Extracting Biological Information from Gene Lists

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Programme

- The theory and practice of gene set enrichment
- Gene set enrichment practical
- Presenting results
- Dealing with artefacts and biases
- Motif analysis
- Motif analysis practical
## Standard Gene List Output

<table>
<thead>
<tr>
<th>Rank</th>
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<th>P-value</th>
<th>GPR Fold Change</th>
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Gene summary sites are useful for single genes

**TNFSF4** - tumor necrosis factor (ligand) superfamily...

Homo sapiens

Synonyms: CD134L, CD252, GP34,

**Blati, E. et al., Godfrey, W.R. et al., Wang, X. et al., Takasawa, N. et al., Ito, T. et al., et al.**

Welcome! If you are familiar with the subject of this article, you can contribute to this open access knowledge base by deleting incorrect information, restructuring or completely rewriting any text. Read more.

**Disease relevance of TNFSF4**

- In two independent human populations, the less common allele of SNP rs3850641 in TNFSF4 was significantly more frequent (P <0.05) in individuals with myocardial infarction than in controls [1].
- However, cytotoxic T lymphocyte (CTL) clones specific for Epstein-Barr virus (EBV)-transformed autologous lymphoblastic cell lines (LGLs) induced bothOX40 andOX40L expression after antigen or T cell receptor (TCR) stimulation [2].
- We have cloned and sequenced a cDNA encoding gp34, a novel glycoprotein expressed in cells bearing human T-cell leukemia virus type 1 (HTLV-I) [2].
- On the other hand, gp34 was not expressed on these cells, although its expression is also known to be associated with HTLV-I infection [4].
- Regulation of T cell activation in vitro and in vivo by targeting theOX40-OX40L ligand interaction: amelioration of ongoing inflammatory bowel disease with anOX40-IgG fusion protein, but not with anOX40IgG fusion protein [5].

**High impact information on TNFSF4**

- We therefore conclude that Tnfsf4 underlies Abh1 in mice and that polymorphisms in its human homolog TNFSF4 increase the risk of myocardial infarction in humans [1].
- The quantitative trait locus region encompasses 11 known genes, including Tnfsf4 (also called Ox40L or Cds134), which encodes OX40 ligand [1].
- When activated in the presence of leukemic CLL B cells, T cells rapidly up-regulate CD28 through an OX40 ligand and Nectin-1 (IL-15) dependent mechanism [6].
- Here we report that TSLP induced human DCs to express OX40 ligand (OX40L) but not IL-12 [2].
- TSLP-induced OX40L on DCs was required for triggering naive CD4+ T cells to produce IL-10, -5, and -13 [7].

**Biological context of TNFSF4**

- This study suggests a possible function of OX40L/OX40, through T cell-T cell interaction, in the reactivation of memory T cells in an autocrine manner, with implications for the pathogenesis of viral infections and neoplasms [2].
- These results indicated that rat OX40L can provide an efficient costimulation for rat T cells and that it may be involved in HTLV-I-associated pathologies in the rat system as has been suggested in the human system [8].
- Enhancing the immunostimulatory function of dendritic cells by transfection with miRNA encoding OX40 ligand [9].
- Cell adhesion assay was performed and in at least three cases, fresh ATL cells exhibited adhesion to human umbilical vein endothelial cells that could be considerably inhibited by either anti-OX40 MoAb or anti-gp34 MoAb [4].
- T cell proliferation by direct cross-talk between OX40Ligand on human mast cells and OX40 on human T cells: comparison of gene expression profiles between human tonsilar and lung cultured mast cells [10].
Functional analysis relates hits to existing knowledge

**Advantages:**

- Biological insight
- Validation of experiment
- Generate new hypotheses

**Limitations:**

- You can only discover what is already known
  - Novel functionality will be missing
  - Existing annotations may be incorrect
  - Many species are poorly supported
Most functional analysis starts from gene lists

• Many considerations
  – Other start points
    • Genomic positions
    • Transcripts / Proteins
  – Gene nomenclature
  – Annotation sources / versions

• Types of list
  – Categorical (hit or not a hit)
  – Ordered
  – Quantitative
A functional gene set provides a group of genes with a common biological relationship

Germ-line stem cell division

The self-renewing division of a germline stem cell to produce a daughter stem cell and a daughter germ cell, which will divide to form the gametes.

<table>
<thead>
<tr>
<th>Gene/product</th>
<th>Gene/product name</th>
<th>Organism</th>
<th>PANTHER family</th>
<th>Type</th>
<th>Source</th>
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<td>protein</td>
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**Functional analysis relates your hits to a set of pre-defined functional groups**

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<th>Flywch1</th>
<th>Mypop</th>
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<tr>
<td>Atl1</td>
<td>Gnpda2</td>
<td>Rnf6</td>
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<td>Cdk19</td>
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<td>Etv5</td>
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Functional analysis relates your hits to a set of pre-defined functional groups

A4galt  Flywch1  Mypop
Atl1    Gnpda2  Rnf6
Cdk19   Hoxc4   Serinc1
Cdon    Ing2    Stra8
Cecr2   Igip1   Trp73
Etv5    Map3k9  Zbtb16

Germ-line stem cell division

The self-renewing division of a germline stem cell to produce a daughter stem cell and a daughter germ cell, which will divide to form the gametes.
Nothing is ever straight forward...

Best hit: “DNA Methylation”  p<2e-10

- name: DNA methylation
- datasource: reactome
- organism: Human
- idtype: hgnc symbol
- Genes:
  - Methyltransferases: DNMT1 DNMT3A DNMT3B DNMT3L
  - Methyltransferase targeting protein: UHRF1
- Histones!!! H2AFB1 H2AFJ H2AFV H2AFX H2AFZ H2BFS H3F3A H3F3B HIST1H2AB HIST1H2AC HIST1H2AD HIST1H2AE HIST1H2AJ HIST1H2AA HIST1H2BA HIST1H2BB HIST1H2BC HIST1H2BD HIST1H2BE HIST1H2BF HIST1H2BG HIST1H2BH HIST1H2BI HIST1H2BJ HIST1H2BK HIST1H2BL HIST1H2BM HIST1H2BN HIST1H2BO HIST1H3A HIST1H3B HIST1H3C HIST1H3D HIST1H3E HIST1H3F HIST1H3G HIST1H3H HIST1H3I HIST1H3J HIST1H4A HIST1H4B HIST1H4C HIST1H4D HIST1H4E HIST1H4F HIST1H4H HIST1H4I HIST1H4J HIST1H4K HIST1H4L HIST2H2AA3 HIST2H2AA4 HIST2H2AC HIST2H2BE HIST2H3A HIST2H3C HIST2H3D HIST2H4A HIST2H4B HIST3H2BB HIST4H4
There are many sources of functional gene lists

- Human curated
  - Gene Ontology
  - Biological Pathways

- Domains / Patterns
  - Protein functional domains
  - Transcription factor regulated

- Experimental
  - Co-expressed genes
  - Interactions
  - Hits from other studies
Gene Ontology is a human curated functional database
GO has three domains and a hierarchical structure.
Genes are placed into each domain as specifically as possible

Nanog homeobox [Source:HGNC Symbol;Acc:HGNC:20857]

- **Cellular Component**
  - GO:0005634 nucleus
  - GO:0005654 nucleoplasm
  - GO:0005730 nucleolus

- **Molecular Function**
  - GO:0003677 DNA binding
  - GO:0003700 transcription factor activity, sequence-specific DNA binding
  - GO:0003714 transcription corepressor activity
  - GO:0005515 protein binding
  - GO:0043565 sequence-specific DNA binding

- **Biological Process**
  - GO:0001714 endodermal cell fate specification
  - GO:0006351 transcription, DNA-templated
  - GO:0006355 regulation of transcription, DNA-templated
  - GO:0007275 multicellular organism development
  - GO:0008283 cell proliferation
  - GO:0019827 stem cell population maintenance
  - GO:0030154 cell differentiation
  - GO:0035019 somatic stem cell population maintenance
  - GO:0045595 regulation of cell differentiation
  - GO:0045944 positive regulation of transcription from RNA polymerase II promoter
  - GO:1903507 negative regulation of nucleic acid-templated transcription
Annotations come with evidence

- **Experimental Evidence**
  - Inferred from Experiment (EXP)
  - Inferred from Direct Assay (IDA)
  - Inferred from Physical Interaction (IPI)
  - Inferred from Mutant Phenotype (IMP)
  - Inferred from Genetic Interaction (IGI)
  - Inferred from Expression Pattern (IEP)
Annotations come with evidence

- Computational Evidence
  - Inferred from Sequence or structural Similarity (ISS)
  - Inferred from Sequence Orthology (ISO)
  - Inferred from Sequence Alignment (ISA)
  - Inferred from Sequence Model (ISM)
  - Inferred from Genomic Context (IGC)
  - Inferred from Biological aspect of Ancestor (IBA)
  - Inferred from Biological aspect of Descendant (IBD)
  - Inferred from Key Residues (IKR)
  - Inferred from Rapid Divergence (IRD)
  - Inferred from Reviewed Computational Analysis (RCA)
Annotations come with evidence

- Publications
  - Traceable Author Statement (TAS)
  - Non-traceable Author Statement (NAS)
- Curators
  - Inferred by Curator (IC)
  - No biological Data available (ND)
- Automated assignment
  - Inferred from Electronic Annotation (IEA)
Annotations come with evidence

- It looks like something which is annotated
- Actual experimental evidence
- Curator Interpretation
- Claimed in a paper
- Mixture of sources
- Annotated based on where it is in the genome
Pathway databases trace metabolic pathways and their regulation
Protein Domain databases map out functional subdomains within proteins
Transcription Factor databases group genes by the motifs in their promoters
Co-expression databases group genes which are expressed together

GeneFriends

[Images of co-expression databases and gene networks]
Interaction databases map out physical interactions between genes and their products
Some databases collate gene sets from many different sources

Pathway Commons
Access and discover data integrated from public pathway and interactions databases.

MSSigDB
Molecular Signatures Database v6.2
Testing for enriched gene sets
There are two basic ways to test for enrichment

• Categorical
  – Start from a list of hit genes
  – Count overlaps between hit list and functional list
  – Find Functional lists where the degree of overlap is statistically unlikely

• Quantitative
  – Start with all genes
  – Associate a value with each gene
  – Look for functional sets with unusual distributions of values
Categorical Enrichment Analysis
Categorical tests for enrichment

13,101 genes on chip

3005 genes related to disease
3005/13,101 = 23.1%

Gene List

747 expression-significant genes

Related to disease
260/747 = 34.8%

Not related to disease
487

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Fisher’s Exact test

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<tr>
<td>annotated</td>
<td></td>
<td>E = 10012.1</td>
<td></td>
</tr>
<tr>
<td>group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>747</td>
<td>13101</td>
<td>13848</td>
</tr>
</tbody>
</table>

\[
\frac{(260/487)}{(3005/10096)}
\]

\[
\text{p-value} = 9.769 \times 10^{-13}
\]

alternative hypothesis: true odds ratio is not equal to 1

95 percent confidence interval:
1.52846 2.10120

sample estimates:
odds ratio
1.793564
Categorical tests are influenced by where you set the cutoff for “interesting” genes

- Function X
  - 3 hits out of 32 in ‘interesting’ list
  - Not significant (p=0.07)
Categorical tests are influenced by where you set the cutoff for “interesting” genes

• Function X
  – 3 hits out of 7 in ‘interesting’ list
  – Significant (p=0.02)
Ordered, but not quantitative lists allow sequential categorical analysis

- Function X
  - Length=1 p=0.60
  - Length=2 p=0.80
  - Length=3 p=0.30
  - Length=4 p=0.35
  - Length=5 p=0.40
  - Length=6 p=0.45
  - Length=7 p=0.05
  - Length=8 p=0.08
  - Length=9 p=0.10
Quantitative Enrichment Analysis
Quantitative comparisons offer more power, if you have a suitable metric

• What quantitation can we use?
  – Differential p-value (normally -10 \( \log(p) \))
  – Fold change
  – Absolute difference

• Measures often have odd distributions and biases
  – Z-scores
  – Ranks
What kind of changes do we expect in an interesting category?

Genes in that category all change, and by about the same amount?
What kind of changes do we expect in an interesting category?

Genes in that category all change in the same direction, but by different amounts?

Kolmogorov Smirnov Test
What kind of changes do we expect in an interesting category?

Genes in that category all change in either direction, but by different amounts?
Kolmogorov Smirnov

- Looks for the biggest point of difference between the background and test lists
Multiple testing correction

• More annotations/functions being tested = more chance of increase in false-positives

**Bonferroni**
- Significance level (e.g. 0.05) / number of tests = new threshold
- Over correction if tests are correlated

**Benjamini-Hochberg**
- Rank the p-values
- Apply more stringent correction to the most significant, and least stringent to the least significant p-values
What do we get back from an enrichment test?

• A p-value
  – Remember that this reflects not only difference but also variance and power (number of observations)

• A difference value
  – Enrichment difference (odds ratio)
  – Mean quantitative difference
  – Remember large differences are easier to obtain with small numbers of observations
Tools for functional gene list analysis

• There are many different tools available, both free and commercial

• Popular tools include:
• Categorical or ordered statistics
• Lots of additional options
• Wide species support
• Interesting presentation
  – Doesn’t scale well to lots of hits
• Categorical or Quantitative statistics
• Part of Gene Ontology Consortium
  – Annotations are up to date
• Simple enrichment analysis
• Functional lists and categorical break down
• Categorical or quantitative statistics
• Pathway focussed
• Simple submission interface (no custom background)
• Really nice visualisations
• Categorical statistics
• Limited species support
• Allows custom backgrounds
• Uses PathwayCommons gene sets
• Innovative detection and presentation of artefacts
• Categorical Statistics
• Most popular system (mostly historic)
• Has been behind the latest annotation
  – Was updated again, but now behind once more
• Lots of support for different IDs and Species
• Configurable gene sets
• Simple output presentation
• Categorical Statistics
• Biggest selection of gene sets
• Simple interface, but limited options
  – No species information
  – No background list option
• Simple interactive visualisation
• Novel scoring scheme to rank hits
• Categorical or ranked analysis
• Mostly GO gene list support
• Interesting visualisation options
GSEA

- Quantitative enrichment
- Designed for expression datasets
- Local application
- Imports tab delimited expression data
• Genes ranked based on correlation to annotation groups
• Genes from a gene set placed onto the ranked lists
• Look for sets where there is unusual grouping at the top or the bottom of the list
• Quantitative enrichment of sequencing datasets
• Local Java application
Gene List
Practical