DNA Microarray Experiments and Analysis

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MBios 503
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Mechanics of DNA Microarrays

complex mixture of mRNA molecules

Gene 1

Gene 2

...Gene 6200

ATGCCGAATCGTCCAGTAACC
UACGGCUUAGCAGGUCAUUGG

Fluorescent Dye
Constructing DNA Microarrays

§ A DNA microarray is a collection of DNA probes separated in a regular array atop a solid support (glass slide, silicon chip, etc)

§ Affymetrix oligonucleotide microarrays
  • Short oligonucleotide probes (25mers) are synthesized directly on a silicon wafer using photolithography methods
  • Multiple oligonucleotide probes per gene including mismatch probe controls

§ cDNA microarrays
  • Full length cDNAs are PCR amplified and then printed using a robot onto coated glass slides
  • Glass slides can be coated with poly-lysine, GAPS, etc to facilitate attachment of DNA to the glass slide

§ Oligonucleotide microarrays
  • Long (70 - 100mers) oligonucleotide probes are printed on coated glass slides
For Yeast:

• Array of 6361 spots, each representing a single yeast gene

• Printed on GAPS-coated glass microscope slide

• Each spot contains 500 pg of PCR product

• Size of PCR products range from 60 bp to 1500 bp; average size: 480 bp
Affymetrix Oligonucleotide Microarrays

- 6181 ORFs + alignment controls
- each ORF: 20 perfect match 25mers (PM)  
  20 single base mismatch (MM)
- mRNA signal = average of differences  
  (PM - MM)
- Sensitivity = 0.1 mRNA molecules/cell
- Dynamic range = 0.1 - 100 mRNAs/cell
- Reproducibility = wt vs wt experiment, 99%  
  of genes within 1.7 fold
The Mechanics of a DNA Microarray Experiment

§ Isolate mRNA from cell cultures

§ Reverse Transcribe mRNA into cDNA

§ Label cDNA or cRNA by incorporating fluorescently-labeled nucleotides

§ Hybridize labeled cDNA to DNA microarray

§ Wash and scan microarray in confocal laser scanner

§ Analyze data
DNA Microarray Experimental Procedure

Experimental yeast culture → Break open cells and purify mRNA → Reverse transcribe w/poly-dT primer → Cy5 → Hybridize to cDNA array

Control yeast culture → → Reverse transcribe w/poly-dT primer → → Cy3 → → Hybridize to cDNA array

Hybridize to cDNA array
- Red: Experimental mRNA
- Green: Control mRNA
- Yellow: Merged
Extracting Data from Scanned Microarray Image

Scanned Image

Gene with altered mRNA levels

§ Grid

- The total amount of fluorescence intensity of Cy5 and Cy3 dyes inside each grid circle is calculated
- Grid can be aligned manually or automatically

- Experimental mRNA (Cy5)
- Control mRNA (Cy3)
- Merge
Initial Microarray Data Analysis

§ Data Normalization
  • Total signal normalization
  • Exogenous (spiked) control mRNAs

§ Filters for identifying genes with altered mRNA levels
  • Absolute intensity change thresholds
  • Fold-change cutoffs
  • T-tests and other significance tests
  • Permutation tests
  • Error model methods

§ Use a Database to normalize, filter, and analyze microarray data
  • Integrate microarray data with data from other sources (gene function, proteomics data, etc)
Microarray Database

Raw data
- Microarray data

Database
- mySQL

Literature
- Gene annotations

Analysis Tools
- Error model analysis
- Cluster analysis
- Chromosome display

Results

Perl

Web interface
Methods for Data Mining and Visualization

§ Data visualization tools
  • Displays microarray data using prior knowledge of gene location/function

§ Clustering
  • Simplifies analysis by grouping together genes with similar expression patterns, but does not infer relationships between genes

§ Boolean Comparisons
  • Compares different microarray data sets to identify significant overlaps in expression patterns

§ Hidden Markov models
  • Useful for clustering time course data

§ Bayesian networks
  • Useful for identifying and testing relationships between genes
## Raw Microarray Data

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<tr>
<th>GCN5 deletion</th>
<th>Gene (parse)</th>
<th>WT1val</th>
<th>WT1call</th>
<th>MT1val</th>
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