Analysis of Microarray Data

Lecture 3: Visualization and Functional Analysis

George Bell, Ph.D.
Senior Bioinformatics Scientist
Bioinformatics and Research Computing
Whitehead Institute
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

{not covered in course}
Review

• Preliminary filtering?
• Measuring differential expression:
  – Fold change, t-test, ANOVA
  – Bonferroni, False Discovery Rate, etc.
• Correcting for multiple hypothesis testing
• 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

Expression in brain: fetal vs adult

WIBR Microarray Course, © Whitehead Institute, 2007
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°
  \[ R \text{ (ratio)} = \log(\text{chip1} / \text{chip2}) \]
  \[ I \text{ (intensity)} = \log(\text{chip1} * \text{chip2}) \]
  \[ M = \log_2(\text{chip1} / \text{chip2}) \]
  \[ A = \frac{1}{2}(\log_2(\text{chip1}\ast\text{chip2})) \]

• 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

p-value from t-test

0.0001
0.001
0.01
0.1
1
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)

WIBR Microarray Course, © Whitehead Institute, 2007
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
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
- ChIP chip data (if available) can be much higher quality
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

Broad Institute
Comparisons with other expression studies

- Array repositories: GEO (NCBI), ArrayExpress (EBI), Stanford (SMD) – and project sites
- 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