

V9 funktionelle Annotation

- **Analyse von Gen-Expression**
- **Funktionelle Annotation: Gene Ontology (GO)**
- **Signifikanz der Annotation: Hypergeometrischer Test**
- **Annotationsanalysen z.B. mit NIH-Tool DAVID**
- **Ähnlichkeit von GO-Termen automatisch bestimmen**
- **OMIM-Datenbank**

Beispiel: differentielle Gen-Expression für ALL-Patienten

Input:

Genexpressionsdaten für 128 Patienten mit akuter lymphatischer Leukämie (ALL).

Alle ALL-Patienten haben chromosomale Veränderungen.

Der Therapieerfolg ist sehr unterschiedlich.

Eine Gruppe von Patienten (ALL1/AF4) hat eine genetische Translokation zwischen den Chromosomen 4 und 11.

Eine zweite Gruppe von Patienten (BCR/ABL) hat eine genetische Translokation zwischen den Chromosomen 9 und 22.

Die Krankheitsursachen + optimale Therapie könnten für die beiden Gruppen verschieden sein.

Ziel:

Identifiziere Gene, die zwischen den beiden Gruppen differentiell exprimiert werden.

Beispiel für die Anwendung der Bioconductor-Software (siehe Ref unten, bisher 6700 mal zitiert).

Gentleman et al. Genome Biology 5, R80 (2004)

Auswahl der differentiell exprimierten Gene

```

> f <- factor(as.character(eset$mol))
> design <- model.matrix(~f)
> fit <- lmFit(eset, design)
> fit <- eBayes(fit)
> topTable(fit, coef = 2)

```

	ID	M	A	t	p-value	B	
	1016	1914_at	-3.1	4.6	-27	5.9e-27	56
	7884	37809_at	-4.0	4.9	-20	1.3e-20	44
	6939	36873_at	-3.4	4.3	-20	1.8e-20	44
	10865	40763_at	-3.1	3.5	-17	7.2e-18	39
	4250	34210_at	3.6	8.4	15	3.5e-16	35
	11556	41448_at	-2.5	3.7	-15	1.8e-15	34
	3389	33358_at	-2.3	5.2	-13	3.3e-13	29
	8054	37978_at	-1.0	6.9	-10	6.5e-10	22
	10579	40480_s_at	1.8	7.8	10	9.1e-10	21
	330	1307_at	1.6	4.6	10	1.4e-09	21

Figure 1

Limma analysis of the ALL data. The leftmost numbers are row indices, ID is the Affymetrix HGU95av2 accession number, M is the log ratio of expression, A is the log average expression, and B is the log odds of differential expression.

Differential expression (D.E.) = $\log(R) / \log(G)$

Log ratio M : $2^M = \log(R) / \log(G)$; M = 1 -> zweifach D.E.

Wie signifikant ist dies? -> bewerte mit statistischem Test.

Vergleiche Gen-Expression in den beiden Gruppen.

Fokussiere auf Gene mit stark unterschiedlicher Expression.

Wähle alle Gene mit p-Wert < 0.05 aus.

Es bleiben 165 Gene übrig.

Gentleman et al. Genome Biology 5, R80 (2004)

Differentielle Gen-Expression als Heatmap visualisieren

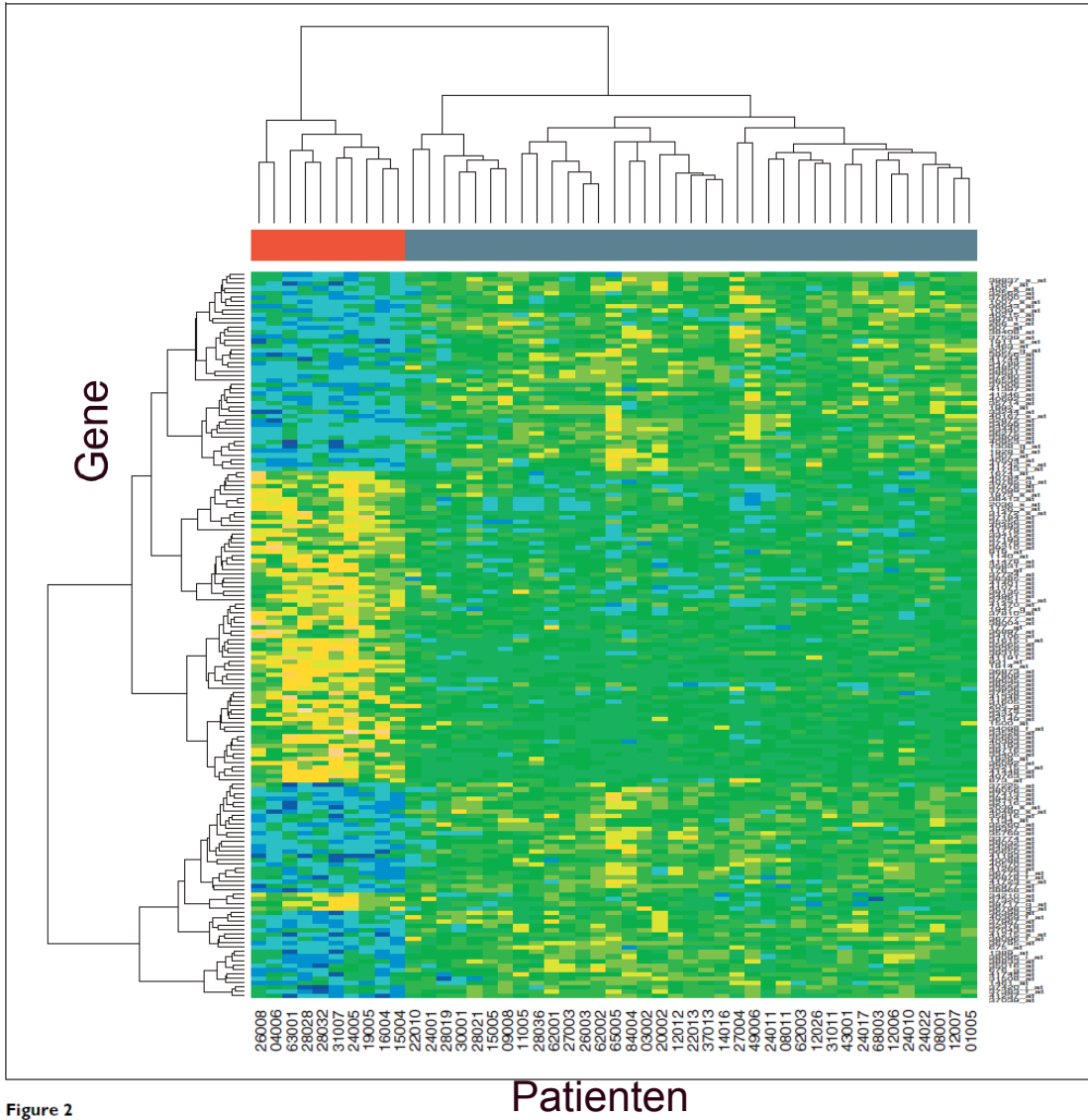


Figure 2
Heat map (produced by the Bioconductor function `heatmap()`) of the ALL leukemia data.

Mit einem Abstandsmaß und einem Cluster-Algorithmus werden die Ähnlichkeiten zwischen den Patienten (x-Achse) und den einzelnen Genen (y-Achse) erfasst.

Die beiden Patienten-Gruppen lassen sich deutlich unterscheiden (rot/grau).

Gelb: stark hochreguliert
Blau: stark runterreguliert

Gentleman et al. Genome Biology 5, R80 (2004)

Zuordnung von Gen-Funktion

```
> f <- factor(as.character(eset$mol))
> design <- model.matrix(~f)
> fit <- lmFit(eset, design)
> fit <- eBayes(fit)
> topTable(fit, coef = 2)
```

Bioconductor
Kommandos

	ID	M	A	t	<i>p</i> -value	B
1016	1914_at	-3.1	4.6	-27	5.9e-27	56
7884	37809_at	-4.0	4.9	-20	1.3e-20	44
6939	36873_at	-3.4	4.3	-20	1.8e-20	44
10865	40763_at	-3.1	3.5	-17	7.2e-18	39
4250	34210_at	3.6	8.4	15	3.5e-16	35
11556	41448_at	-2.5	3.7	-15	1.8e-15	34
3389	33358_at	-2.3	5.2	-13	3.3e-13	29
8054	37978_at	-1.0	6.9	-10	6.5e-10	22
10579	40480_s_at	1.8	7.8	10	9.1e-10	21
330	1307_at	1.6	4.6	10	1.4e-09	21

Figure 1

Limma analysis of the ALL data. The leftmost numbers are row indices, ID is the Affymetrix HGU95av2 accession number, M is the log ratio of expression, A is the log average expression, and B is the log odds of differential expression.

Links gezeigt ist dieselbe Tabelle wie zwei Folien zuvor.

Neue Frage: welche Funktionen üben diese Gene in der Zelle aus?

Verwende dazu die Gene Ontology.

Gentleman et al. Genome Biology 5, R80 (2004)

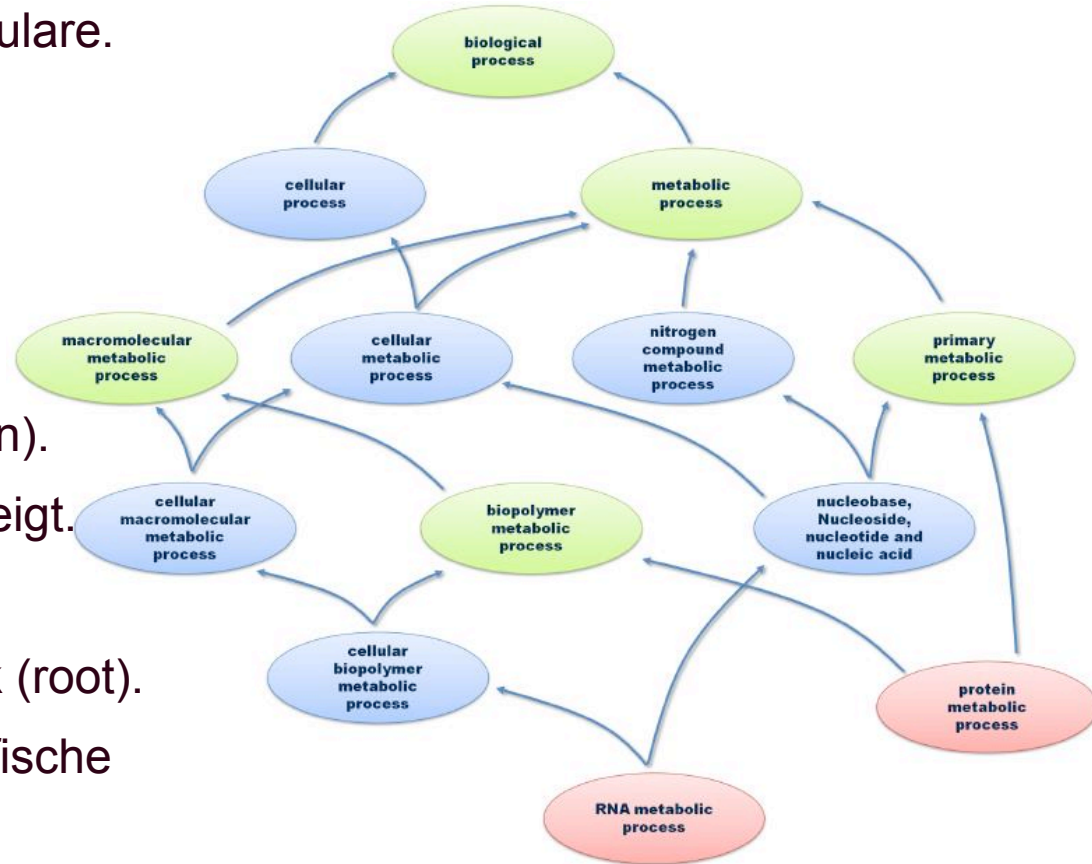
Die Gene Ontology (GO)

Ontologien sind strukturierte Vokabulare.

Die Gene Ontology hat 3 Bereiche:

- biologischer Prozess (BP)
- molekulare Funktion (MF)
- zelluläre Komponente (Lokalisation).

Hier ist ein Teil des BP-Baums gezeigt.



Oben ist der allgemeinste Ausdruck (root).

Rot: Blätter des Baums (sehr spezifische GO-Terme)

Grün: gemeinsame Vorgänger.

Blau: andere Knoten.

Linien: „Y ist in X enthalten“-Beziehungen

Dissertation Andreas Schlicker (UdS, 2010)

Gene Ontology (GO) - Konsortium

Berkeley Bioinformatics Open-source Project (BBOP)

British Heart Foundation - University College London (BHF-UCL)

dictyBase

EcoliWiki

FlyBase

GeneDB

UniProtKB-Gene Ontology Annotation @ EBI (UniProtKB-GOA)

GO Editorial Office at the European Bioinformatics Institute

Gramene

Institute of Genome Sciences, Univ. of Maryland

J Craig Venter Institute

Mouse Genome Informatics (MGI)

Rat Genome Database (RGD)

Reactome

Saccharomyces Genome Database (SGD)

The Arabidopsis Information Resource (TAIR)

WormBase

The Zebrafish Information Network (ZFIN)

Woher stammen die Gene Ontology Annotationen?

Evidence code	Evidence code description	Source of evidence	Manually checked	Current number of annotations*
IDA	Inferred from direct assay	Experimental	Yes	71,050
IEP	Inferred from expression pattern	Experimental	Yes	4,598
IGI	Inferred from genetic interaction	Experimental	Yes	8,311
IMP	Inferred from mutant phenotype	Experimental	Yes	61,549
IPI	Inferred from physical interaction	Experimental	Yes	17,043
ISS	Inferred from sequence or structural similarity	Computational	Yes	196,643
RCA	Inferred from reviewed computational analysis	Computational	Yes	103,792
IGC	Inferred from genomic context	Computational	Yes	4
IEA	Inferred from electronic annotation	Computational	No	15,687,382
IC	Inferred by curator	Indirectly derived from experimental or computational evidence made by a curator	Yes	5,167
TAS	Traceable author statement	Indirectly derived from experimental or computational evidence made by the author of the published article	Yes	44,564
NAS	Non-traceable author statement	No 'source of evidence' statement given	Yes	25,656
ND	No biological data available	No information available	Yes	132,192
NR	Not recorded	Unknown	Yes	1,185

*October 2007 release

Rhee et al. Nature Reviews Genetics 9, 509-515 (2008)

Woher stammen die Gene Ontology Annotationen?

Species (NCBI taxon ID)	Genes* with experimental annotations [†]	Total annotated genes*	Percentage of genes* with at least one experimental annotation	Total genes*	Percentage annotated [§]	Percentage known in genome
<i>Schizosaccharomyces pombe</i> (4896)	4,482	4,930	90.9%	4,930	100%	90.9%
<i>Saccharomyces cerevisiae</i> (4932)	4,947	5,794	85.4%	5,794	100%	85.4%
Mouse (10090)	10,621	18,386	57.8%	27,289	67.4%	38.9%
<i>Caenorhabditis elegans</i> (6239)	4,614	14,154	32.6%	20,163	70.2%	22.9%
Human [§] (9606)	4,780	17,021	28.1%	20,887	81.5%	22.9%
<i>Arabidopsis thaliana</i> [¶] (3702)	5,530	26,637	20.8%	27,029	98.5%	20.5%
Rat (10116)	3,566	17,243	20.7%	17,993	95.8%	19.8%
Fruitfly (7227)**	2,790	9,563	29.2%	14,141	67.6%	19.7%
<i>Candida albicans</i> (5476)	806	3,756	21.4%	6,166	60.9%	13.0%
<i>Pseudomonas aeruginosa</i> PAO1 (208964)	491	2,506	19.6%	5,568	45.0%	8.82%
Slime mold (44689)	797	6,892	11.6%	13,625	50.6%	5.9%
<i>Trypanosoma brucei</i> (5691)	449	3,914	11.5%	9,154	42.8%	4.92%
Zebrafish (7955)	1,235	13,574	5.8%	21,322	63.7%	3.7%
<i>Plasmodium falciparum</i> (5833)	188	3,243	5.8%	5,420	59.8%	3.47%
Rice (39947)	654	29,877	2.2%	41,908	71.3%	1.57%
Chicken [§] (9031)	75	6,063	1.2%	16,737	36.2%	0.4%
Cow [§] (9913)	96	8,536	1.1%	21,756	39.2%	0.4%

*Total genes in genomes include only those that encode proteins. These numbers were obtained from the databases that contribute annotations to GO and are listed on the GO annotations download page (<http://www.geneontology.org/GO.current.annotations.shtml>). [†]Experimental annotations include those only with the following evidence codes: IDA (inferred from direct assay), IEP (inferred from expression pattern), IGI (inferred from genetic interaction), IMP (inferred from mutant phenotype) and IPI (inferred from physical interaction). [‡]Percentage annotated is determined by dividing the number of genes annotated by total genes. [§]Percentage known in genome is determined by multiplying the percentage of experimentally derived annotations by the percentage of the genome annotated. This is an approximation of the extent of knowledge about the portion of the genome that encodes proteins in an organism with a complete genome sequence that is captured by annotation. [¶]Numbers are from the GO annotation project at the European Bioinformatics Institute, human data last updated 14 September 2007, cow data last updated 17 January 2007, chicken data last updated 10 July 2007. ^{**}Numbers are from The Arabidopsis Information Resource (TAIR), last updated 14 December 2007. ^{***}Numbers are based on release 5.4 of the *Drosophila melanogaster* genome and GO annotations from FlyBase release FB2007_03 (dated 11 January 2007). NCBI, National Center for Biotechnology Information.

Rhee et al. Nature Reviews Genetics 9, 509-515 (2008)

Beispiel: GO-Annotation für humanes BRCA1-Gen

BRCA1

Breast cancer type 1 susceptibility protein

protein from *Homo sapiens* (human)

Term associations ↓ Gene product information → Peptide Sequence → Sequence information →

Term Associations

Download all association information in: [gene association format](#) [RDF/XML](#)

Filter associations displayed ?

Filter Associations

Ontology	Evidence Code
All	All
biological process	IC
cellular component	IDA
molecular function	IEA

Set filters
Remove all filters

1 2 View all results

Select all Clear all Perform an action with this page's selected terms... Got

Accession, Term	Ontology	Qualifier	Evidence	Reference	Assigned by
162 gene products GO:0030521 : androgen receptor signaling pathway	biological process	NAS		PMID:15572661	UniProtKB
6411 gene products GO:0006915 : apoptosis	biological process	TAS		PMID:10918303	UniProtKB
1997 gene products GO:0007420 : brain development	biological process	IEA With Ensembl:ENSRNOP00000028109		GO REF:0000019	Ensembl (via UniProtKB)
10144 gene products GO:0007049 : cell cycle	biological process	IEA With SP KW:KW-0131		GO REF:0000004	UniProtKB
26 gene products	biological process	IDA		PMID:10868478	UniProtKB

Einzelne GO-Terme, mit denen das Brustkrebs-Gen BRCA1 annotiert ist.

Signifikanz von GO-Annotationen

Sehr allgemeine GO-Terme wie z.B. “cellular metabolic process“ werden vielen Genen zugeordnet.

Sehr spezielle Terme gehören jeweils nur zu wenigen Genen.

Man muss also vergleichen, wie signifikant das Auftreten jedes GO-Terms in einer Testmenge an Genen im Vergleich zu einer zufällig ausgewählten Menge an Genen derselben Größe ist.

Dazu verwendet man meist den **hypergeometrischen Test**.

Vorbemerkung

Zieht man aus einer Urne mit n Kugeln insgesamt k Kugeln **ohne Beachtung der Reihenfolge**, so gibt es hierfür genau

$$\frac{n \cdot (n-1) \cdot (n-2) \cdot \dots \cdot (n-k+1)}{k!} = \frac{n!}{k! \cdot (n-k)!} = \binom{n}{k} \text{ Möglichkeiten}$$

Hypergeometrischer Test

$$\text{p-Wert} = \sum_{i=k_{\pi}}^{\min(n, K_{\pi})} \frac{\binom{K_{\pi}}{i} \binom{N-K_{\pi}}{n-i}}{\binom{N}{n}}$$

Der hypergeometrische Test ist ein statistischer Test, der z.B. überprüft, ob in einer vorgegebenen Testmenge an Genen eine biologische Annotation π gegenüber dem gesamten Genom statistisch signifikant angereichert ist.

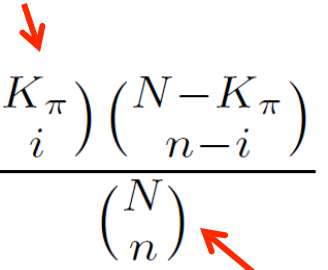
- Sei N die Anzahl an Genen im Genom.
- Sei n die Anzahl an Genen in der Testmenge.
- Sei K_{π} die Anzahl an Genen im Genom mit der Annotation π .
- Sei k_{π} die Anzahl an Genen in der Testmenge mit der Annotation π .

Der hypergeometrische p-Wert drückt die Wahrscheinlichkeit aus, dass k_{π} oder mehr **zufällig** aus dem Genom ausgewählte Gene auch die Annotation π haben.

Hypergeometrischer Test

Wähle $i = k_\pi$ Gene mit
Annotation π aus dem Genom.
Davon gibt es genau K_π .

Die anderen $n - i$ Gene in der
Testmenge haben dann nicht die
Annotation π . Davon gibt es im Genom
genau $N - K_\pi$.

$$\text{p-Wert} = \sum_{i=k_\pi}^{\min(n, K_\pi)} \frac{\binom{K_\pi}{i} \binom{N - K_\pi}{n - i}}{\binom{N}{n}}$$


Die Summe läuft von mindestens
 k_π Elementen bis zur maximal
möglichen Anzahl an Elementen.

Eine Obergrenze ist durch die
Anzahl an Genen mit Annotation π
im Genom gegeben (K_π).

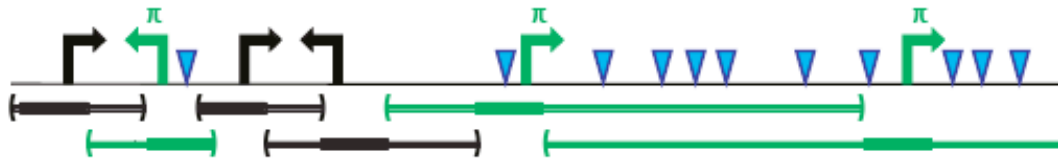
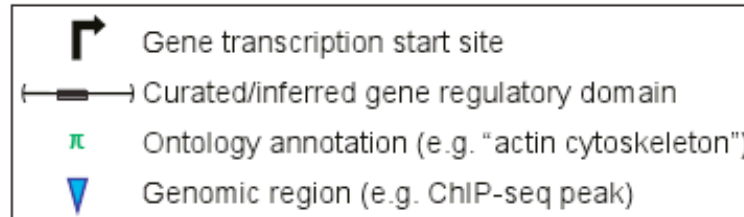
Die andere Obergrenze ist die Zahl
der Gene in der Testmenge (n).

Korrigiert für die kombinatorische
Vielfalt an Möglichkeiten um n
Elemente aus einer Menge mit N
Elementen auszuwählen.

N.B. dies gilt für den Fall, dass
die Reihenfolge der Elemente
egal ist.

Beispiel

$$\text{p-Wert} = \sum_{i=k_{\pi}}^{\min(n, K_{\pi})} \frac{\binom{K_{\pi}}{i} \binom{N-K_{\pi}}{n-i}}{\binom{N}{n}}$$



Hypergeometric test over genes

N = 6 total genes

K_{π} = 3 genes annotated with π

n = 3 genes with an associated genomic region

k_{π} = 3 genes annotated and with a genomic region

P-value = 0.05

Frage: ist die Annotation π in der Testmenge von 3 Genen signifikant angereichert?

Ja! $p = 0.05$ ist signifikant.

<http://great.stanford.edu/>

Anwendung auf ALL-Beispiel

```
> ll <- mget(geneNames(esetSel),  
            hgu95av2LOCUSID)      Bioconductor  
                                Kommandos  
> ll <- unique(unlist(ll))  
> mf <- as.data.frame(GOHyperG(ll))[, 1:3]  
> mf <- mf[mf$pvalue < 0.01, ]  
> mf
```

	<i>p</i> -values	goCounts	intCounts
GO:0045012	1.1e-08	12	6
GO:0030106	6.4e-03	8	2
GO:0004888	7.0e-03	523	16
GO:0004872	9.7e-03	789	21

```
> as.character(unlist(mget(row.names(mf),  
                          GOTERM)))
```

```
[1] "MHC class II receptor activity"  
[2] "MHC class I receptor activity"  
[3] "transmembrane receptor activity"  
[4] "receptor activity"
```

Figure 3

Hypergeometric analysis of molecular function enrichment of genes selected in the analysis described in Figure 1.

Die signifikanteste Anreicherung ergibt sich für MHC Klasse 2 Rezeptoraktivität. 6 von 12 Genen im Genom mit dieser Annotation sind in den 2 ALL-Klassen differentiell exprimiert.

Gentleman et al. Genome Biology 5, R80 (2004)

NIH Tool David: Tool für Annotation der Genfunktion

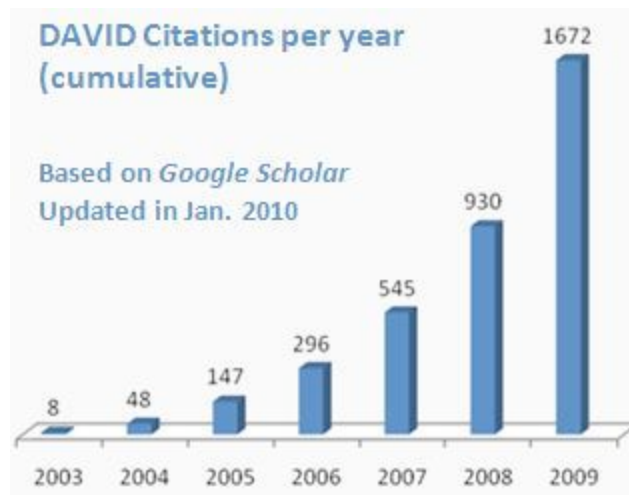
PROTOCOL

Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources

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Published online 18 December 2008; doi:10.1038/nprot.2008.211



NIH Tool David

DAVID 2006 Functional Annotation Bioinformatics (LIB, NIAID/NIH, SAIC-Frederick) - Microsoft Internet Explorer

File Edit View Favorites Tools Help

Back Forward Stop Home Search Favorites

Address <http://david.abcc.ncifcrf.gov/home.jsp> Go Links

Google Bookmarks PageRank Popups okay Check AutoLink Settings

DAVID Bioinformatic Resources 2006

National Institute of Allergy and Infectious Diseases (NIAID), NIH

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Shortcut to DAVID Tools

- Functional Annotation**
Gene-annotation enrichment analysis, functional annotation clustering, BioCarta & KEGG pathway mapping, gene-disease association, homologue match, ID translation, literature match and [more](#)
- Gene Functional Classification**
Provide a rapid means to reduce large lists of genes into functionally related groups of genes to help unravel the biological content captured by high throughput technologies. [More](#)
- Gene ID Conversion**
Convert list of gene ID/accessions to others of your choice with the most comprehensive gene ID mapping repository. The ambiguous accessions in the list can also be determined semi-automatically.
- List Gene Names in Batch** new!

Welcome to DAVID Bioinformatic Resources

The Database for Annotation, Visualization and Integrated Discovery (DAVID) 2006 is an expanded version of our original web-accessible programs of DAVID 2.1, 2.0 & 1.0. DAVID provides a comprehensive set of functional annotation tools for investigators to understand biological meaning behind large list of genes. For any given gene list, DAVID tools are able to:

- Identify enriched biological themes, particularly GO terms
- Discover enriched functional-related gene groups
- Visualize genes on BioCarta & KEGG pathway maps
- Search for other functionally related genes not in the list
- List interacting proteins
- Explore gene names in batch
- Link gene-disease associations
- Highlight protein functional domains and motifs
- Redirect to related literatures

What's New in DAVID 2006?

- [Functional Annotation Clustering](#)
- [Pre-built Affy gene backgrounds](#)
- [User's customized gene background](#)
- [Updated annotation databases](#)
- [Enhanced calculating speed](#)

DAVID Bioinformatic Forum

- [Technical notes & help](#)
- [Ask questions & get answers](#)
- [Share experiences](#)
- [Comments and feedback](#)
- [Bug report](#)

Statistics About DAVID

Done Internet

Submit gene list or use built-in demo_lists

DAVID 2006: functional annotation result summary - Microsoft Internet Explorer

Address: http://david.abcc.ncifcrf.gov/tools.jsp

Analysis Wizard

DAVID Bioinformatic Resources 2006, NIAID/NIH

Home Start Analysis Shortcut to DAVID Tools Technical Center Archives Term of Service DAVID Forum Credits About Us

Upload List Background

Upload Gene List

[Demolist 1](#) [Demolist 2](#)
[Upload Help](#)

Step 1: Enter Gene List

A: Paste a list

Or

B: Choose From a File

Step 2: Select Identifier

AFFY_ID

Step 3: List Type

Gene List
Background

Step 4: Submit List

Analysis Wizard

[Tell us how you like the tool](#)
[Contact us for questions](#)

← Step 1. Submit your gene list through left panel.

An example:

Copy/paste IDs to "box A" -> Select Identifier as "AFFY_ID" -> List Type as "Gene List" -> Click "Submit" button

```
1007_s_at
1053_at
117_at
121_at
1255_g_at
1294_at
1316_at
1320_at
1405_i_at
1431_at
1438_at
1487_at
1494_f_at
1598_g_at
```

Select the DAVID Gene Functional Classification Tool

The screenshot shows the DAVID 2006 website in a Microsoft Internet Explorer browser window. The page title is "DAVID 2006: functional annotation result summary". The browser address bar shows "http://david.abcc.ncifcrf.gov/tools.jsp". The website header includes the "Analysis Wizard" title and "DAVID Bioinformatic Resources 2006, NIAID/NIH". A navigation menu contains links for Home, Start Analysis, Shortcut to DAVID Tools, Technical Center, Archives, Term of Service, DAVID Forum, Credits, and About Us.

The main content area is titled "Analysis Wizard" and displays the following steps:

- Step 1. Successfully submitted gene list**
Current Gene List: Demo_List_2
Current Background: HOMO SAPIENS
- Step 2. Analyze above gene list with one of DAVID tools**

Under Step 2, a blue arrow points to a list of tools:

- [Functional Annotation Tool](#)
- [Functional Annotation Clustering](#)
 - [Functional Annotation Chart](#)
 - [Functional Annotation Table](#)
- [Gene Functional Classification Tool](#) (indicated by a red arrow)
- [Gene ID Conversion Tool](#)
- [Show Gene List Tool](#)

On the left side of the page, there is a "Gene List Manager" section with a dropdown menu showing "HOMO SAPIENS(403)" and "SYNTHETIC CONSTRUCTS". Below it are buttons for "Select", "List Manager", "Use", "Rename", "Remove", "Combine", and "Show Gene List^{new!}".

Select the DAVID Gene Functional Classification Tool

DAVID 2006: Gene Functional Classification

Current Gene List: Demo_List_2
Current Background: HOMO SAPIENS
394 DAVID IDs

Options Classification Stringency Medium

Rerun using options Create Sublist Heatmap

16 Clusters

Download File

Gene Group 1		Enrichment Score: 3.37	RG	T	G
1	<input type="checkbox"/> 34375_at, 875_g_at	chemokine (c-c motif) ligand 2			
2	<input type="checkbox"/> 40385_at	chemokine (c-c motif) ligand 20			
3	<input type="checkbox"/> 36103_at	chemokine (c-c motif) ligand 3			
4	<input type="checkbox"/> 36674_at	chemokine (c-c motif) ligand 4			
5	<input type="checkbox"/> 408_at	chemokine (c-x-c motif) ligand 1 (melanoma growth stimulating activity, alpha)			
6	<input type="checkbox"/> 1369_s_at, 35372_r_at	interleukin 8			

Gene Group 2		Enrichment Score: 2.89	RG	T	G
1	<input type="checkbox"/> 1857_at	smad, mothers against dpp homolog 7 (drosophila)			
2	<input type="checkbox"/> 39421_at	runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene)			
3	<input type="checkbox"/> 36999_at	jumonji, at rich interactive domain 1a (rbbp2-like)			
4	<input type="checkbox"/> 1994_at	activating transcription factor 2			
5	<input type="checkbox"/> 1895_at, 32583_at	v-jun sarcoma virus 17 oncogene homolog (avian)			
6	<input type="checkbox"/> 35768_at	ring finger protein 40			
7	<input type="checkbox"/> 36226_r_at	splicing factor proline/glutamine-rich (polypyrimidine tract binding protein associated)			
8	<input type="checkbox"/> 789_at	early growth response 1			

Select the DAVID Gene Functional Annotation Tool

DAVID 2006: functional annotation result summary - Microsoft Internet Explorer

Address: http://david.abcc.ncifcrf.gov/summary.jsp

Functional Annotation Tool
DAVID Bioinformatic Resources 2006, NIAID/NIH

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Upload List Background

Gene List Manager

Select to limit annotations by one or more species [Help](#)

- Use All Species -
HOMO SAPIENS(403)
SYNTHETIC CONSTRUCT(4)

Select

List Manager [Help](#)

Demo_List_2

Select List to:

Use Rename
Remove Combine
Show Gene List^{new!}

Annotation Summary Results

Current Gene List: Demo_List_2 394 DAVID IDs

Current Background: HOMO SAPIENS Check Defaults Clear All

- Main Accessions (0 selected)
- Other Accessions (0 selected)
- Gene Ontology (3 selected)
- Protein Domains (3 selected)
- Pathways (3 selected)
- General Annotations (0 selected)
- Functional Categories (3 selected)
- Protein Interactions (0 selected)
- Literature (0 selected)
- Disease (2 selected)






Combined View for Selected Annotation

Functional Annotation Clustering^{new!} ←

Functional Annotation Chart

Functional Annotation Table

Funktionelles Clustering von angereicherten GO-Termen

Options		Classification Stringency	Custom			
Rerun using options		Create Sublist	Heatmap	Cluster Comparison		
Functional Group 1		2.9E-4	RG	T		
1	<input type="checkbox"/> 31506_s_at, 31793_at	defensin, alpha 1				
2	<input type="checkbox"/> 34546_at	defensin, alpha 4, corticostatin				
3	<input type="checkbox"/> 34623_at	defensin, alpha 5, paneth cell-specific				
Functional Group 2		7.0E-4	RG	T		
1	<input type="checkbox"/> 35566_f_at	immunoglobulin heavy constant gamma 1 (g1m marker)				
2	<input type="checkbox"/> 35566_f_at	immunoglobulin heavy locus				
3	<input type="checkbox"/> 1355_g_at	neurotrophic tyrosine kinase, receptor, type 2				
4	<input type="checkbox"/> 1786_at	c-mer proto-oncogene tyrosine kinase				
5	<input type="checkbox"/> 1901_s_at	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)				
6	<input type="checkbox"/> 1112_g_at	neural cell adhesion molecule 1				
7	<input type="checkbox"/> 32469_at	carcinoembryonic antigen-related cell adhesion molecule 3				
8	<input type="checkbox"/> 35038_at	myosin binding protein c, cardiac				
9	<input type="checkbox"/> 35090_g_at, 35091_at	neuregulin 2				
10	<input type="checkbox"/> 37968_at	natural cytotoxicity triggering receptor 3				
11	<input type="checkbox"/> 33530_at	carcinoembryonic antigen-related cell adhesion molecule 8				
12	<input type="checkbox"/> 35956_s_at	pregnancy specific beta-1-glycoprotein 4				
13	<input type="checkbox"/> 31987_at	kin of irre like (drosophila)				
14	<input type="checkbox"/> 35956_s_at	pregnancy specific beta-1-glycoprotein 2				
Functional Group 3		2.7E-3	RG	T		
1	<input type="checkbox"/> 37454_at	chemokine (c-c motif) ligand 13				
2	<input type="checkbox"/> 36703_at	chemokine (c-c motif) ligand 25				
3	<input type="checkbox"/> 1403_s_at	chemokine (c-c motif) ligand 5				
Functional Group 4		3.5E-3	RG	T		
1	<input type="checkbox"/> 31687_f_at	hemoglobin, beta				
2	<input type="checkbox"/> 33516_at	hemoglobin, delta				
3	<input type="checkbox"/> 31525_s_at	hemoglobin, alpha 1				
Functional Group 5		4.1E-3	RG	T		
1	<input type="checkbox"/> 40317_at	amiloride-sensitive cation channel 1, neuronal (denegerin)				

XXXX_at sind die Kürzel für einzelne Proben auf Affymetrix-Microarray-Chip

Huang *et al.* *Genome Biology* 2007 **8**:R183

David: Genes-to-terms 2D view

Gene-Term 2D Heat Map View

[SVG version](#)

■ corresponding gene-term association positively reported

■ corresponding gene-term association not reported yet



- chemokine (C-C motif) ligand 3-like 1
- chemokine (C-X-C motif) ligand 1 (melanoma growth stimula
- chemokine (C-C motif) ligand 4-like
- chemokine (C-C motif) ligand 20
- chemokine (C-C motif) ligand 3
- chemokine (C-C motif) ligand 2
- interleukin 8
- defensin, beta 4

- NEGATIVE REGULATION OF CELLULAR PHYSIOLOGICAL PROCESS -- (GOTERM_BP_ALL)
- REGULATION OF CELLULAR PHYSIOLOGICAL PROCESS -- (GOTERM_BP_ALL)
- MACROPHAGE INFLAMMATORY PROTEIN SIGNALING PATHWAY -- (GOTERM_BP_ALL)
- G-PROTEIN COUPLED RECEPTOR INTERACTION -- (KEGG_PATHWAY)
- 169.Chemokine families -- (BRID)
- IPR000227:SMALL CHEMOKINE, C-C -- (GOTERM_BP_ALL)
- G-PROTEIN COUPLED RECEPTOR, LINKED SIGNAL
- CELL-CELL SIGNALING -- (SP_PIR_KEYWORDS)
- CELL-CELL CYTOKINE-CYTOKINE RECEPTOR INTERACTION -- (GOTERM_BP_ALL)
- HEA04060:CYTOKINE-SEQUENCING -- (SP_PIR_KEYWORDS)
- CHEMOTAXIS -- (SP_PIR_KEYWORDS)
- DIRECT PROTEIN RESPONSE -- (GOTERM_BP_ALL)
- INFLAMMATORY RESPONSE -- (GOTERM_BP_ALL)
- INFLAMMATORY RECEPTOR BINDING -- (GOTERM_BP_ALL)
- 3D-STRUCTURE -- (SMART_NAME)
- CITOKINE -- (SP_PIR_KEYWORDS)
- SMO0159:SCY -- (SMART_FEATURE)
- HELVX -- (UP_SEQ_FEATURE) (GOTERM_BP_ALL)
- STRAND -- (UP_SEQ_FEATURE) (GOTERM_BP_ALL)
- TURN -- (UP_SEQ_FEATURE) (GOTERM_BP_ALL)
- RESPONSE TO WOUNDING -- (GOTERM_BP_ALL)
- SIGNAL TRANSDUCTION -- (GOTERM_BP_ALL)
- 1PR00181:SMALL CHEMOKINE, INTERLEUKIN-P-LIKE -- (INTERPRO_NAME)
- SIGNAL TRANSDUCTION -- (GOTERM_MF_ALL)
- CHEMOKINE ACTIVITY -- (GOTERM_MF_ALL)
- CHEMOKINE RECEPTOR BINDING -- (GOTERM_MF_ALL)
- CHEMOKINE RECEPTOR BINDING -- (GOTERM_MF_ALL)
- CHEMOKINE ACTIVITY -- (GOTERM_MF_ALL)
- CHEMOKINE COUPLED RECEPTOR BINDING -- (GOTERM_MF_ALL)
- CITOKINE RESPONSE -- (GOTERM_BP_ALL)
- G-PROTEIN RESPONSE -- (GOTERM_BP_ALL)
- 1MMUNE RESPONSE -- (UP_SEQ_FEATURE) (GOTERM_BP_ALL)
- SIGNAL -- (SP_PIR_KEYWORDS) (GOTERM_BP_ALL)
- DISULFIDE BOND -- (UP_SEQ_FEATURE) (GOTERM_BP_ALL)
- SIGNAL PEPTIDE -- (UP_SEQ_FEATURE) (GOTERM_BP_ALL)
- SIGNAL TO ABIOTIC STIMULUS -- (GOTERM_BP_ALL)
- RESPONSE TO BIOTIC SUBSTANCE -- (GOTERM_BP_ALL)
- RESPONSE TO CHEMICAL SUBSTANCE -- (GOTERM_BP_ALL)
- RESPONSE TO EXTERNAL STIMULUS -- (GOTERM_BP_ALL)
- RESPONSE TO EXTERNAL STIMULUS -- (GOTERM_BP_ALL)
- RESPONSE TO PATHOGEN OR PARASITE -- (GOTERM_BP_ALL)
- RESPONSE TO PEST -- (GOTERM_BP_ALL)
- RESPONSE TO STRESS -- (GOTERM_BP_ALL)
- RESPONSE TO STRESS -- (GOTERM_BP_ALL)
- TAXIS -- (GOTERM_BP_ALL)
- CHEMOTAXIS -- (GOTERM_BP_ALL)

Was ist in einem Cluster zusammengefasst?
 Grün: positive Assoziation des GO-Terms und einem Gen;
 Schwarz : Beziehung ist unbekannt.

Huang et al. *Genome Biology* 2007 **8**:R183

Messe funktionelle Ähnlichkeit von GO-Termen

Die **Wahrscheinlichkeit eines Knoten** t kann man auf 2 Arten ausdrücken:

Wieviele Gene besitzen die
Annotation t relativ zur Häufigkeit
der Wurzel?

Anzahl an GO-Termen im bei t
startenden Unterbaum relativ zu der
Anzahl an GO-Termen im Gesamtbaum.

$$P_{anno}(t) = \frac{occur(t)}{occur(root)}$$

$$P_{graph}(t) = \frac{D(t)}{D(root)}$$

Die Wahrscheinlichkeit hat Werte zwischen 0 und 1 und nimmt zwischen den Blättern bis zur Wurzel monoton zu.

Aus der Wahrscheinlichkeit p berechnet man den **Informationsgehalt** jedes Knotens:

$$IC(t) = -\log p(t)$$

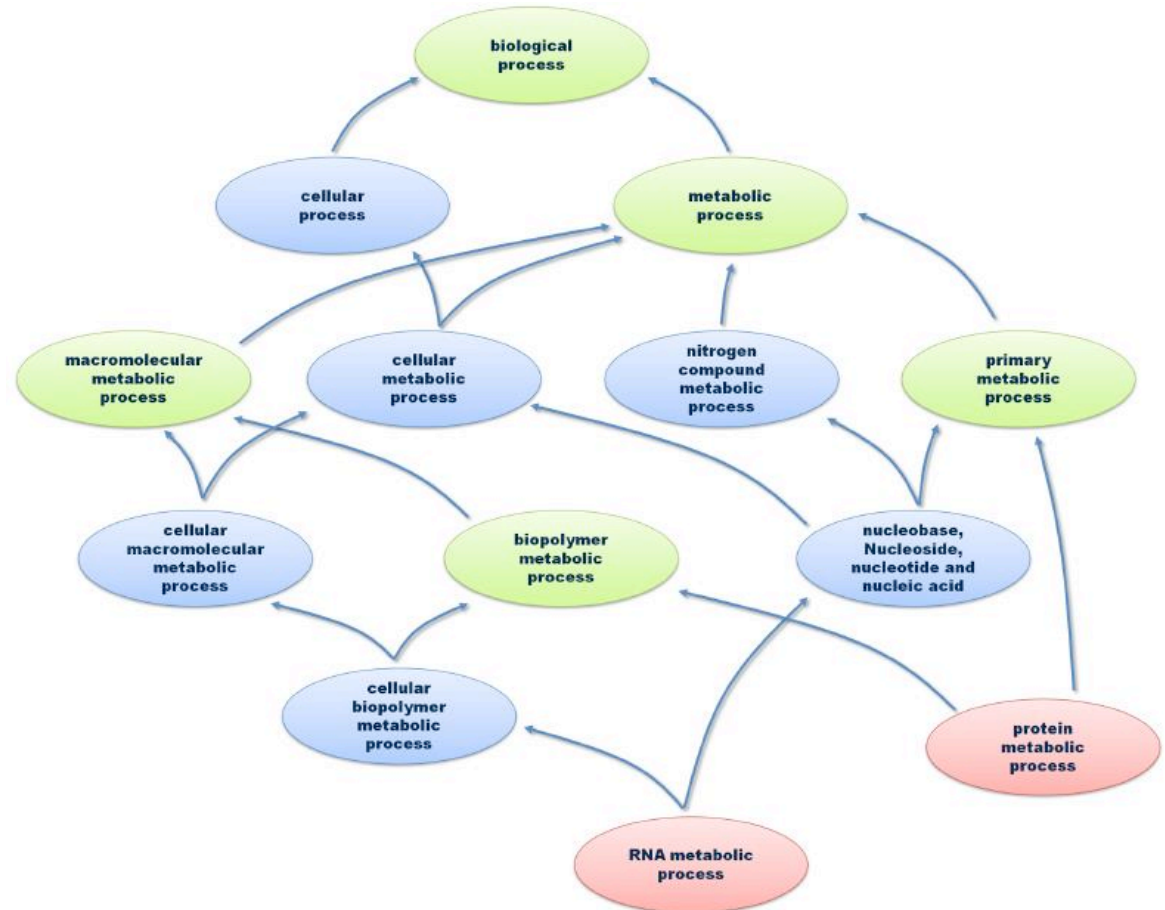
Je seltener ein Knoten ist, desto höher sein Informationsgehalt.

Messe funktionelle Ähnlichkeit von GO-Termen

Die Menge an gemeinsamen Vorgängern (common ancestors (CA)) zweier Knoten t_1 und t_2 enthält alle Knoten, die auf einem Pfad von t_1 zum Wurzelknoten **UND** auf einem Pfad von t_2 zum Wurzelknoten liegen.

Der **most informative common ancestor (MICA)** der Terme t_1 und t_2 ist der Term mit dem höchsten Informationsgehalt in CA.

Normalerweise ist das der gemäß dem Abstand nächste gemeinsame Vorgänger.



Messe funktionelle Ähnlichkeit von GO-Termen

Schlicker et al. definierten aus dem Abstand zum **most informative common ancestor** (MICA) die Ähnlichkeit der Terme t_1 und t_2

$$sim_{Rel}(t_1, t_2) = \frac{2 \cdot IC(MICA)}{IC(t_1) + IC(t_2)} \cdot (1 - p(MICA))$$

Der hintere Faktor gewichtet die Ähnlichkeit mit der Häufigkeit $p(MICA)$. Dies ergab Vorteile in der Praxis.

Messe funktionelle Ähnlichkeit von GO-Termen

Zwei Gene oder zwei Mengen an Genen A und B haben jedoch meist jeweils mehr als eine GO-Annotation. Betrachte daher die Ähnlichkeit aller Terme i und j :

$$s_{ij} = \text{sim}(GO_i^A, GO_j^B), \forall i \in 1, \dots, N, \forall j \in 1, \dots, M.$$

und wähle daraus in den Reihen und Spalten jeweils die Maxima

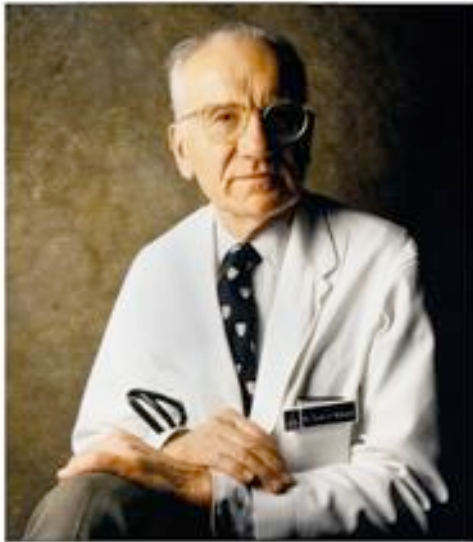
$$\text{rowScore}(A, B) = \frac{1}{N} \sum_{i=1}^N \max_{1 \leq j \leq M} s_{ij}, \quad \text{GOscore}_{\text{avg}}^{\text{BMA}}(A, B) = \frac{1}{2} \cdot (\text{rowScore}(A, B) + \text{columnScore}(A, B))$$

$$\text{columnScore}(A, B) = \frac{1}{M} \sum_{j=1}^M \max_{1 \leq i \leq N} s_{ij}. \quad \text{GOscore}_{\text{max}}^{\text{BMA}}(A, B) = \max(\text{rowScore}(A, B), \text{columnScore}(A, B))$$

Aus den Scores für den BP-Baum und den MF-Baum wird der *funsim*-Score berechnet.

$$\text{funsim}(A, B) = \frac{1}{2} \cdot \left[\left(\frac{\text{BPscore}}{\max(\text{BPscore})} \right)^2 + \left(\frac{\text{MFscore}}{\max(\text{MFscore})} \right)^2 \right]$$

OMIM-Datenbank



OMIM® , Online Mendelian Inheritance in Man® .
OMIM is a comprehensive, authoritative, and timely compendium of human genes and genetic phenotypes.

NCBI Online Mendelian Inheritance in Man (OMIM) search interface. Search term: OMIM. Results: All: 1, OMIM UniSTS: 0, OMIM dbSNP: 0.

Victor McKusick (1921-2008),
Johns Hopkins Universität,
- begründete das Gebiet
Medical genetics
- gründete die Datenbank
Mendelian Inheritance in Man

MIM ID #211980 LUNG CANCER

[GeneTests, Links](#)

Other entities represented by this entry

ALVEOLAR CELL CARCINOMA, INCLUDED
ADENOCARCINOMA OF LUNG, INCLUDED
NONSMALL CELL LUNG CANCER, INCLUDED
LUNG CANCER, PROTECTION AGAINST, INCLUDED

Gene map locus: [17q21.1](#), [12p12.1](#), etc.

[Clinical Synopsis](#)

Text

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A number sign (#) is used with this entry because mutations in several different genes are associated with lung cancer. Both germline and somatic mutations have been identified in the EGFR ([131550](#)) and p53 (TP53; [191170](#)) genes, and somatic mutations have been identified in the KRAS ([190070](#)), BRAF ([164757](#)), ERBB2 ([164870](#)), MET ([164860](#)), STK11 ([602216](#)), PIK3CA ([171834](#)), and PARK2 ([602544](#)) genes. Amplification of several genes,

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Improving disease gene prioritization using the semantic similarity of Gene Ontology terms

Andreas Schlicker[†], Thomas Lengauer and Mario Albrecht*

Max Planck Institute for Informatics, Department of Computational Biology and Applied Algorithmics, Campus E1.4, 66123 Saarbrücken, Germany

ONIM-Datenbank & UniProt Datenbank: GO-Annotationen für bekannte Krankheitsgene.

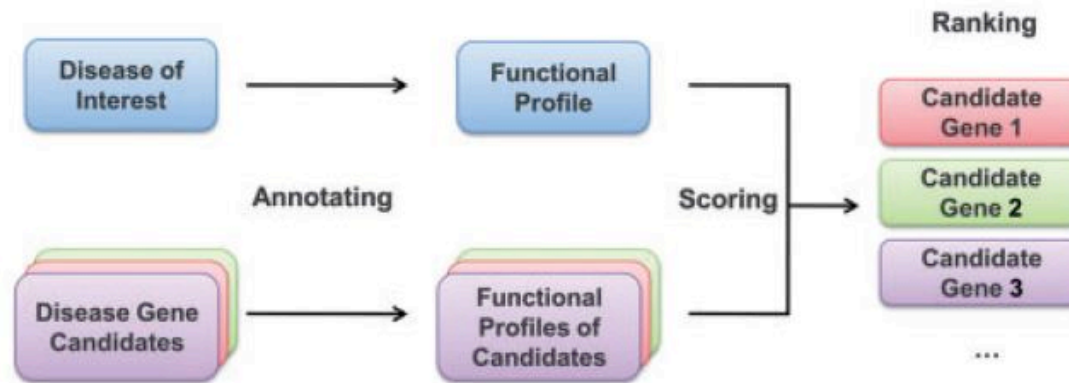
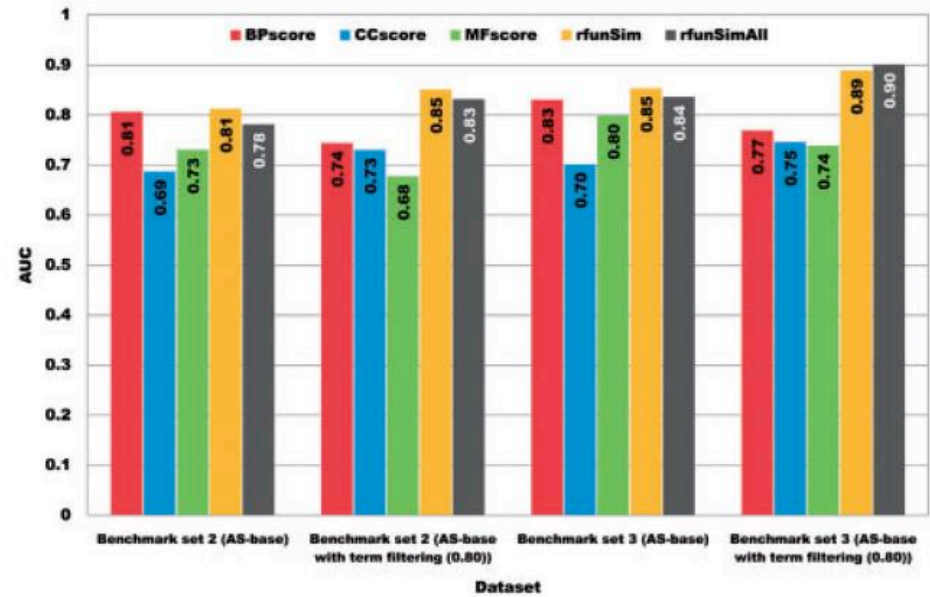


Fig. 1. Flow chart of the MedSim approach. First, the functional profiles of the disease of interest and the disease gene candidates are created using one of the annotation strategies. Afterwards, the functional profile of the disease is scored against each functional profile of a candidate, and the candidates are ranked according to this functional similarity score.

Schlicker et al. Bioinformatics 26, i561 (2010)

Die Methode liefert recht genaue Vorhersagen, mit welchen Krankheiten Gene in Verbindung stehen könnten.

Die Sensitivität, d.h. die Anzahl der korrekten Vorhersagen relativ zur Anzahl aller Vorhersagen, beträgt 73%.



Schlicker et al. Bioinformatics 26, i561 (2010)

Zusammenfassung

Daten aus Microarray-Analyse wurden ursprünglich als sehr „verrauscht“ angesehen.

Mittlerweile wurden jedoch sowohl die experimentellen Schritte wie auch die Datenauswertung gründlich verfeinert.

Microarray-Analyse ist daher heute eine (zwar teure, aber zuverlässige) Routine-Methode, die in allen großen Firmen verwendet wird.

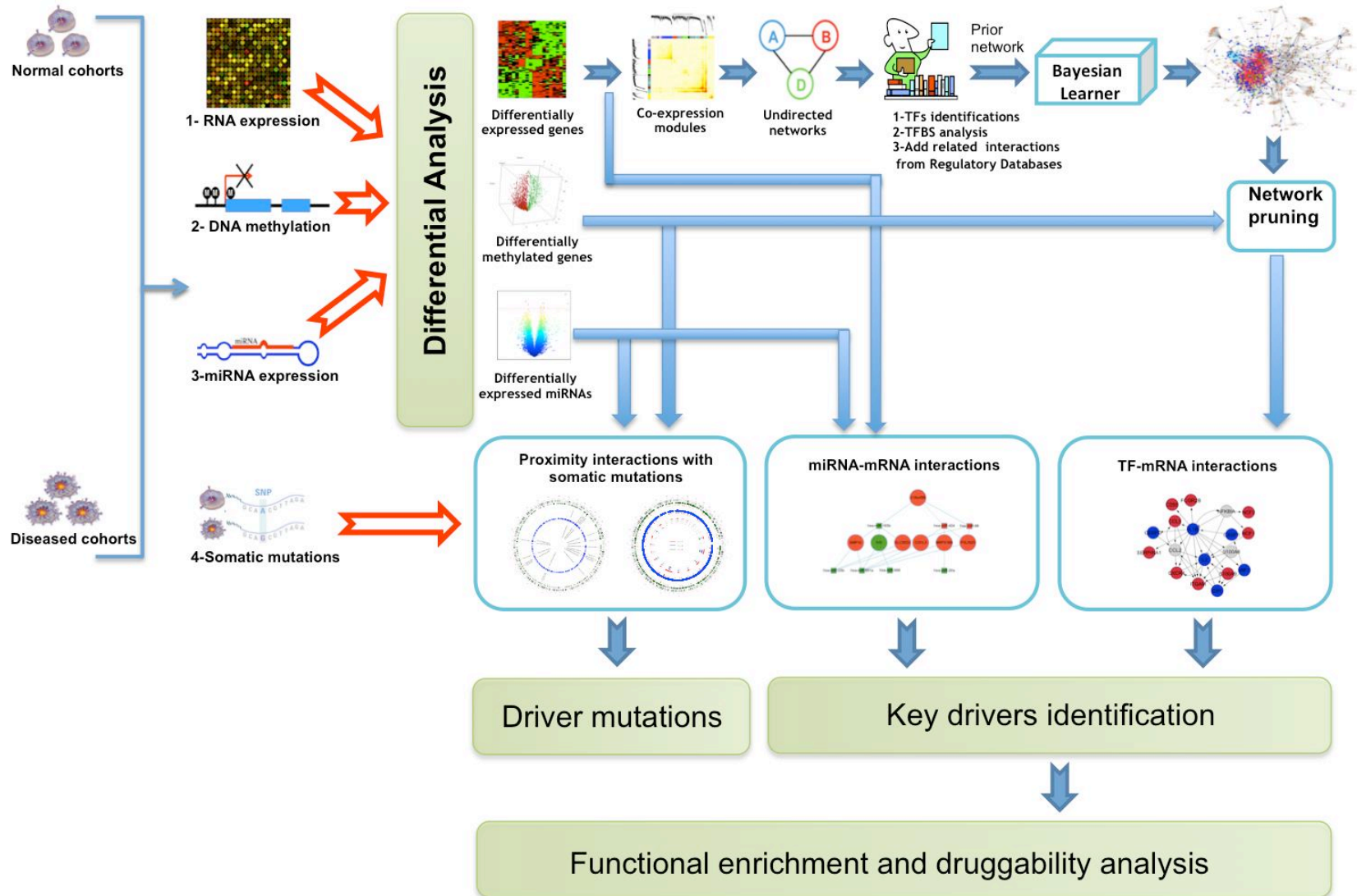
Die Datenaufbereitung kann folgende Schritte enthalten:

Normalisierung, Logarithmierung, Clustering, evtl. Ko-Expressionsanalyse, Annotation der Genfunktion

Sehr wichtig ist es, die Signifikanz der Ergebnisse zu bewerten.

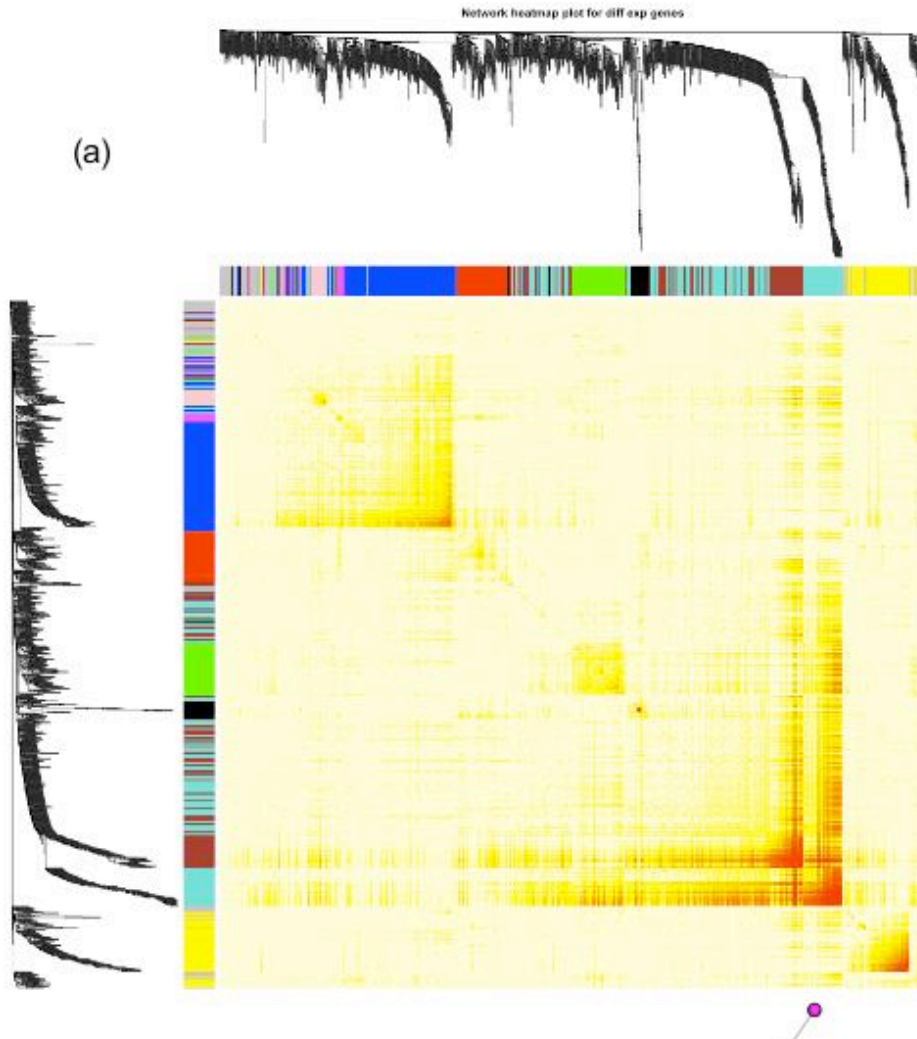
Gentleman et al. Genome Biology 5, R80 (2004)

funktionelle Annotation von OMICS-Daten für Brustkrebs



Hamed et al. BMC Genomics (accepted)

Analyse von Ko-Expression



Ko-Expression der 1317 differenziell exprimierten Gene (Krebs vs. Normal)

Hierarchisches Clustern

-> 10 Module mit 26 – 295 Genen

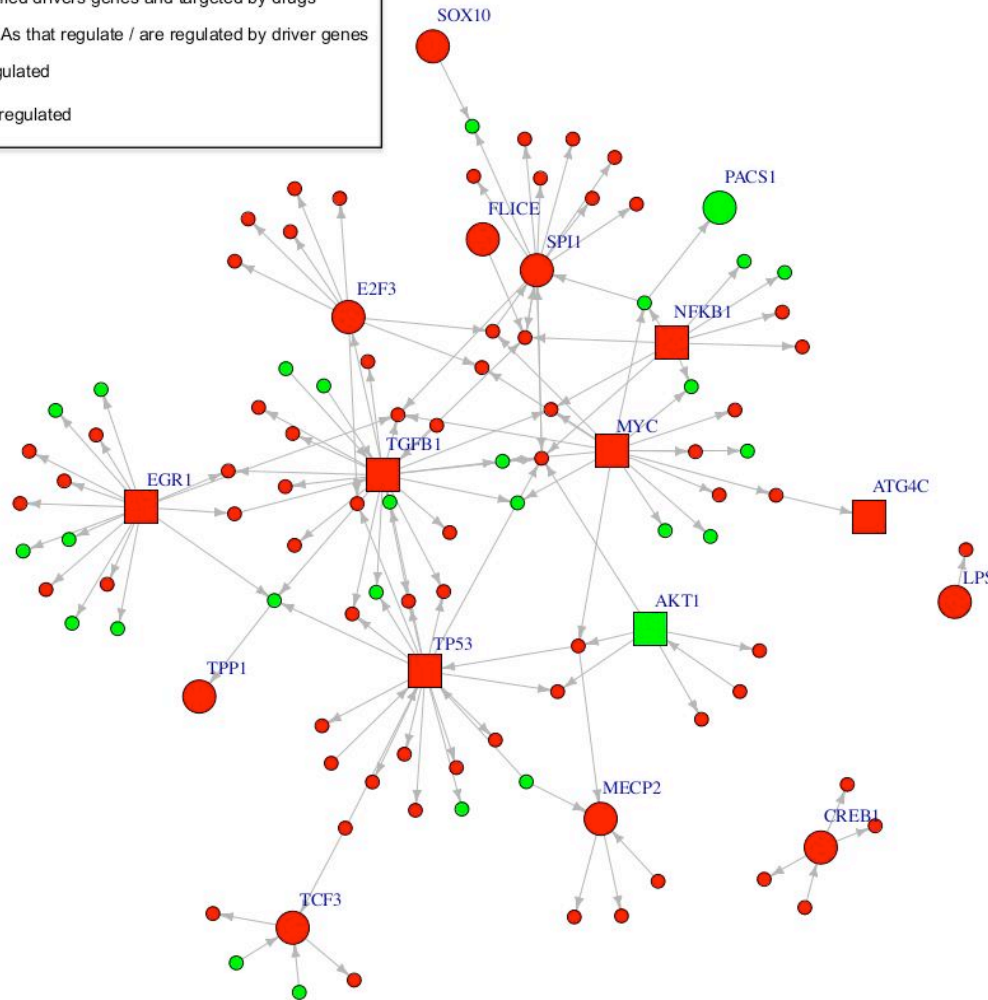
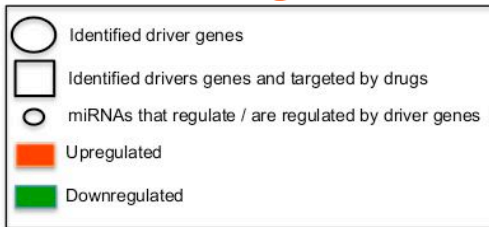
Gibt es angereicherte Genfunktionen in diesen Modulen?

	Module	Gene count	Top GO category	Top KEGG categories	Key driver count	Key drivers
TF- mRNA interactions	black	41	Regulation of transcription	Pathways in cancer, Renal cell carcinoma	5	SORBS3, ZNF43, ZNF681, RBMX, POU2F1
	blue	247	Nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	Cell cycle, Prostate cancer, Melanoma	9	AR , BRCA1 , ESR1 , JUN , MYB , RPN1, E2F1, E2F2, PPARD
	brown	195	Anatomical structure morphogenesis	Leukocyte transendothelial migration	5	TMOD3, CREB1, POU5F1, SP3, TERT
	green	110	Cellular macromolecule metabolic process	Endometrial cancer, Insulin signaling pathway	15	B4GALT7 , OS9 , CDC34 , MAN2C1, MYO1C, SH3GLB2, INPP5E, PLXNB1, USF2, PPP1R12C, CDK9, DAP, E4F1, E2F4, USF1
	grey	148	Anatomical structure development	Sulfur metabolism	18	AHCTF1 , NQO2 , FGFR2 , CCDC130 , ABCG4 , BIRC6 , CA6 , SP4, RNF2, SPRR1B, C16orf65, DNAJC5G, SNCAIP, GRIK5, SLC6A4, SMAD1, DAD1, POU4F2
	magenta	26	Regulation of metabolic process	p53 signaling pathway, Alzheimer's disease	3	ATF6 , NGEF, POGK
	pink	30	Transcription initiation from RNA polymerase II promoter	Basal transcription factors	4	CCDC92 , TMEM70, RNF139, E2F5
	red	93	Regulation of cellular process	Endometrial cancer, Neurotrophin signaling pathway	14	ATP1B1 , STAT3 , ABC88 , MYC , TGFB1 , SP1 , TP53 , PCGF1, SUMF2, GTF3A, IPO13, GMPPA, HTR6, TGIF1
	turquoise	295	Regulation of cellular metabolic process	p53 signaling pathway, Pancreatic cancer, Apoptosis	2	UBL5, RNF111
	yellow	132	Immune system process	Chemokine signaling pathway, Natural killer cell mediated cytotoxicity	19	APOC1 , CD2 , CD79B , LRRC28 , DAPK1 , FAM124B, EML2, LAP3, TSPAN2, FCRL3, ELMO1, SLC7A7, RASSF5, SLC31A2, TRAF3IP3, GALNT12, ITGA4, SPI1, TFAP2A
	Total	1317				

Module hängen mit Prozessen zusammen, die bereits mit Brustkrebs in Verbindung gebracht werden (endometrical cancer, p53, Prostatakrebs ...)

Hamed et al. BMC Genomics (accepted)

Ergänze regulatorische Information + driver genes



Differenziell experimentierte Gene eines Moduls

-> extrahiere regulatorische Interaktionen (TF -> Gen) aus den öffentlichen Datenbanken JASPAR, TRED, MSigDB

Driver genes sind Transkriptionsfaktoren, die möglichst viele Gene des Moduls regulieren.

31% der Driver genes kodieren für Proteine, die Targets für bekannte Krebs-Medikamente sind!

Hamed et al. BMC Genomics (accepted)

Ausblick auf den 3. Teil der Vorlesung

- Protein-Protein-Interaktionsnetzwerke – Analyse mit Cytoscape
- metabolische Netzwerke – Simulation mit Copasi
- Ko-Expression / Go-Annotation – Prozessierung mit Bioconductor