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www.cis.fiu.edu/~giri/teach/BioinfS13.html
Gene Expression

- Process of transcription and/or translation of a gene is called gene expression.
- Every cell of an organism has the same genetic material, but different genes are expressed at different times.
- Patterns of gene expression in a cell is indicative of its state.
Hybridization

- If two complementary strands of DNA or mRNA are brought together under the right experimental conditions they will \textit{hybridize}.

- \textit{A hybridizes to B} \implies
  - A is reverse complementary to B, or
  - A is reverse complementary to a subsequence of B.

- It is possible to experimentally verify whether A hybridizes to B, by \textit{labeling} A or B with a radioactive or fluorescent tag, followed by excitation by laser.
Gene expression for a single gene can be measured by extracting mRNA from the cell and doing a simple hybridization experiment.

Given a sample of cells, gene expression for every gene can be measured using a single microarray experiment.
Microarray/DNA chip technology

- High-throughput method to study gene expression of thousands of genes simultaneously.

- Many applications:
  - Genetic disorders & Mutation/polymorphism detection
  - Study of disease subtypes
  - Drug discovery & toxicology studies
  - Pathogen analysis
  - Differing expressions over time, between tissues, between drugs, across disease states
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Gene Chips
DNA Chips & Images
<table>
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<tr>
<th>Probe 1</th>
<th>Probe 2</th>
<th>...</th>
<th>Probe N</th>
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</table>

Gene g
Microarray/DNA chips (Simplified)

- Construct probes corresponding to reverse complements of genes of interest.
- Microscopic quantities of probes placed on solid surfaces at defined spots on the chip.
- Extract mRNA from sample cells and label them.
- Apply labeled sample (mRNA extracted from cells) to every spot, and allow hybridization.
- Wash off unhybridized material.
- Use optical detector to measure amount of fluorescence from each spot.
Affymetrix DNA chip schematic

www.affymetrix.com
What’s on the slide?

RNA fragments with fluorescent tags from sample to be tested

Shining a laser light at GeneChip® array causes tagged DNA fragments that hybridized to glow

RNA fragment hybridizes with DNA on GeneChip® array

Non-hybridized DNA

Hybridized DNA
Microarrays: competing technologies

- **Affymetrix & Agilent**
- **Differ in:**
  - Method to place DNA: Spotting vs. photolithography
  - Length of probe
  - Complete sequence vs. series of fragments
Sample

Treated Sample(t1)

Treated Sample(t2)

Treated Sample(t3)

...  

Treated Sample(tn)

Study effect of treatment over time
2-color DNA microarray

- Treated mRNA → Cy5 Probe
- Control mRNA → Cy3 Probe

Normalization → Data extraction → Scanning → Simultaneous hybridization
How to compare 2 cell samples with Two-Color Microarrays?

- mRNA from sample 1 is extracted and labeled with a red fluorescent dye.
- mRNA from sample 2 is extracted and labeled with a green fluorescent dye.
- Mix the samples and apply it to every spot on the microarray. Hybridize sample mixture to probes.
- Use optical detector to measure the amount of green and red fluorescence at each spot.
Sources of Variations & Experimental Errors

- Variations in cells/individuals
- Variations in mRNA extraction, isolation, introduction of dye, variation in dye incorporation, dye interference
- Variations in probe concentration, probe amounts, substrate surface characteristics
- Variations in hybridization conditions and kinetics
- Variations in optical measurements, spot misalignments, discretization effects, noise due to scanner lens and laser irregularities
- Cross-hybridization of sequences with high sequence identity
- Limit of factor 2 in precision of results
- Variation changes with intensity: larger variation at low or high expression levels

Need to Normalize data
Clustering

- Clustering is a general method to study patterns in gene expressions.
- Several known methods:
  - Hierarchical Clustering (Bottom-Up Approach)
  - K-means Clustering (Top-Down Approach)
  - Self-Organizing Maps (SOM)
Hierarchical Clustering: Example
A Dendrogram
Hierarchical Clustering [Johnson, SC, 1967]

- Given $n$ points in $\mathbb{R}^d$, compute the distance between every pair of points.
- While (not done)
  - Pick closest pair of points $s_i$ and $s_j$ and make them part of the same cluster.
  - Replace the pair by an average of the two $s_{ij}$

Try the applet at:
http://home.dei.polimi.it/matteucc/Clustering/tutorial_html/AppletH.html
Distance Metrics

For clustering, define a distance function:

- **Euclidean distance metrics**
  \[ D_k(X, Y) = \sqrt{\frac{1}{d} \sum_{i=1}^{d} (X_i - \bar{X})^k (Y_i - \bar{Y})^k} \]  
  \( k=2: \) Euclidean Distance

- **Pearson correlation coefficient**
  \[ x_{xy} = \frac{1}{d} \sum_{i=1}^{d} \frac{X_i - \bar{X}}{\sigma_x} \cdot \frac{Y_i - \bar{Y}}{\sigma_y} \]  
  \(-1 \leq \rho_{xy} \leq 1\)
EXHIBIT 3.4  Joint Probability Model for the Ratings of Two People

(a) $\rho_{xy} = 0$

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(b) $\rho_{xy} = \frac{1}{2}$

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(c) $\rho_{xy} = -\frac{1}{2}$

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(d) $\rho_{xy} = \frac{1}{3}$

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(e) $\rho_{xy} = -\frac{1}{3}$

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(f) $\rho_{xy} = \frac{2}{3}$

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(g) $\rho_{xy} = -\frac{2}{3}$

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Clustering of gene expressions

- Represent each gene as a vector or a point in $d$-space where $d$ is the number of arrays or experiments being analyzed.
Clustering Random vs. Biological Data

Example from Andrew Moore’s tutorial on Clustering.
K-means
1. Ask user how many clusters they’d like. (e.g., k=5)
2. Randomly guess k cluster center locations
3. Each datapoint finds out which center it’s closest to.
4. Each cluster finds the centroid of the points it owns.

Start
K-means
Start
Advance apologies: in Black and White this example will deteriorate
Example generated by Dan Pelleg's super-duper fast K-means system:
Dan Pelleg and Andrew Moore. Accelerating Exact K-means Algorithms with
Geometric Reasoning. Proc. Conference on Knowledge Discovery in Databases 1999,

K-means continues
...

K-means continues
...

K-means continues
...

K-means continues
...
K-means
1. Ask user how many clusters they’d like. (e.g. k=5)
2. Randomly guess k cluster center locations

K-means continues ...

K-means terminates

Copyright © 2001, Andrew W. Moore
K-Means Clustering [McQueen ’67]

Repeat
- Start with randomly chosen cluster centers
- Assign points to give greatest increase in score
- Recompute cluster centers
- Reassign points

until (no changes)

Try the applet at:
http://home.dei.polimi.it/matteucc/Clustering/tutorial_html/AppletH.html
Comparisons

- **Hierarchical clustering**
  - Number of clusters not preset.
  - Complete hierarchy of clusters
  - Not very robust, not very efficient.

- **K-Means**
  - Need definition of a mean. Categorical data?
  - More efficient and often finds optimum clustering.
Functionally related genes behave similarly across experiments

Figure 1: Expression profiles of the cytoplasmic ribosomal proteins. Figure (a) shows the expression profiles from the data in [Eisen et al., 1998] of 121 cytoplasmic ribosomal proteins, as classified by MYGD [MYGD, 1999]. The logarithm of the expression ratio is plotted as a function of DNA microarray experiment. Ticks along the X-axis represent the beginnings of experimental series. They are, from left to right, cell division cycle after synchronization with alpha factor arrest (alpha), cell division cycle after synchronization by centrifugal elutriation (elu), cell division cycle measured using a temperature sensitive cdc15 mutant (cdc), sporulation (spo), heat shock (he), reducing shock (re), cold shock (co), and diauxic shift (di). Sporulation is the generation of a yeast spore by meiosis. Diauxic shift is the shift from anaerobic (fermentation) to aerobic (respiration) metabolism. The medium starts rich in glucose, and yeast cells ferment, producing ethanol. When the glucose is used up, they switch to ethanol as a source for carbon. Heat, cold, and reducing shock are various ways to stress the yeast cell. Figure (b) shows the average, plus or minus one standard deviation, of the data in Figure (a).
Self-Organizing Maps [Kohonen]

- Kind of neural network.
- Clusters data and find complex relationships between clusters.
- Helps reduce the dimensionality of the data.
- Map of 1 or 2 dimensions produced.
- Unsupervised Clustering
- Like K-Means, except for visualization
SOM Architectures

- 2-D Grid
- 3-D Grid
- Hexagonal Grid
SOM Algorithm

- Select SOM architecture, and initialize weight vectors and other parameters.

- **While** (stopping condition not satisfied) do for each input point \( x \)
  - Winning node \( q \) has weight vector closest to \( x \).
  - Update weight vector of \( q \) and its neighbors.
  - Reduce neighborhood size and learning rate.
SOM Algorithm Details

- **Distance between x and weight vector:** \[ \| x - w_i \| \]
- **Winning node:** 
  \[ q(x) = \min_{i} \| x - w_i \| \]
- **Weight update function (for neighbors):** 
  \[ w_i(k+1) = w_i(k) + (k, x, i)[x(k) - w_i(k)] \]
- **Learning rate:** 
  
  \[ (k, x, i) = \sigma(k) \exp \left( \frac{\| r_i - r_{q(x)} \|^2}{2} \right) \]

39 indicators considered e.g., health, nutrition, educational services, etc.

The complex joint effect of these factors can be visualized by organizing the countries using the self-organizing map.
World Poverty SOM
Viewing SOM Clusters on PCA axes
SOM Example [Xiao-rui He]
Neural Networks

Input $X$ \rightarrow \Sigma \rightarrow f(\cdot) \rightarrow Output y

Synaptic Weights $W$

Bias $\theta$
Learning NN

Input $X$

Weights $W$

Adaptive Algorithm

Error

Desired Response
Types of NNs

- Recurrent NN
- Feed-forward NN
- Layered

Other issues

- Hidden layers possible
- Different activation functions possible
Application: Secondary Structure Prediction

- Identical for all positions in the window
- Identical for all positions in the window
PCR and Sequencing
Polymerase Chain Reaction (PCR)

- For testing, large amount of DNA is needed
  - Identifying individuals for forensic purposes
    - (0.1 microliter of saliva contains enough epithelial cells)
  - Identifying pathogens (viruses and/or bacteria)
- PCR is a technique to amplify the number of copies of a specific region of DNA.
- Useful when exact DNA sequence is unknown
- Need to know “flanking” sequences
- Primers designed from “flanking” sequences
PCR

DNA

Flanking Regions with known sequence

Region to be amplified

Forward Primer

Reverse Primer

Millions of Copies
Polymerase Chain Reaction (PCR)
Schematic outline of a typical PCR cycle

- Target DNA
- Primers
- dNTPs
- DNA polymerase
Polymerase Chain Reaction

1. DNA is denatured. Primers attach to each strand. A new DNA strand is synthesized behind primers on each template strand.

2. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

3. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

4. Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

5. Continued rounds of amplification swiftly produce large numbers of identical fragments. Each fragment contains the DNA region of interest.

Picture Copyright: AccessExcellence @ the National Museum of Health
Gel Electrophoresis

- Used to measure the lengths of DNA fragments.
- When voltage is applied to DNA, different size fragments migrate to different distances (smaller ones travel farther).
Gel Electrophoresis: Measure sizes of fragments

- The phosphate backbone makes DNA a highly negatively charged molecule.
- DNA can be separated according to its size.
- **Gel**: allow hot 1% solution of purified agarose to cool and solidify/polymerize.
- DNA sample added to wells at the top of a gel and voltage is applied. Larger fragments migrate through the pores slower.
- Varying concentration of agarose makes different pore sizes & results.
- Proteins can be separated in much the same way, only acrylamide is used as the crosslinking agent.
Gel Electrophoresis
Gel Electrophoresis
Why sequencing?

- Useful for further study:
  - Locate gene sequences, regulatory elements
  - Compare sequences to find similarities
  - Identify mutations
  - Use it as a basis for further experiments

Next 4 slides contains material prepared by Dr. Stan Metzenberg. Also see: http://stat-www.berkeley.edu/users/terry/Classes/s260.1998/Week8b/week8b/node9.html
History

- Two methods independently developed in 1974
  - Maxam & Gilbert method
  - Sanger method: became the standard
- Nobel Prize in 1980
Original Sanger Method

- (Labeled) Primer is annealed to template strand of denatured DNA. This primer is specifically constructed so that its 3' end is located next to the DNA sequence of interest. Once the primer is attached to the DNA, the solution is divided into four tubes labeled "G", "A", "T" and "C". Then reagents are added to these samples as follows:
  - “G” tube: ddGTP, DNA polymerase, and all 4 dNTPs
  - “A” tube: ddATP, DNA polymerase, and all 4 dNTPs
  - “T” tube: ddTTP, DNA polymerase, and all 4 dNTPs
  - “C” tube: ddCTP, DNA polymerase, and all 4 dNTPs

- DNA is synthesized, & nucleotides are added to growing chain by the DNA polymerase. Occasionally, a ddNTP is incorporated in place of a dNTP, and the chain is terminated. Then run a gel.

- All sequences in a tube have same prefix and same last nucleotide.

- [http://www.wellcome.ac.uk/Education-resources/Teaching-and-education/Animations/DNA/WTDV026689.htm](http://www.wellcome.ac.uk/Education-resources/Teaching-and-education/Animations/DNA/WTDV026689.htm)
Example of sequences seen in gel from “G” tube:

5′-GAATGTCCTTTCTCTAAGTCCTAAAG
3′-GGAGACTTACAGGAAAGAGATTCCAGGATTCAGGAGGCTTACCCTGATGAAGATCAAG-5′

5′-GAATGTCCTTTCTCTAAGTCCTAAAG
3′-GGAGACTTACAGGAAAGAGATTCCAGGATTCAGGAGGCTTACCCTGATGAAGATCAAG-5′

5′-GAATGTCCTTTCTCTAAGTCCTAAAG
3′-GGAGACTTACAGGAAAGAGATTCCAGGATTCAGGAGGCTTACCCTGATGAAGATCAAG-5′

5′-GAATGTCCTTTCTCTAAGTCCTAAAG
3′-GGAGACTTACAGGAAAGAGATTCCAGGATTCAGGAGGCTTACCCTGATGAAGATCAAG-5′

5′-GAATGTCCTTTCTCTAAGTCCTAAAG
3′-GGAGACTTACAGGAAAGAGATTCCAGGATTCAGGAGGCTTACCCTGATGAAGATCAAG-5′

5′-GAATGTCCTTTCTCTAAGTCCTAAAG
3′-GGAGACTTACAGGAAAGAGATTCCAGGATTCAGGAGGCTTACCCTGATGAAGATCAAG-5′
**Modified Sanger**

- Reactions performed in a single tube containing all four ddNTP's, each labeled with a different color dye.

![Dye-labeled nucleotides diagram]
Sequencing Gels: Separate vs Single Lanes

GCCAGGTGAGCCTTTTGGCA

A   C   G   T
Sequencing

**FIGURE 13.3** A sample chromatogram, as viewed with the vtrace program (Ewing, 2002). Signal intensities corresponding to fragments ending with A (green), C (blue), G (black), and T (red) are shown out to approximately 722 bases.
Shotgun Sequencing

From http://www.tulane.edu/~biochem/lecture/723/humgen.html
Sequencing

FIGURE 13.1 Shotgun cloning. Genomic DNA sequencing begins with isolated genomic DNA in green at the top of the figure. In the hierarchical clone-based shotgun approach on the left, DNA is sheared and the size is selected for large fragments on the order of 200 Kb, then ligated to a suitable vector, such as a BAC vector shown in blue. Individually isolated clones in turn are sheared independently, generating fragments of approximately 4 Kb, which are then ligated to a small-scale vector, typically a plasmid (red bar) suitable for sequencing reactions. The whole genome shotgun approach bypasses the intermediate large-insert clone and generates large numbers of small fragments, typically 4 Kb and 10 Kb.
Sequencing: Generate Contigs

- Short for “contiguous sequence”. A continuously covered region in the assembly.

  - **Dove-tail overlap**
  - **Collapsing into a single sequence**

Scaffold (supercontig): formed when two contigs with no sequence overlap can be linked

- Data from paired end reads help create scaffolds with known gaps
  - If two reads end up in two different contigs, then we can link contigs to form scaffold.
Shotgun Sequencing

From http://www.tulane.edu/~biochem/lecture/723/humgen.html
Human Genome Project

- Many videos available on youtube.com, dnatube.com, and elsewhere.
- Find some and watch them.
Assembly: Simple Example

- ACCGT, CGTGC, TTAC, TACCGT
- Total length = ~10

- --ACCGT--
- ----CGTGC
- TTAC-----
- -TACCGT-
- TTACCGTGCT
Assembly: Complications

- Errors in input sequence fragments (~3%)
  - Indels or substitutions
- Contamination by host DNA
- Chimeric fragments (joining of non-contiguous fragments)
- Unknown orientation
- Repeats (long repeats)
  - Fragment contained in a repeat
  - Repeat copies not exact copies
  - Inherently ambiguous assemblies possible
  - Inverted repeats
- Inadequate Coverage
Assembly: Complications

\[ w = AGTATTTGGCAATC \]
\[ z = AATCGATG \]
\[ u = ATGCAAACT \]
\[ x = CTTTTTGG \]
\[ y = TTGGCAATCACT \]

\[
\begin{array}{c}
AGTATTTGGCAATC
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\end{array}
\begin{array}{c}
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\begin{array}{c}
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AGTATTTGGCAATCACTAATCGATGCAAAACCTTTTTGG
\end{array}
\]

**FIGURE 4.20**
A bad solution for an assembly problem, with a multiple alignment whose consensus is a shortest common superstring. This solution has length 36 and is generated by the Greedy algorithm. However, its weakest link is zero.

\[
\begin{array}{c}
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\end{array}
\begin{array}{c}
TTGGCAATCACT
\end{array}
\begin{array}{c}
-------
\end{array}
\begin{array}{c}
ATGCAAACT
\end{array}
\begin{array}{c}
-------
\end{array}
\begin{array}{c}
AGTATTTGGCAATCGATGCAAAACCTTTTGGCAATCACT
\end{array}
\]

**FIGURE 4.21**
Solution according to the unique Hamiltonian path. This solution has length 37, but exhibits better linkage. Its weakest link is 3.
Assembly: Complications

**FIGURE 4.8**
Target sequence leading to ambiguous assembly because of repeats of the form XXX.

**FIGURE 4.9**
Target sequence leading to ambiguous assembly because of repeats of the form XXXY.

**FIGURE 4.10**
Target sequence with inverted repeat. The region marked $\bar{X}$ is the reverse complement of the region marked $X$. 