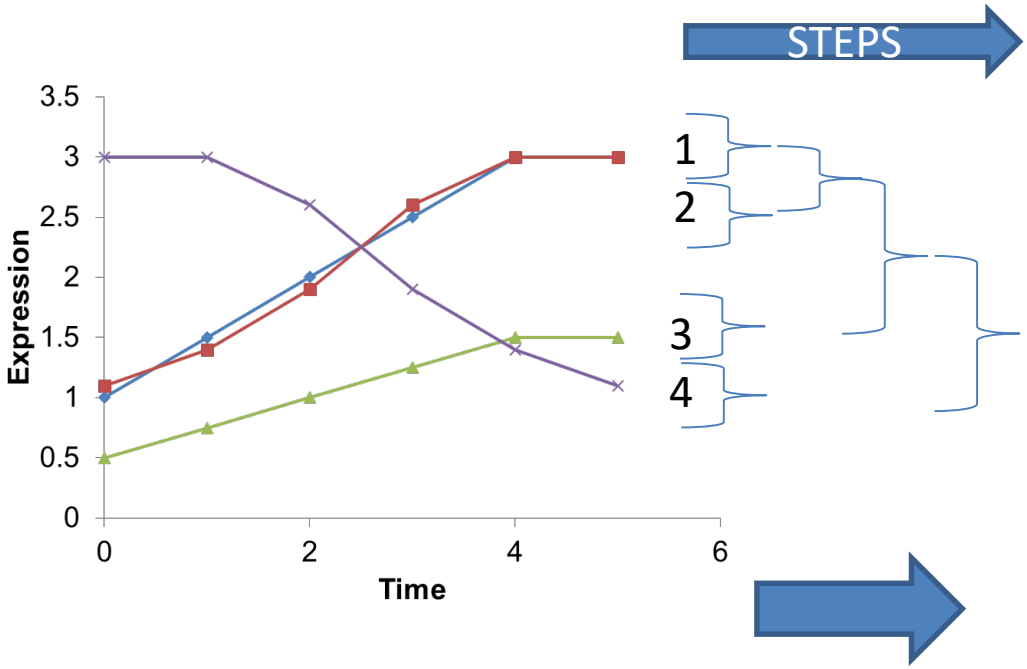
- Cluster samples (columns)
  - e.g., expression levels of thousands of genes for each tumor sample

Similar expression patterns may suggest biological relationship among samples

Two types of approaches: Agglomerative & Divisive

Agglomerative:

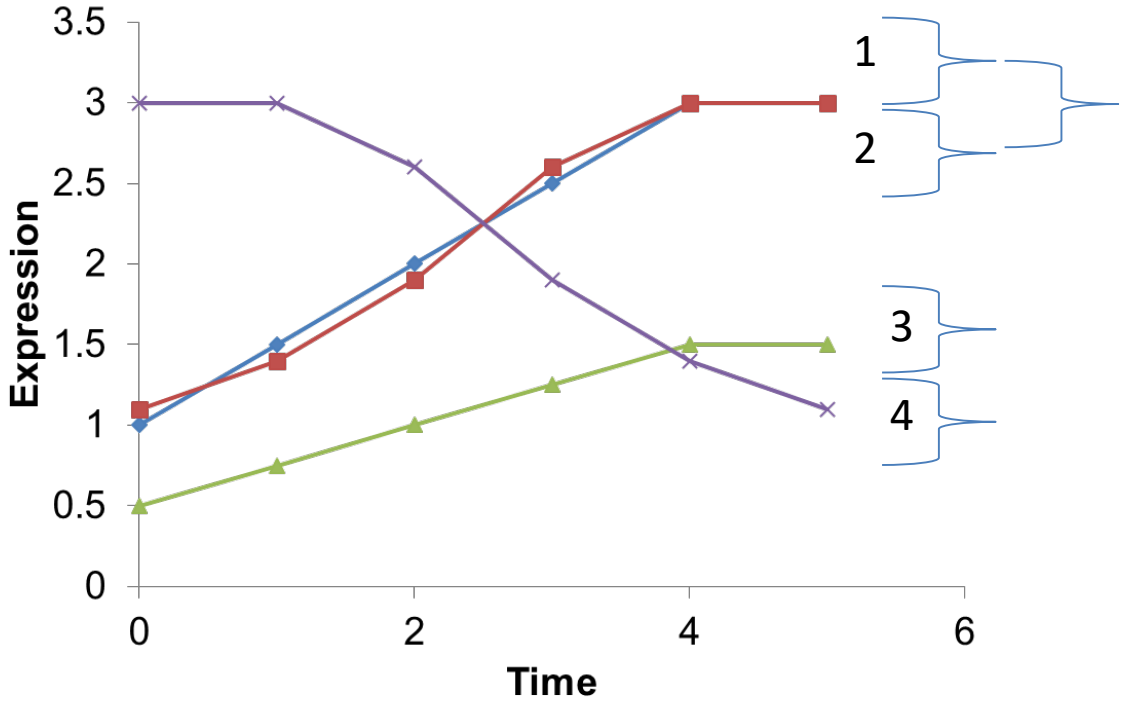
- Initialize: Each vector is in its own cluster
- Repeat until there is only one cluster:
  - Merge the two most similar clusters.



Step 1: each gene is its own cluster
Step 2: combine the two most similar genes
Step 3: find the two most similar clusters

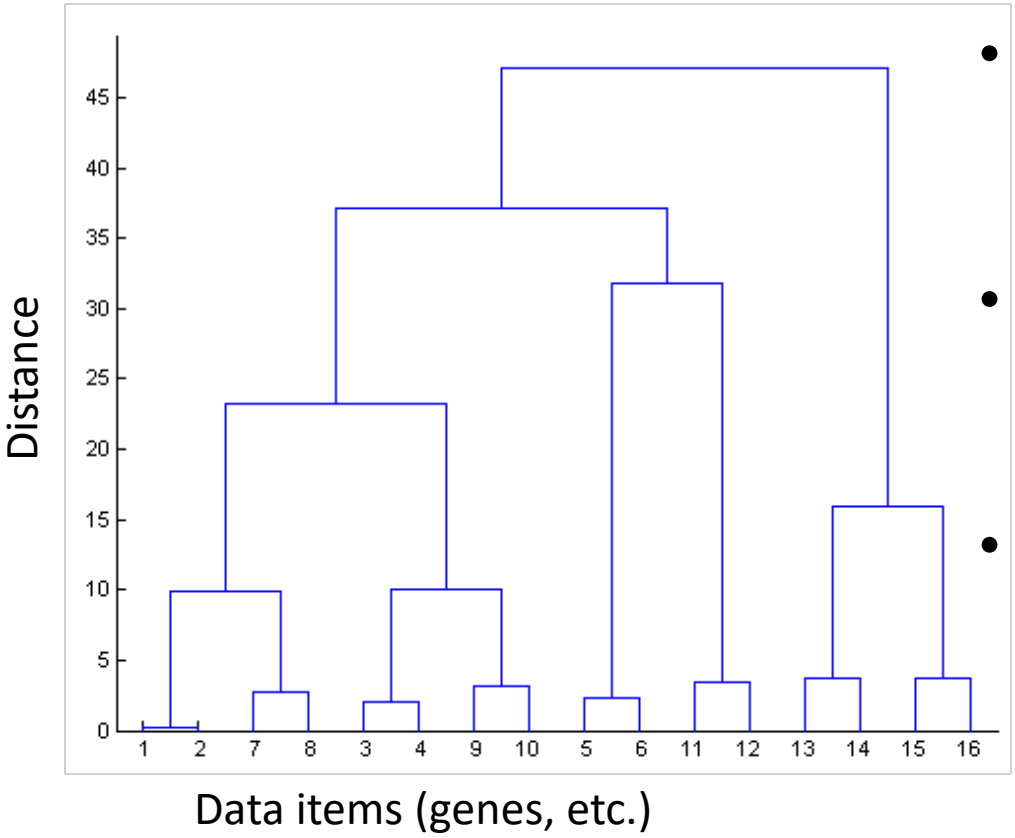
Several options:

minimum distance between members of cluster A,B
maximum distance between members of cluster A,B
average distance between members of cluster A,B



… but I have not told you how to compute distance between the two genes in the new cluster with individual genes

Dendrograms



- The final cluster is the root and each data item is a leaf
- The heights of the bars indicate how close the items are
- Can ‘slice’ the tree at any distance cutoff to produce discrete clusters
- The results will always be hierarchical, even if the data are not.
- The order of the leaf nodes is not meaningful