Microarray Annotation

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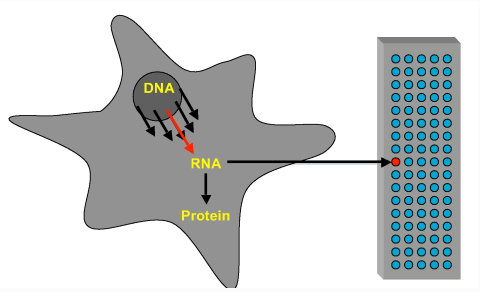
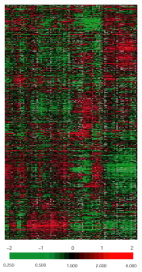
Biological Setting



There might be a correlation, but

- measured signal of DNA-microarrays $\not\Rightarrow$ amount of protein
- amount of protein $\not\Rightarrow$ activation status or effect of protein

Information in microarray data



Different levels and types of information

- Genes expression levels
- Genes annotations
- Samples annotations

Why do we need microarray clone annotation?

- Often, the result of microarray data analysis is a list of genes.
- The list has to be summarized with respect to its biological meaning. For this, information about the genes and the related proteins has to be gathered.
- If the list is small (let's say, 1–30), this is easily done by reading database information and/or the available literature.
- Sometimes, lists are longer (100s or even 1000s of genes). Automatic parsing and extracting of information is needed.
- To get complete information, you will need the help of an experienced computational biologist (aka bioinformatician). However, there is a lot that you can do on your own.

Primary databases

- Sequence databases
  Contain information about genes and the encoded proteins, e.g. database accession number, nucleotide and protein sequences, database cross references, and a sequence name that may or may not give a hint to the function. To find a sequence in another database, use sequence comparison tools like BLAST.
- There are large repositories for sequence data, the most prominent being the redundant databases **EMBL** , **GenBank** and **DDBJ** . They cover whole genome sequencing data, directly submitted sequences, sequences reported in support of patent applications and much more. Because they are so large, nobody cares about the quality of the data. Everybody having internet access can deposit sequence information there. Errors introduced long time ago will stay there forever.

Curated databases

- In contrast, some databases are curated. That means that biologists will get the information first and compare them with literature before it goes into the database. Thus, the database is of high quality, but it takes some time until a newly discovered sequence is entered.
- Because information is only entered by curators, annotation can be unified. Rules can be put in place that say, e.g., that all enzymes cutting off phosphates are called phosphatases, not 'phosphate hydrolases'. A very famous curated database is Amos Bairoch's SWISSPROT (http://www.expasy.org/sprot).

Some further database examples

Meta databases collect further information and relate them to primary databases.
Examples are:

- **OMIM** (online mendelian inheritance in man) for disease-related genes
- **EntrezGene** for genomic location (integrates information from LocusLink and from genes annotated on Reference Sequences from completely sequenced genomes)
- **PFAM** for protein domain structure
- **GeneCards** for comprehensive information from other databases on human genes.

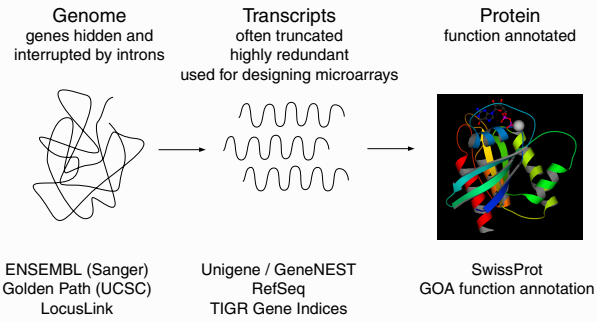
The relation of clone information to genes and proteins

- Microarrays are produced using information on *expressed sequences* as EST clones, cDNAs, partial cDNAs etc.
- At the other end, functional information is generated (and available) for *proteins*. Hence, there is a need to map a clone sequence ID to a protein ID. This is non-trivial.
- First, there are usually hundreds of ESTs (and several cDNA sequences) that map to the same gene. The Database *Unigene* tries to resolve this clustering by sequence clustering.

The relation of clone information to genes and proteins II

- An alternative approach is taken by *Locus Link*. This is a quite stable repository of genomic loci, supposed to be a single gene. Since the emphasis is on well-characterised loci, Locus Link is not complete.
  N.B. Locus Link has been replaced by *Entrez Gene*, which contains similar information. The Bioconductor meta packages, since Release 1.6 (3-2005) link to Entrez Gene.

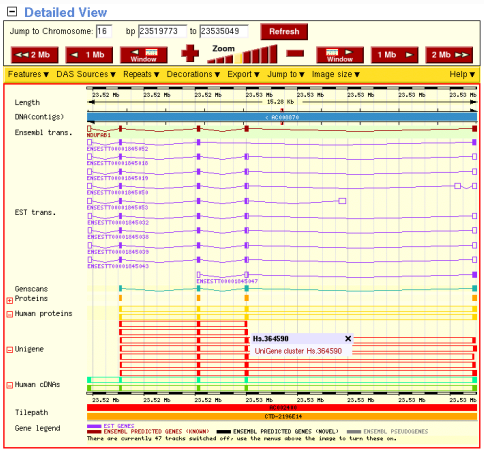
- There are other projects like RefSeq (NCBI) or TIGR Gene Indices. According to the cross-references available for a certain microarray, one or the other may be advantageous.



The Human Genome Sequence

- With the completion of the human genome sequence, you'd think that such ambiguities can be resolved. In fact, that is not the case.
- Part of the problem is due to the fact that it is hard to predict gene structure (intron/exon) without knowing the entire mRNA sequence, which happens for about two-thirds of all genes.
- Then, there are errors in the assembly (putting together the sequence snippets). A typical symptom is that a gene appears to map to multiple loci on the same chromosome, with very high sequence similarity.
- But there are also sequences that are nearly indentical, but duplicated. This has happened not long ago in evolution by means of transposable elements.

Genomic mapping: ENSEMBL Browser



Some figures

- Currently, it's estimated that the human genome contains about 25,000 – 30,000 genes that code for 50,000 – 100,000 different transcripts (and thus, proteins).
- Unigene (human section) contains 54,576 clusters, but 18,064 of them are of size 2 or less.
- RefSeq DNA contains 28,118 human sequences (3,295 EST's, 11,972 predicted seq., 17,708 mRNA's).
- ENSEMBL contains 24,194 predicted genes, 35,845 predicted transcripts. Fully computational methods like Genscan produce more than 65,000 predictions.
- Entrez Gene contains 32,941 genes.

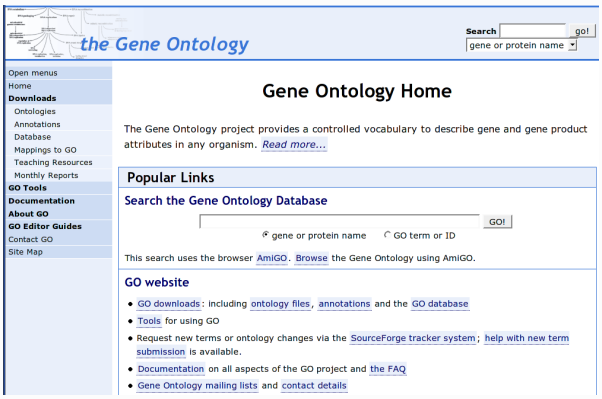
Function annotation

- Probably, the most important thing you want to know is what the genes or their products are concerned with, i.e. their **function**.
- Function annotation is difficult: Different people use different words for the same function, or may mean different things by the same word. The context in which a gene was found (e.g. "TGFβ-induced gene") may not be particularly associated with its function.
- Inference of function from sequence alone is error-prone and sometimes unreliable. The best function annotation systems (GO, SwissProt) use human beings who read the literature before assigning a function to a gene.

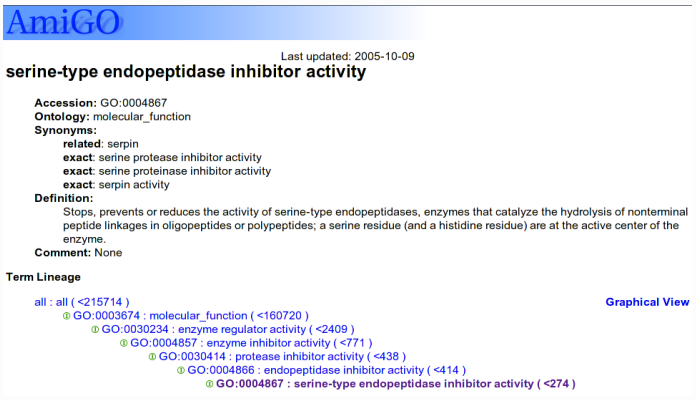
The Gene Ontology system

- To overcome some of the problems, an annotation system has been created: Gene Ontology (http://www.geneontology.org). Ontology means here the art (or science) of giving everything its correct name.
- It represents a unified, consistent system, i.e. terms occur only once, and there is a dictionary of allowed words.
- Furthermore, terms are related to each other: the hierarchy goes from very general terms to very detailed ones.

The Gene Ontology site



The Gene Ontology hierarchy



Actual annotation

- Gene Ontology by itself is only a system for annotating genes and proteins. It does not relate database entries to a special annotation value.
- Luckily, research communities for several model organisms have agreed on entering Gene Ontology information into the databases. As this is done 'by hand', GO annotation for most organisms is far from complete.

Available Gene Ontology information

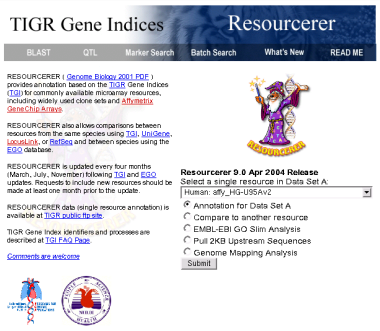
	Biological Process		Molecular Function		Cellular Component		Total Gene Products Associated	Total References Included as Evidence	TAB Delimited File of Associations & Last Update
	All codes	non-IEA codes	All codes	non-IEA codes	All codes	non-IEA codes			
GO Annotations @ EBI Chicken README	14053	70	21684	89	10396	70	22976	109	Download Nov 22, 2005
GO Annotations @ EBI Human README	21307	8408	25250	8208	18692	7316	28042	14978	Download Nov 26, 2005
GO Annotations @ EBI PDB README	14019	0	15302	0	4560	0	16359	1	Download Nov 22, 2005
GO Annotations @ EBI UniProt README	1126105	3254	1285618	3216	724063	2776	1504042	4216	Download Nov 26, 2005

The NetAffx System

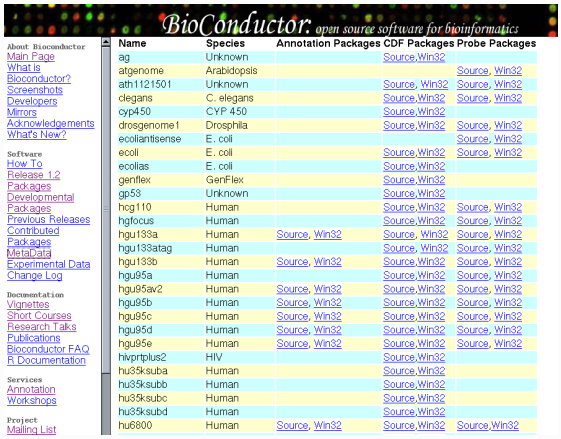
- For Affymetrix arrays, annotation is provided by the supplier via the NetAffx system (`http://www.affymetrix.com/analysis/netaffx/`)

Alternative pre-compiled annotation

- The Institute of Genomic Research (TIGR) has its own pre-compiled annotation for most commercial arrays (Affymetrix, Agilent, Incyte etc.): `http://www.tigr.org/tigr-scripts/magic/r1.pl`



Data packages in Bioconductor



Bioconductor metadata packages

- These packages contain one-to-one and one-to-many mappings for frequently used chips, especially Affymetrix arrays.
- Information available includes gene names, gene symbol, database accession numbers, Gene Ontology function description, enzmye classification number (EC), relations to PubMed abstracts, and others.
- The data use the framework of the `annotate` package, so I will briefly explain how it works.

Environments in R

- To quickly find information on one subject in a long list, a data structure called *hash table* is frequently used in computer science.
- A hash table is a list of key/value pairs, where the key is used to find the corresponding value. To go the other way round, you have to use pattern matching, which is much slower.
- In R, hash tables are implemented as *environments*. For the moment, we do not care about the philosophy behind it and simply treat it as another word for hash table.

Setting up environments

To set up a new environment:

```
symbol.hash = new.env(hash=TRUE)
```

To create a key/value pair:

```
assign("1234_at", "EphA3", env=symbol.hash)
```

To list all keys of an environment:

```
ls(env=symbol.hash)
```

To get the value for a certain key:

```
get("1234_at", env=symbol.hash)
```

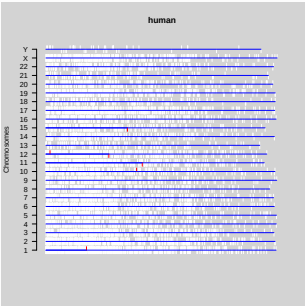
The annotate package

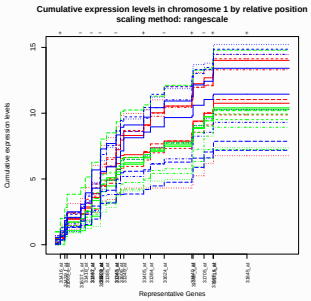
- That's all standard R. The annotate package gives one further function, `mget`, which retrieves more than one entry at a time, and definitions for special data, e.g. PubMed abstracts, or chromosomal location objects.
- ChromLoc objects are quite useful if you want to associate gene expression with certain positions on a chromosome, e.g. if aberration occurs in your samples.
- You can construct a ChromLoc object on your own (→ Vignette), or use the function `buildChromLocation`. For chip HGU95a_v2:

```
library(hgu95av2)
cl.95a = buildChromLocation("hgu95av2")
```

Plots for ChromLocation objects

- Plotting methods are available via library `geneplotter`





How to get annotation for a set of genes

- Suppose you have found some interesting genes. The index in the matrix is in `index.int`. To get the gene names:

```
gnam.int = geneNames(exprset)[index.int]
```

- To find the description:

```
mget(gnam.int, env=hgu95av2GENENAME)
```

- To get EC Numbers (relating to KEGG pathways):

```
mget(gnam.int, env=hgu95av2ENZYME)
```

Some caveats

- Because of the non-unique matching of sequences to the genome, array features are sometimes annotated with more than one position:

```
a = ls(env=hgu95av2CHRLOC)
table(sapply(mget(a, env=hgu95av2CHRLOC),
length))
    1     2     3     4     5     6     7     9
11551   825   160    53    20     9     4     3
```

- For the 800 or so sequences with more than one location, only the first one is used, although there is no warning. It should be desirable to resolve the ambiguities by hand, but nobody has done yet.

Some caveats

- Looking at the number of chromosomal annotations

```
table(sapply(mget(a,env=hgu95av2CHRLOC),
      function(x){length(unique(names(x)))}))
  0     1     2     3
997 11574    53     1
```

There are even 54 probe sets on HGU95A_v2 that map to 2 or more chromosomes; however, most of these are located on some special extrachromosomal segment and annotated with "X" and "Y".

annaffy

- Special annotation package for Affymetrix arrays
- Provides simplified mappings between Affymetrix IDs and annotation data
- Relies on chip-level annotation packages created by Annbuilder
- Supplies functions to produce mappings for almost all environments in a given annotation

biomaRt

- Enables to query the BioMart databases Ensembl, VEGA (Vertebrate Genome Annotation), dbSNP, sequence mart (Ensembl genome sequences)
- Two sets of functions
  - Information retrieval from BioMart databases `http://www.biomart.org`
  - Functions to access Ensembl `http://www.ensembl.org`
- Supplies annotation of features on arrays concerning affy ids, locuslink, RefSeq, entrezgene, gene names, GO, OMMIN, ...

Pattern matching

- To find something in character vectors or character lists, some pattern matching is required.
- If you have real full names, use `match`, e.g.

  ```
  match("1234_at", rownames(exprs(exprset)) )
  ```
- This will give you the index of ‘‘`1234_at`’’. It works also with more than one gene:

  ```
  match(gnam.int, rownames(exprs(exprset)) )
  ```

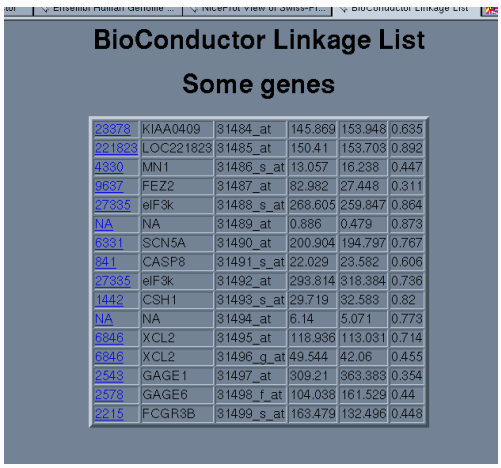
  will give all indeces for genes in `gnam.int`.
- If you want to use regular expression matching, use `grep`.

Export of annotation to HTML

- `annotate` is able to export tables of gene annotations to HTML, which is much nicer to browse than text tables
- Suppose, from a t-test you have for some genes `igenes`: mean of genes in class 1, `igenes.gp1`, mean in class 2, `igenes.gp2`, and P-value `igenes.pval`. To construct pretty HTML output:

```
igenes.ll = mget(igenes, env=hgu95av2LOCUSID)
igenes.sym = mget(igenes, env=hgu95av2SYMBOL)
ll.htmlpage(igenes.ll, "HOWTO.igenes", "Some genes",
list(igenes,sym, igenes, round(igenes.gp1,3),
round(igenes.gp2,3),round(igenes.pval,3)))
```

The result



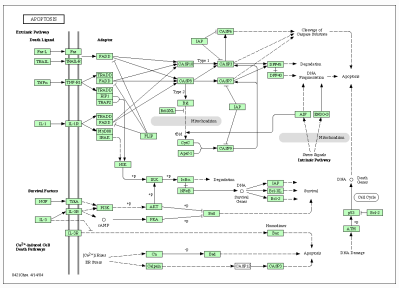
23378	KIAA0409	31484_at	145.869	153.948	0.635
221823	LOC221823	31485_at	150.41	153.703	0.892
4330	MN1	31486_s_at	13.057	16.238	0.447
9637	FEZ2	31487_at	82.982	27.448	0.311
27335	eIF3k	31488_s_at	268.605	259.847	0.864
NA	NA	31489_at	0.886	0.479	0.873
6331	SCN5A	31490_at	200.904	194.797	0.767
841	CASP8	31491_s_at	22.029	23.582	0.606
27335	eIF3k	31492_at	293.814	318.384	0.736
1442	CSH1	31493_s_at	29.719	32.583	0.82
NA	NA	31494_at	6.14	5.071	0.773
6846	XCL2	31495_at	118.936	113.031	0.714
6846	XCL2	31496_g_at	49.544	42.06	0.455
2543	GAGE1	31497_at	309.21	363.383	0.354
2578	GAGE6	31498_f_at	104.038	161.529	0.44
2215	FCGR3B	31499_s_at	163.479	132.496	0.448

Pathways

- For biological interpretation of function, most people want to use *pathways*
- A pathway is something like a bunch of interacting proteins and/or nucleic acids that allow for mass flux (metabolism) or information flux (signal transduction)
- The problem is that interaction information for proteins is quite rare (except for yeast)
- Some textbook pathways exist, but only few in computer-readable format

Pathway databases

- For metabolic pathways, some databases exist: KEGG (`http://www.genome.ad.jp/kegg/`), and EcoCyc (`http://ecocyc.org`), HumanCyc (`http://humancyc.org`) from SRI



Signal transduction information

- KEGG has some very limited information on signal transduction
- The database TRANSPATH wants to cover signal transduction. But information is incomplete, and you have to pay for part of the information (available via HNB)
- Other sources are `www.biocarta.com` and `www.stke.org` (requires registration)

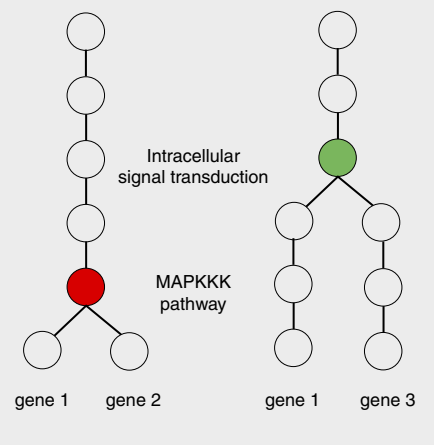
Some software packages for function analysis

- There are some packages that allow to map gene expression profiles to biological information, like pathways.
- One example is GeneMAPP (`www.genmapp.org`) which also has a collection of user-contributed pathways.
- GoMiner (`http://discover.nci.nih.gov/gominer`) tries to find statistically significantly enriched terms in a gene list. This is, however, very crude and tends to favor annotations with very few total number of associated genes.
- Ingenuity (`http://www.ingenuity.com`) has its own database with interaction information, and software to infer pathways from microarray experiments. It seems to be quite capable, but is also expensive.

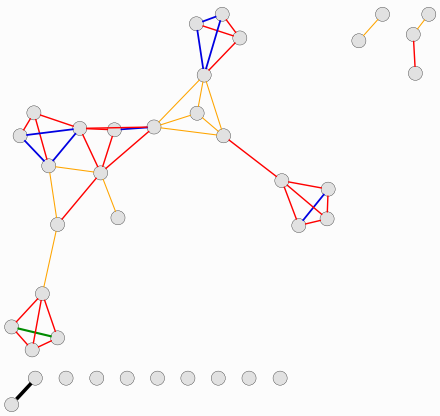
Dealing with GO annotations

- Since the annotation system is hierarchical, i.e. for each term there is a hierarchical list of more general terms, we can compare functions of genes on every level we wish.
- Technically, this amounts to the problem of finding the least common parent node between to genes of interest.
- This can be used to find clusters of functionally related genes in a list that comes out of some other analysis.

Comparing GO-annotated genes



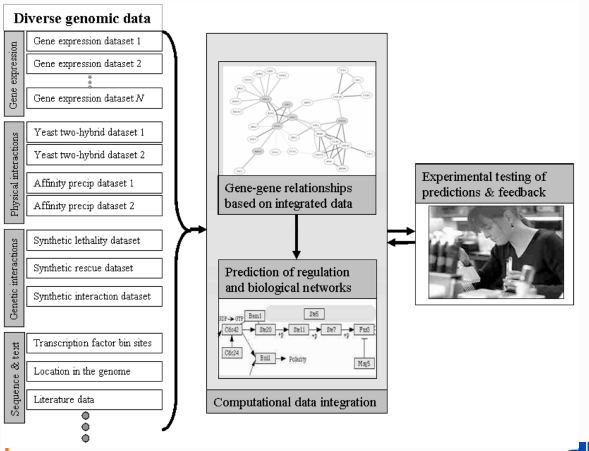
GO functional clusters as a graph



Graphs as analysis tools

- Graphs are quite useful for bioinformatic analysis, and have a long-standing history in sequence analysis.
- Recently, some functionality has been built into R to deal with graphs (`graph, Rgraphviz, RBGL`). Certainly, the most useful capability is to visualize graphs via `Rgraphviz`. The R package is an interface to the external program `graphviz` (from AT&T). Big graphs should be visualized by means of `ggobi`, however.
- Some other immediate use is to construct PubMed co-citation graphs for genes of interest. Functions for this exist. However, for many other applications the meaning of graphs or graph-theoretic algorithms is not clear, so a lot of work remains to be done.

Outlook: Integrated Analysis



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Thank you for your attention!