Microarrays Sequencing

Section 4

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Oct. 14th, 2003

Outline

- Logistics
  - Final Project Deadlines
- RNA1 Topics
  - Traditional RNA analysis
  - Microarrays
    - Types and Construction
    - Overview and Terminology
    - Usage and Analysis
  - Sequencing

Final Project Deadlines

- **Project ideas due Tues. 10/21/03**
  - 1-2 paragraph description
  - Team members listed (if any)
  - Please submit to your section TF
- **Proposal due Tues. 11/4/03**
  - Length: about 1 page
  - Should include description on overall goals, planned approach, and any progress
  - Please submit to your section TF

Final Project Deadlines

- **Project due Tues. 12/2**
  - Includes BOTH written report and powerpoint presentation
  - 1MB email limit on file attachments
  - 5% penalty per late day
- **Project presentations 12/2, 12/9, & 12/16**
  - (Problem Set 5 is due 12/9, so plan carefully)

Important Terminology

- **Nucleic acid hybridization**
  - The binding of complementary nucleic acid strands (A pairs with T/U, G to C). Two hybridized strands are said to form a duplex.
- **Nucleic acid denaturation (melting)**
  - The opposite of hybridization; when two complementary sequences come apart into single strands. This can be accomplished by heating, extremes of pH, or reducing the salt concentration.
- **Melting temperature (T_m)**
  - The temperature, under a standard condition, at which a double stranded DNA molecule denatures.
  - Major factors in determining T_m are the length of the duplex and the GC content (GC base pairs are more stable than ATs).

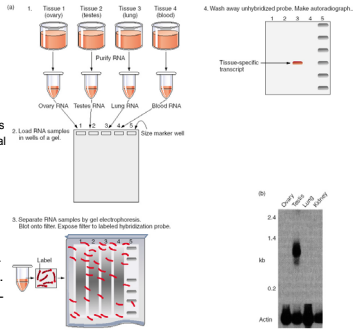
Important Terminology

- **Nucleic acid probe**
  - A short single-stranded nucleic acid sequence whose sequence allows it to hybridize to a sequence of interest. It will also be "labeled" in some way, e.g. have a radioactive or fluorescent attachment, so it can be detected.
  - Probe design involves optimization of melting temperature, secondary structure, and probe-probe sequence similarity.
- **cDNA (complementary DNA)**
  - Created from an enzyme called reverse transcriptase, which can copy RNA molecules into DNA. cDNA sequence is the reverse complement of the original RNA template.
  - cDNA is commonly used to make probes that represent an RNA sample.

Traditional RNA Analysis

Northern Blots

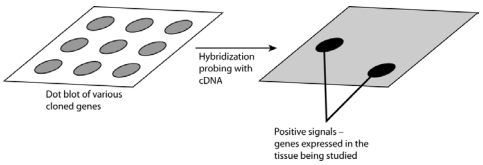
- Used to detect the presence of a particular RNA transcript
- RNA purification
  - Chemically extracted with phenol/chloroform from homogenized cells and tissues
  - mRNA transcripts (<5% of total RNA) have a poly-A tail and can be isolated with a poly-dT matrix
- An RNA sample is separated by size on a gel, transferred to a membrane and then allowed to hybridize to a radioactively-labeled nucleic acid 'probe'.
- Northern blots can be semi-quantitative but aren't very precise



From Hartwell L. H. et al, *Genetics: From Genes to Genomes* (2000) p. 362

Traditional RNA Analysis

Dot Blots



- **Microarray precursor**
  - **Note – it doesn't inform you of RNA size**

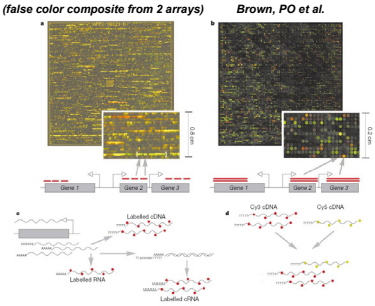
Key Principle of all hybridization techniques:

Over a certain nucleic acid concentration range, the amount of nucleic acid which is hybridized is proportional to its concentration in the hybridization solution.

DNA Microarrays

Affymetrix
(false color composite from 2 arrays)

Spotted
Brown, PO et al.

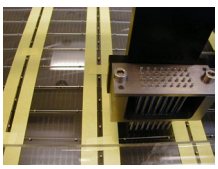


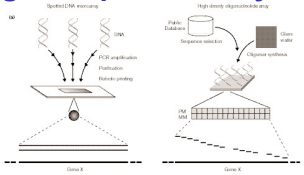
- Microarrays were initially developed to enable genome-scale gene expression analysis.
- The utility of microarrays lie in their ability to perform thousands of simultaneous measurements of a nucleic acid sample.
- Two major classes of DNA microarrays are ***high density oligonucleotide arrays*** (Affymetrix) and ***spotted arrays*** (developed by PO Brown et al.).

From Lockhart D.J. & Winzeler E.A., "Genomics, gene expression and DNA arrays". *Nature*. Vol. 405, no. 6788, 15 June 2000, p. 828.

Microarray Construction

Ways of getting DNA put on arrays





From Harrington et al., *Current Opinion in Microbiology*, 3(3): 285-91.

Spotted microarray (Brown, PO et al.) **Affymetrix microarray**

- **Spotted arrays**
  - Whole-gene or fragments of DNA physically deposited on glass slides
  - Easily customizable with a wide variety of different kinds of DNAs (next slide)
  - Robotic contact printing (shown) or piezoelectric printing (like today's inkjet printers)
- **High density oligonucleotide arrays (Affymetrix)**
  - Chemically synthesized *in situ* on glass wafers using lithographic processes
  - Short oligonucleotides (25-mers) tiled across gene
  - Paired PM (Perfect Match) and MM (MisMatches) format

Microarray Construction

What kind of DNA to use?

- **Regions of the genome**
  - ORFs, or Open Reading Frames: regions that are actually translated into proteins. Most common type used in DNA microarrays, and is used to measure global gene expression via mRNA transcript abundances
  - Intergenic regions: regions between different genes.
    - These regions are most useful in "Chip^{2nd} studies (Chromatin immunoprecipitation of protein-DNA compexes put on DNA Chips, or microarrays).
    - These studies examine protein binding (e.g. transcription factors, histone tail-binding proteins) to regulatory and non-transcribed regions of genes
  - ESTs, or Expressed Sequence Tags: transcribed sequences which are converted into DNA and partially sequenced. Most are of unknown function.
  - Clone libraries: Assortments of DNA fragments collected by a variety of means.
    - Sequences can be inserted into bacteria or viruses. If a sequence turns out to be interesting, the clone harboring that sequence can be grown up to produce enough to study.
    - A common clone library is a cDNA library, where each clone contains a single cDNA reverse transcribed from an RNA in an RNA sample of interest.
  - Tiling arrays: oligonucleotide arrays which contain oligonucleotides corresponding to sequences spaced at short intervals across the region of interest in the genome

Microarray Construction

What kind of DNA to use?

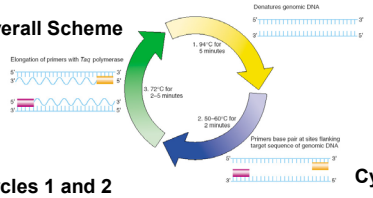
- **PCR products vs oligonucleotides**
  - PCR products
    - Derived from cells/tissues as starting template
    - The first microarrays consisted of all ORFs in the yeast genome, spotted as PCR products
    - Tend to be long (0.5-3 kb or longer)
    - Produce more stable duplexes (hybridized strands)
    - Averages signal across the entire gene
    - Low initial cost but variable quality
  - Oligonucleotides
    - Chemically synthesized
    - Tend to be short (25-70 bases)
    - Duplexes less stable (hence Affymetrix's PM/MM system)
    - Can target specific regions of a gene and test splice variants
    - High initial cost but frequently high quality

PCR (for non-biologists)

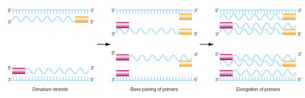
- **The Polymerase Chain Reaction (PCR)**
  - This Nobel-prize winning enzymatic reaction can make a large amount of DNA from a very small amount of starting material.
  - In principle only a single molecule is needed.
  - By designing 'primers' which surround a region of interest, the region between the primers can be copied exponentially.
  - Sets of primers can be designed to copy any region of the genome in quantities large enough to spot on a microarray.

PCR (for non-biologists)

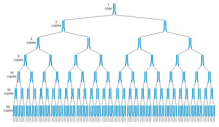
Overall Scheme



Cycles 1 and 2



Cycles 1-7, etc.

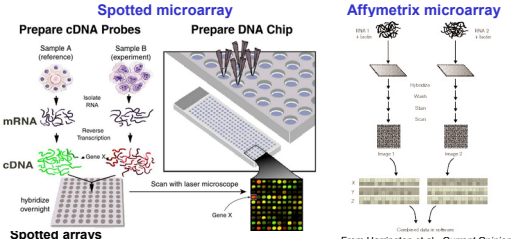


From Hartwell L. H. et al, *Genetics: From Genes to Genomes* (2000) pp. 294-5

Using Microarrays

for gene expression profiling

Spotted microarray | **Affymetrix microarray**



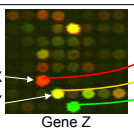
From Harrington et al., *Current Opinion in Microbiology*, 3(3): 285-91.

- **Spotted arrays**
  - A competitive hybridization of two RNA samples
  - cDNA Probe is labeled with fluorescent dyes (direct incorporation or amino-allyl coupling)
    - (typically cyanine 3 = reference, and cyanine 5 = experiment)
  - Data obtained is measured as a ratio of one color to the other (e.g. Red/Green ratio) and provides *relative* abundance information
- **High density oligonucleotide arrays (Affymetrix)**
  - A non-competitive hybridization of one single RNA sample (per array)
  - Data obtained is measured as absolute intensity units if it passes PM/MM and provides *absolute* abundance information

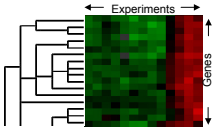
Analysis of Raw Microarray Data

Raw spotted microarray image → Signal intensity analysis → Color-balance normalization

Net signal intensity → Red/Green ratio calculation → Log transformation



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



Clustering preview (Section 6)

Repressed (downregulated)

Gene Expression

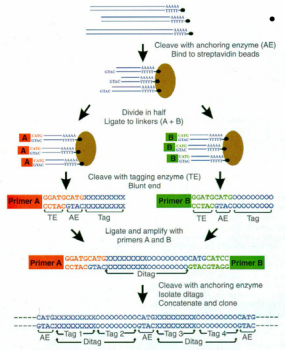
Induced (upregulated)

Interpreting Microarray Data

Spotted Microarrays

- Only measures relative abundances
  - Each spot on an array has a different hybridization efficiency; only self-comparisons are valid
  - Normalization is necessary to compare identical spots across separate arrays
- Cross-hybridization
  - Similarity between sequences or high abundances of other transcripts with low but significant affinities for the spot can hybridize to the 'wrong' spot.
  - Washing disrupts duplexes of given stabilities depending on stringency of the conditions used.
  - However, washing cannot completely eliminate cross-hybridization, especially when stabilities of specific and non-specific duplexes can overlap.
    - (consider definitions of T_m and denaturation and what the properties of a stringent wash might be)
  - Intelligent oligonucleotide design for spotted oligonucleotide arrays can greatly reduce cross-hybridization

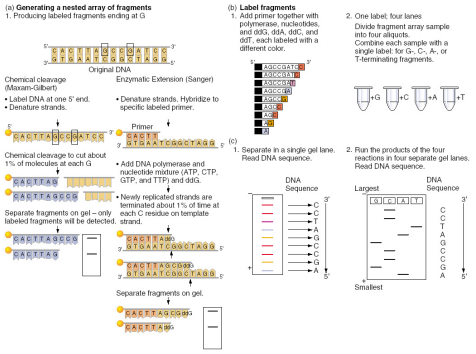
SAGE



From Velculescu, et.al, "Serial Analysis of Gene Expression", *Science* 270: 484-487 (1995).

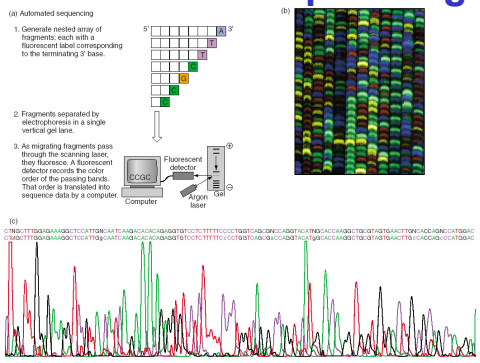
- **Serial Analysis of Gene Expression**
  - Employs sequencing to quantify RNA abundance on a global scale
  - RNA samples are processed to produce small sequence tags (10-14 bp) from each one
  - Tags are concatenated along with tags from other RNAs.
  - These stretches of tags are sequenced and the number of tags from each transcript is counted
  - Genome sequences enable identification of tag sequence to the corresponding gene
  - This method is digital (discrete counting events), whereas hybridization methods are analog (continuous response to changes in RNA concentration)
  - SAGE is currently more labor-intensive than microarrays

DNA Sequencing Methods



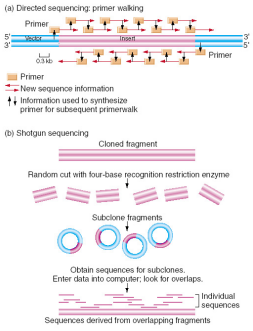
From Hartwell L. H. et al, *Genetics: From Genes to Genomes* (2000) p. 288

Automated Sequencing



From Hartwell L. H. et al, *Genetics: From Genes to Genomes* (2000) p. 292

Directed and Shotgun Sequencing



- **Directed Sequencing**
  - Involves "primer walking"
  - Slow and laborious, but more reliable
- **Shotgun Sequencing**
  - Randomly cut region being sequence
  - Reassemble region into contig via sequence alignment of overlaps
  - Much more rapid but less reliable
  - Requires several-fold coverage of region

Large sequencing projects usually use both methods

From Hartwell L. H. et al, *Genetics: From Genes to Genomes* (2000) p. 293

Next Week

- Population Genetics

Acknowledgement / References

Harrington et al., "Monitoring gene expression using DNA microarrays". *Current Opinion in Microbiology*, 3(3): 285-91.

Hartwell L. H. ed. et al. *Genetics: From Genes to Genomes* (2000). McGraw-Hill Companies, Inc.

Lockhart D.J. & Winzeler E.A., "Genomics, gene expression and DNA arrays". *Nature* 405 (6788): 827-836.

Velculescu, et.al, "Serial Analysis of Gene Expression", *Science* 270: 484-487 (1995).

This handout includes material written by Suzanne Komili, Yonatan Grad, Doug Selinger, and Zhou Zhu.

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