Analysis of Microarray Data

Lecture 3:
Visualization and Functional Analysis

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Outline

• Review
• Visualizing all the data
• What to do with a set of interesting genes?
  – Basic annotation
  – Comparing lists
  – Genome mapping
  – Obtaining and analyzing promoters
  – Gene Ontology and pathway analysis
  – Other expression data
Generic Microarray Pipeline

- Design experiment
- Prepare samples and perform hybridizations
- Quantify scanned slide image
- Calculate expression values
- Normalize
- Handle low-level expression values
- Merge data for replicates
- Determine differentially expressed genes
- Cluster interesting data
Review

• Preliminary filtering?
• Measuring differential expression:
• Correcting for multiple hypothesis testing
  – Fold change, t-test, ANOVA
  – Bonferroni, False Discovery Rate, etc.
• Filtering; identifying “interesting” genes
• Distance measures for clustering
• Clustering/segmentation types and methods
• What is the best analysis pipeline?
  – Why are you doing the experiment?
  – Are you being reasonable with the statistics?
Why draw figures?

• Get a global perspective of the experiments
• Quality control: check for low-quality data and errors
• Compare raw and normalized data
• Compare controls: are they homogeneous?
• Help decide how to filter data
• Look at a subset of data in detail
Intensity histogram

Histogram of fetal brain expression (raw Affymetrix data)

Median = 6.6

Median = 100
Intensity histogram

- Most genes have low expression levels
- Using $\log_2$ scale to transform data
  - more normal distribution
  - more helpful interpretation
- One way to observe overall intensity of chip
- How to choose genes with “no” expression?
Intensity scatterplot
Intensity scatterplot

• Compares intensity on two colors or chips
• Genes with similar expression are on the diagonal
• Use log-transformed expression values
• Genes with lower expression
  – noisier expression
  – harder to call significant
R-I and M-A plots

R-I plot for brain: fetal vs adult

\[ R = \log_2 (\text{fetal} / \text{adult}) \]

\[ I = \log_2 (\text{fetal} \times \text{adult}) \]
**R-I and M-A plots**

- Compares intensity on two colors or chips
- Like an intensity scatterplot rotated 45°
  
  \[
  R \text{ (ratio)} = \log(\text{chip1} / \text{chip2}) \\
  I \text{ (intensity)} = \log(\text{chip1} \times \text{chip2})
  \]

  \[
  M = \log_2(\text{chip1} / \text{chip2}) \\
  A = \frac{1}{2}(\log_2(\text{chip1}\times\text{chip2}))
  \]

- Popularized with lowess normalization
- Easier to interpret than an intensity scatterplot
Volcano plot

Brain volcano plot: ratio vs p-value

- Not so interesting
- Down
- Up

log2 expression ratio: adult vs fetal
Volcano plot

• Scatterplot showing differential expression statistics and fold change
• Visualize effects of filtering genes by both measures
• Using fold change vs. statistical measures for differential expression produce very different results
Boxplots

Raw and median-normalized log₂ (expression values)
Boxplots

- Display summary statistics about the distribution of each chip:
  - Median
  - Quartiles (25% and 75% percentiles)
  - Extreme values (>3 quartiles from median)
  - Note that mean-normalized chips wouldn’t have the same median
  - Easy in R; much harder to do in Excel
Chip images

- Affymetrix U95A chip hybridized with fetal brain
- Image generated from .cel file
- Helpful for quality control
Heatmaps
Using distance measurements

Genes with most similar profiles to GPR37

| Expression profiles in Novartis human set (U95) closest to 34297_at |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | Normal | gland | Caudate | nucleus | Striatum | caudate | colliculus | caudate | hippocampus | cingulate | occipital | temporal | parietal | frontal | insular | temporal | parietal | occipital |
| Gene Symbol      |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| GPR37            |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| d = 4              |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| h = 2.11           |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| OLTC6            |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| d = 2925         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| h = 2.27          |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| HBB              |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| d = 2946         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| h = 2.10          |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| KLRK             |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| d = 2941         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| h = 2.17          |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| L11orf9          |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| 33522_at         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| d = 2864         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| h = 2.2           |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| FGFR2            |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| d = 2972         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| h = 5.12         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| GPR38B           |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| 46291_at         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| d = 3196         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| h = 4.2          |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| TM4SF11          |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| 45686_at         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| d = 2092         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| h = 2.12         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| HHE              |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| 37989_at         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| d = 2985         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| h = 4.1           |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| HHE              |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| 37987_at         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| d = 2917         |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
| h = 4.2           |        |       |         |         |          |         |           |         |            |           |          |          |         |         |      |          |           |         |          |
Functional Analysis: intro

- After data is normalized, compared, filtered, clustered, and differentially expressed genes are found, what happens next?
- Driven by experimental questions
- Specificity of hypothesis testing increases power of statistical tests
- One general question: what’s special about the differentially expressed genes?
Annotation using sequence databases

- Gene data can be “translated” into IDs from a wide variety of sequence databases:
  - LocusLink, Ensembl, UniGene, RefSeq, genome databases
  - Each database in turn links to a lot of different types of data
  - Use Excel or programming tools to do this quickly
- Web links, instead of actual data, can also be used.
- What’s the difference between these databases?
- How can all this data be integrated?
Venn diagrams

- Show intersection(s) between at least 2 sets

Typical figure     More informative figure
Mapping genes to the genome

Genomic locations of differentially expressed genes

Human genome, May 2004
Promoter extraction

• Prerequisite of any promoter analysis
• Requires a sequenced genome and a complete, mapped cDNA sequence
• “Promoter” is defined in this context as upstream regulatory sequence
• Extract genomic DNA using a genome browser: UCSC, Ensembl, NCBI, GBrowse, etc.
• Functional promoter needs to be determined experimentally
Promoter analysis

- TRANSFAC contains curated binding data
- Transcription factor binding sites can be predicted
  - matrix (probabilities of each nt at each site)
  - pattern (fuzzy consensus of binding site)
- Functional sites tend to be evolutionarily conserved
- Functional promoter activity needs to be verified experimentally
Gene Ontology

- GO is a systematic way to describe protein (gene) function
- GO comprises ontologies and annotations
- The ontologies:
  - Molecular function
  - Biological process
  - Cellular component
- Ontologies are like hierarchies except that a “child” can have more than one “parent”.
- Annotation sources: publications (TAS), bioinformatics (IEA), genetics (IGI), assays (IDA), phenotypes (IMP), etc.
Gene Ontology enrichment analysis

• Unbiased method to ask question, “What’s so special about my set of genes?”
• Obtain GO annotation (most specific term(s)) for genes in your set
• Climb an ontology to get all “parents” (more general, “induced” terms)
• Look at occurrence of each term in your set compared to terms in population (all genes or all genes on your chip)
• Are some terms over-represented?
  Ex: sample: 10/100  pop1: 600/6000  pop2: 15/6000
Pathway enrichment analysis

• Unbiased method to ask question, “Is my set of genes especially involved in specific pathways?”
• First step: Link genes to pathways
• Are some pathways over-represented?
• Caveats
  – What is meant by “pathway”?
  – Multiple DBs with varied annotations
  – Annotations are very incomplete
Enrichment analysis on sorted expression data

• Input 1: complete sorted gene list
  – no threshold value or definition of significance
• Input 2: set of biologically meaningful gene sets
  – pathway, genome location, function, ...
• Is the rank of genes from any gene set in your sorted list non-random?
• Example: GSEA
Comparisons with other expression studies

- Array repositories: GEO (NCBI), ArrayExpress (EBI), WADE (WIBR)
- Search for genes, chips, types of experiments, species
- View or download data
- Normalize but still expect noise
  - Check medians and distribution of data
- It’s much easier to make comparisons within an experiment than between experiments
Summary

• Plots: histogram, scatter, R-I, volcano, box
• Other visualizations: whole chip, heatmaps, bar graphs, Venn diagrams
• Annotation to sequence DBs
• Genome mapping
• Promoter extraction and analysis
• GO and pathway enrichment analysis
• Comparison with published studies
More information

• Course page:

• Bioconductor short courses: http://www.bioconductor.org/

• BaRC analysis tools:
  – http://jura.wi.mit.edu/bioc/tools/

• Gene Ontology Consortium website:
  – http://www.geneontology.org/


Exercises

• Graphing all data
  – Scatterplot
  – R-I (M-A) plot
  – Volcano plot

• Functional analysis
  – Annotation
  – Comparisons
  – Genome mapping
  – Promoter extraction and analysis
  – GO and pathway analysis
  – Using other expression studies