# Gene List Enrichment Analysis

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BaRC Hot Topics
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## Outline

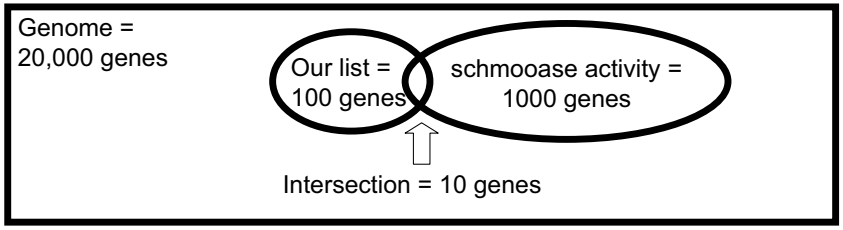
- Why do enrichment analysis?
- Main types
- Selecting or ranking genes
- Annotation sources
- Statistics
- Remaining issues
- Presenting findings
- Recommended tools

## Why do enrichment analysis?

- Most array, sequencing, and screens produce
  - A measurement for most or all genes
  - List(s) of "interesting" genes
- Most cellular processes involve sets of genes.
- Can we compare the above two datasets?
- Is the overlap different than expected?
- Does this tell us something about cellular mechanisms?

## Why not just link genes to physiology?

- Too many genes to examine in detail.
- Are we biased?
- How do we know that what we're seeing is surprising?

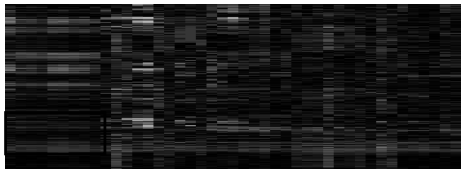


# Main types of enrichment analysis

- List-based: inputs are
  - A subset of all genes chosen by some relevant method
  - A list of annotations, each linked to genes
- Rank-based: inputs are
  - A set of all genes ranked by some metric (ratio, fold change, etc.)
  - A list of annotations, each linked to genes
- List-based with relationships: inputs are
  - A subset of all genes
  - A list of annotations, each linked to genes, organized in some relationship (e.g., a hierarchy)

# Getting your list

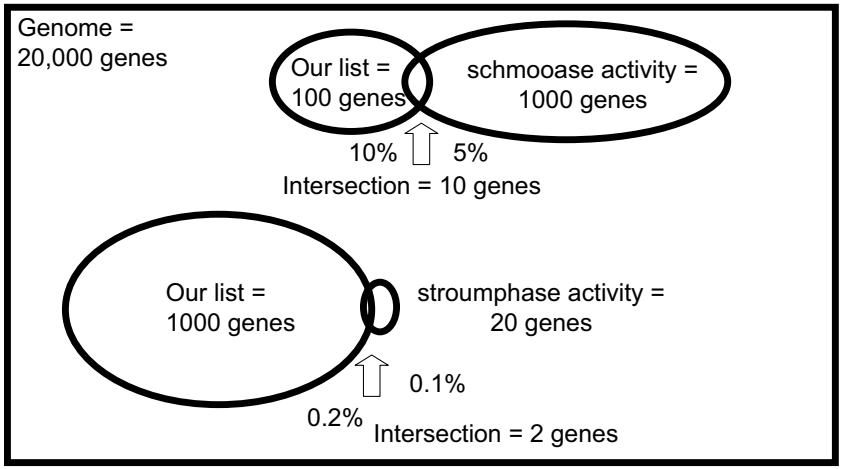
- Goal: Identify a list of genes (or probes) that appear to be working together in some way.
- What identifiers to use?
- Most common method: Get a list of differentially expressed genes
  - P-value or fold change?
  - Threshold?
- Alternatives:
  - Define a cluster
  - Sort data and/or apply a model to rank genes
- Recommendations:
  - Try lists of varying length
  - Try to maximize signal / noise (What produces the smallest p-values for enrichment?)



# Annotation sources

- Gene Ontology (most popular)
  - biological process, molecular function, cellular component
  - Terms may have >1 “parent” (more general term)
  - GO Slim: includes only general categories
- KEGG; REACTOME pathways
- Genes sharing a motif of regulated by the same protein/miRNA
- Genes found on the same chromosome
- Also … see Broad’s Molecular Signatures Database (MSigDB)
- [any grouping that is biologically sensible]

# Statistics to test for enrichment



## Tests for enrichment

- Fisher's exact
- Hypergeometric
- Binomial
- Chi-squared
- Z
- Kolmogorov-Smirnov
- Permutation

## Statistics to test for enrichment

- What is the chance of observing enrichment at least this extreme due to chance?
- Different tests produce very different ranges of p-values
- All look for over-enrichment; some look for under-enrichment
- Recommendation: Use p-values as a tool to rank genes but don't take them literally
- Most methods correct for multiple testing (e.g., with FDR), which is necessary

## Other statistical issues

- Goal: Identifying theme(s) of maximal biological significance
  - but this is not perfectly correlated with statistical significance
- What is your background gene set?
  - All genes that could appear in your list
- What about sparse annotation groups?
- Some annotation terms may be subsets of other terms.

## Practicalities

- Choose a tool that
  - Includes your species
  - Includes your gene / probe identifiers
  - Has up-to-date annotation
  - Lets you define your background (if possible)
- Get recommendations from the usual sources.
- Try at least a few tools.

## Presenting results

- Generally ignore enriched categories which
  - Contain very few genes
  - Show high overlap with other categories
- When in doubt, select more general category.
- Simplify complex results.
- Graphical or text summary?
- Plan to share your gene lists when you publish.

## Enrichment tools

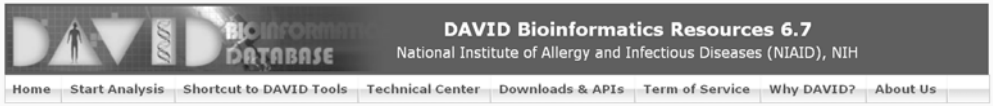

- See http://www.geneontology.org/GO.tools.shtml

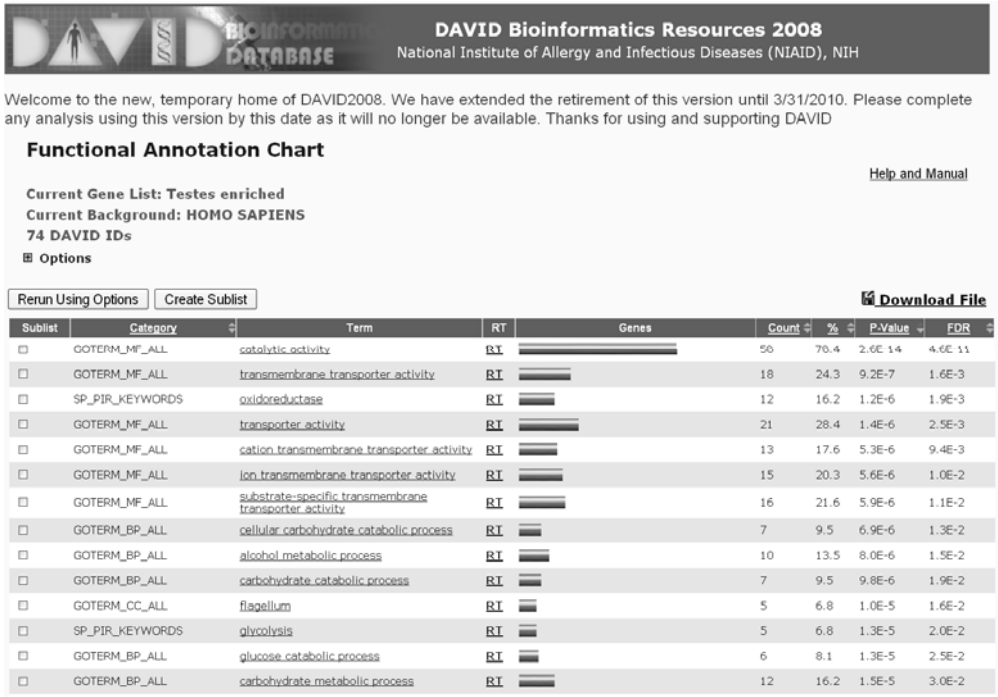
## Some recommended tools

- DAVID
- GSEA
- BIOBASE (Whitehead has license)
- BiNGO (uses Cytoscape)
- GoMiner: http://discover.nci.nih.gov/gominer
- GOstat: http://gostat.wehi.edu.au

## DAVID

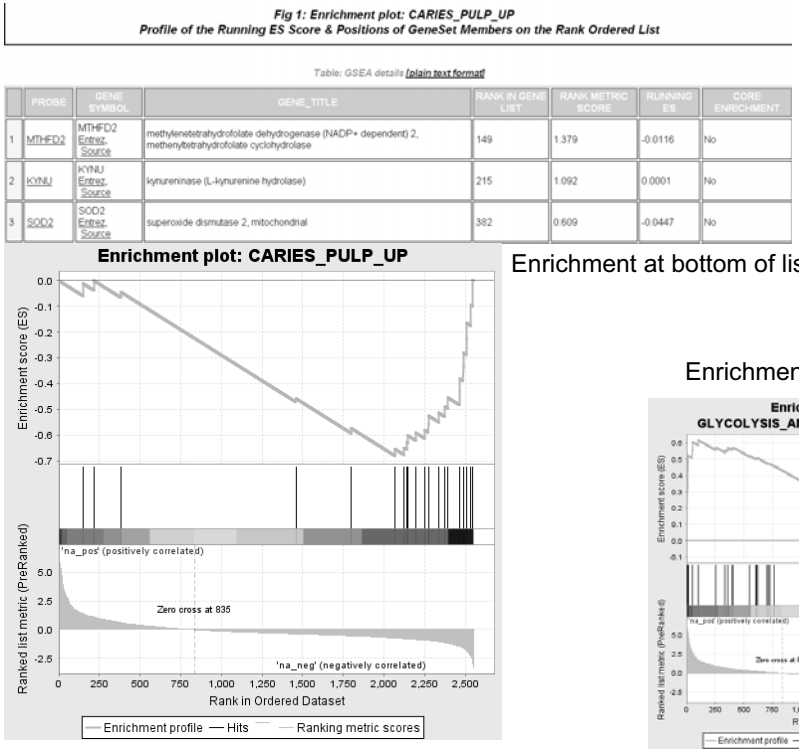
- Database for Annotation, Visualization and Integrated Discovery (NIAID)
- List-based
- http://david.abcc.ncifcrf.gov/
- Lots of identifiers; lots of species
- Allows background definition
- Statistic is a modified Fisher exact test






# GSEA

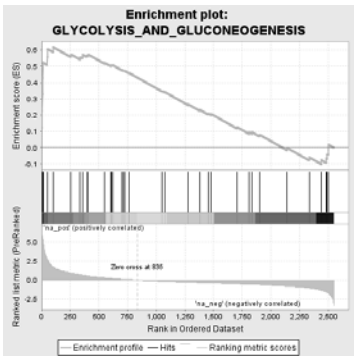
- Gene Set Enrichment Analysis
- Rank-based
- http://www.broadinstitute.org/gsea/
- As a Java Web Start or desktop application
- Linked to MSigDB (annotated gene lists)
- Also permits custom annotation




Input: pre-ranked gene list

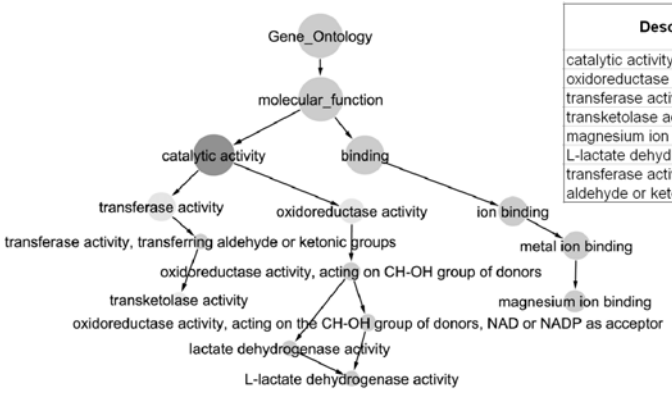
Enrichment at bottom of list

Enrichment at top of list




# BiNGO

- BiNGO: A Biological Network Gene Ontology tool
- http://www.psb.ugent.be/cbd/papers/BiNGO/
- Works with Cytoscape network visualization tool
- Also permits custom annotation

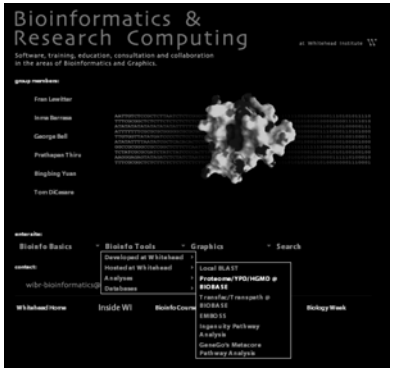



| Description | corrected p-value | Number of genes in list |
|---|---|---|
| catalytic activity | 2.0E-13 | 48 |
| oxidoreductase activity | 1.0E-03 | 12 |
| transferase activity | 1.2E-03 | 18 |
| transketolase activity | 2.4E-03 | 2 |
| magnesium ion binding | 4.5E-03 | 8 |
| L-lactate dehydrogenase activity | 4.5E-03 | 2 |
| transferase activity, transferring aldehyde or ketonic groups | 4.5E-03 | 2 |

Shows relationship between annotation categories

# BIOBASE

- BIOBASE Knowledge Library
- Use Internet Explorer
- Go to “Gene Set Analysis”




| | | | |
|---|---|---|---|
| **Legend:** | BP = GO biological process | GF = gene family | PP = plant phenotype |
| | CC = GO cellular component | IN = protein interaction | PT = canonical pathway |
| | DI = disease | MD = protein modification | PX = expression in plants |
| | DG = pharmaceutical | MF = GO molecular function | RE = regulators of fungal genes |
| | DO = protein domain | PH = mouse phenotype | SP = species & chromosomes |
| | EX = expression in mammals | PM = yeast or worm phenotype | WX = expression in worms |

| P-value | Term | Protein count | Expected Protein count |
|---|---|---|---|
| 5.03e-45 | SP Human | 58 | 10.7 |
| 8.62e-21 | SP Mammal | 58 | 28.3 |
| 2.3e-12 | EX testis | 27 | 7.73 |
| 3.09e-11 | MF catalytic activity | 45 | 21.3 |
| 1.03e-10 | SP Human chromosome 17 | 6 | 0.594 |

# References


- Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. (PMID: 19033363) *Review*
- Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. (PMID: 19131956) *DAVID*
- Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. (PMID: 16199517) *GSEA*