Gene Ontology and Functional Enrichment

Genome 559: Introduction to Statistical and Computational Genomics

Elhanan Borenstein
The parsimony principle:
- Find the tree that requires the fewest evolutionary changes!

A fundamentally different method:
- Search rather than reconstruct

Parsimony algorithm
1. Construct all possible trees
2. For each site in the alignment and for each tree count the minimal number of changes required
3. Add sites to obtain the total number of changes required for each tree
4. Pick the tree with the lowest score
A quick review – cont’

- **Small vs. large parsimony**

- **Fitch’s algorithm:**
  1. **Bottom-up phase:** Determine the set of possible states
  2. **Top-down phase:** Pick a state for each internal node

- **Searching the tree space:**
  - Exhaustive search, branch and bound
  - Hill climbing with Nearest-Neighbor Interchange

- **Branch confidence and bootstrap support**
From sequence to function

Which molecular processes/functions are involved in a certain phenotype - disease, response, development, etc.

(what is the cell doing vs. what it could possibly do)

Gene expression profiling
Gene expression profiling

- Measuring gene expression:
  - (Northern blots and RT-qPCR)
  - Microarray
  - RNA-Seq

- Experimental conditions:
  - Disease vs. control
  - Across tissues
  - Across time
  - Across environments
  - Many more …
Different techniques, same structure
1. Find the set of differentially expressed genes.
2. Survey the literature to obtain insights about the functions that differentially expressed genes are involved in.
3. Group together genes with similar functions.
4. Identify functional categories with many differentially expressed genes.

Conclude that these functions are important in disease/condition under study
The good old days were not so good!

*Time-consuming*

*Not systematic*

*Extremely subjective*

*No statistical validation*
What do we need?

- A shared functional vocabulary
- Systematic linkage between genes and functions
- A way to identify genes relevant to the condition under study
- Statistical analysis (combining all of the above to identify cellular functions that contributed to the disease or condition under study)
- A way to identify “related” genes
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Gene Ontology Annotation

- Fold change, Ranking, ANOVA
- Clustering, classification
- Enrichment analysis, GSEA
The Gene Ontology (GO) Project

- A major bioinformatics initiative with the aim of standardizing the representation of gene and gene product attributes across species and databases.

- Three goals:
  1. Maintain and further develop its controlled **vocabulary** of gene and gene product attributes
  2. **Annotate** genes and gene products, and assimilate and disseminate annotation data
  3. Provide **tools** to facilitate access to all aspects of the data provided by the Gene Ontology project
The Gene Ontology (GO) is a **controlled vocabulary**, a set of standard **terms** (words and phrases) used for indexing and retrieving information.

### Term Information

<table>
<thead>
<tr>
<th>Accession</th>
<th>GO:0006348</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ontology</td>
<td>Biological Process</td>
</tr>
</tbody>
</table>
| Synonyms    | exact: heterochromatic silencing at telomere  
|             | exact: telomere chromatin silencing  
|             | exact: telomeric silencing |
| Definition  | Repression of transcription of telomeric DNA by altering the structure of chromatin.  
| Comment     | None |
| Subset      | None |
| Community   | Add usage comments for this term at [GONUTS](http://www.gonuts.org). |
GO also defines the **relationships** between the terms, making it a **structured** vocabulary.

GO is structured as a **directed acyclic graph**, and each term has defined relationships to one or more other terms.
GO domains

- Three ontology domains:
  1. **Molecular function**: basic activity or task
     *e.g. catalytic activity, calcium ion binding*
  2. **Biological process**: broad objective or goal
     *e.g. signal transduction, immune response*
  3. **Cellular component**: location or complex
     *e.g. nucleus, mitochondrion*

- Genes can have multiple annotations:

  *For example, the gene product cytochrome c can be described by the molecular function term oxidoreductase activity, the biological process terms oxidative phosphorylation and induction of cell death, and the cellular component terms mitochondrial matrix and mitochondrial inner membrane.*
Go domains

**Biological process**

- DNA degradation
- DNA packaging
- Mitochondrial genome maintenance
- DNA replication
- DNA repair
- DNA-dependent DNA replication
- Pre-replicative complex formation and maintenance

**Cellular component**

- Nucleus
- Nucleoplasm
- Pre-replicative complex

**Molecular function**

- Nucleic acid binding
- DNA binding
- Helicase
- ATP-dependent helicase
- DNA-dependent adenosine triphosphatase
- Lamin/chromatin binding

**Others**

- DNA ligase
- DNA pol-α-180

**Proteins**

- SACCHAROMYCES
- DIROSPHILA
- MUS
Ontology and annotation databases

“The nice thing about standards is that there are so many to choose from”

Andrew S. Tanenbaum
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A way to identify "related" genes
Picking “relevant” genes

- In most cases, we will consider differential expression as a marker:
  - Fold change cutoff (e.g., > two fold change)
  - Fold change rank (e.g., top 10%)
  - Significant differential expression (e.g., ANOVA) (don’t forget to correct for multiple testing, e.g., Bonferroni or FDR)
Enrichment analysis

Signalling category contains 27.6% of all genes in the study set - **by far the largest category.** Reasonable to conclude that signaling may be important in the condition under study.

<table>
<thead>
<tr>
<th>Functional category</th>
<th># of genes in the study set</th>
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<tr>
<td>Signaling</td>
<td>82</td>
<td>27.6</td>
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<td>Metabolism</td>
<td>40</td>
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Enrichment analysis – the wrong way

- Signaling category contains 27.6% of all genes in the study set - by far the largest category.
- Reasonable to conclude that signaling may be important in the condition under study.
Enrichment analysis – the wrong way

- What if ~27% of the genes on the array are involved in signaling?
  - The number of signaling genes in the set is what expected by chance.
  - We need to consider not only the number of genes in the set for each category, but also the total number on the array.

- We want to know which category is over-represented (occurs more times than expected by chance).

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Enrichment analysis – the right way

Say, the microarray contains 50 genes, 10 of which are annotated as ‘signaling’. Your expression analysis reveals 8 differentially expressed genes, 4 of which are annotated as ‘signaling’. Is this significant?

A statistical test, based on a null model

Assume the study set has nothing to do with the specific function at hand and was selected randomly, would we be surprised to see this number of genes annotated with this function in the study set?

The “urn” version: You pick a random set of 8 balls from an urn that contains 50 balls: 40 white and 10 blue. How surprised will you be to find that 4 of the balls you picked are blue?
A quick review: Modified Fisher's exact test

Do I have a surprisingly **high** number of blue genes?

Null model: the 8 genes/balls are selected randomly

So, if you have 50 balls, 10 of them are blue, and you pick 8 balls randomly, what is the probability that $k$ of them are blue?
A quick review:
Modified Fisher's exact test

Hypergeometric distribution

\[ P(\sigma_t = k) = \frac{\binom{m_i}{k} \binom{m-m_i}{n-k}}{\binom{m}{n}} \]

m=50, \( m_t = 10 \), n=8

So ... do I have a surprisingly high number of blue genes?

What is the probability of getting at least 4 blue genes in the null model?

\[ P(\sigma_t \geq 4) \]
Modified Fisher's Exact Test

- Let $m$ denote the total number of genes in the array and $n$ the number of genes in the study set.

- Let $m_t$ denote the total number of genes annotated with function $t$ and $n_t$ the number of genes in the study set annotated with this function.

- We are interested in knowing the probability of seeing $n_t$ or more annotated genes!

\[
P(\sigma_t \geq n_t) = \sum_{k=n_t}^{\min(m_t, n)} \frac{(m_t)}{k!} \frac{(m-m_t)}{(n-k)!} \frac{(m)}{(n)}
\]

(This is equivalent to a one-sided Fisher exact test)
So ... what do we have so far?

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Still far from being perfect!

- A shared functional vocabulary
- Systematic linkage between genes and functions
  - Arbitrary!
  - Considers only a few genes
  - Ignores links between GO categories
  - Limited hypotheses
  - Simplistic null model!
- A way to identify genes relevant to the condition under study
- Statistical analysis
  (combining all of the above to identify cellular functions that contributed to the disease or condition under study)
- A way to identify “related” genes