Introduction To Bioconductor

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Bioconductor Basics

- Bioconductor (www.bioconductor.org) is a software project aimed at providing high quality, innovative software tools appropriate for computational biology

- We rely mainly on R (www.r-project.org) as the computational basis

- We welcome contributions

Some basics

- for microarray data analysis we have assembled a number of R packages that are appropriate to the different types of data and processing

- some issues:
  - data complexity
  - data size
  - data evolution
  - meta-data

Software Design

- to overcome complexity we use two strategies: Abstract Data Types and object oriented programming
- to deal with data evolution we have separated the biological meta-data from the experimental data

Pedagogy

- among the many choices we made in the Bioconductor project is to try and develop better teaching materials

- in large part this is because we are between two disciplines (Biology and Statistics) and most users are familiar with only one of these

Vignettes

- we have adopted a new type of documentation: the *vignette*

- a vignette is an integrated collection of text and code – the code is runnable and using Sweave it is possible to replace the code with its output

- these documents are short and explicit directions on how to perform specific tasks

Vignettes – HowTo's

- a good way to find out how to use Bioconductor software is to read the relevant Vignette

- then extract the code (`tangleToR`) and examine it

- HowTo documents are shorter (one or two pages)

- please write and contribute these

Vignettes

- in Bioconductor 1.1 we introduced two new methods to interact with Vignettes

- `openVignette`() – gives you a menu to select from

- `vExplorer`() – our first attempt at turning Vignettes into interactive documents

Bioconductor packages
Release 1.1,Nov. 18, 2002

- General infrastructure:
  Biobase, **rhdf5**, **tkWidgets, reposTools**.

- Annotation:
  annotate, **AnnBuilder** → data packages.

- Graphics:
  geneplotter, hexbin.

- Pre-processing for Affymetrix oligonucleotide chip data:
  affy, CDF packages, vsn.

- Pre-processing for cDNA microarray data:
  marrayClasses, **marrayInput**, **marrayNorm**, **marrayPlots, vsn**.

- Differential gene expression:
  edd, **genefilter**, **multtest**, **ROC**.

Outline

- **Biobase and the basics**

- **annotate** and **AnnBuilder** packages

- **genefilter** package

- **multtest** package

- R clustering and classification packages

Biobase: exprSet class

exprs — Matrix of expression measures, genes x samples

se.exprs — Matrix of SEs for expression measures

phenoData — Sample level covariates, instance of class **phenoData**

annotation — Name of annotation data

description — Object of class MIAME

notes — Any notes

```
> golubTest

Expression Set (exprSet) with
        7129 genes
        34 samples
                phenoData object with 11 variables and 34 cases
          varLabels
                Samples: Sample index
                ALL.AML: Factor, indicating ALL or AML
                BM.PB: Factor, sample from marrow or peripheral blood
                T.B.cell: Factor, T cell or B cell leuk.
                FAB: Factor, FAB classification
                Date: Date sample obtained
                Gender: Factor, gender of patient
                pctBlasts: pct of cells that are blasts
                Treatment: response to treatment
                PS: Prediction strength
                Source: Source of sample
```

exprSet

- the set is closed under subsetting operations (either x[,1] or x[1,]) both produce new exprSets

- the first subscript is for genes, the second for samples

- the software is responsible for maintaining data integrity

exprSet: accessing the phenotypic data

- phenotypic data is stored in a special class: `phenoData`

- this is simply a dataframe and a set of associated labels describing the variables in the dataframe

Meta-data packages

- One of the largest challenges in analyzing genomic data is associating the experimental data with the available metadata, e.g. sequence, gene annotation, chromosomal maps, literature.

- The **annotate** and **AnnBuilder** packages provides some tools for carrying this out.

- These are very likely to change, evolve and improve, so please check the current documentation - things may already have changed!

Meta-data packages

- meta-data packages;
- Matching IDs using environments;
- Searching and processing queries from WWW databases
  - LocusLink,
  - GenBank,
  - PubMed;
- HTML reports.

WWW resources

- Nucleotide databases: e.g. GenBank.
- Gene databases: e.g. LocusLink, UniGene.
- Protein sequence and structure databases: e.g. SwissProt, Protein DataBank (PDB).
- Literature databases: e.g. PubMed, OMIM.
- Chromosome maps: e.g. NCBI Map Viewer.
- Pathways: e.g. KEGG.
- Entrez is a search and retrieval system that integrates information from databases at NCBI (National Center for Biotechnology Information).

NCBI Entrez

www.ncbi.nlm.nih.gov/Entrez
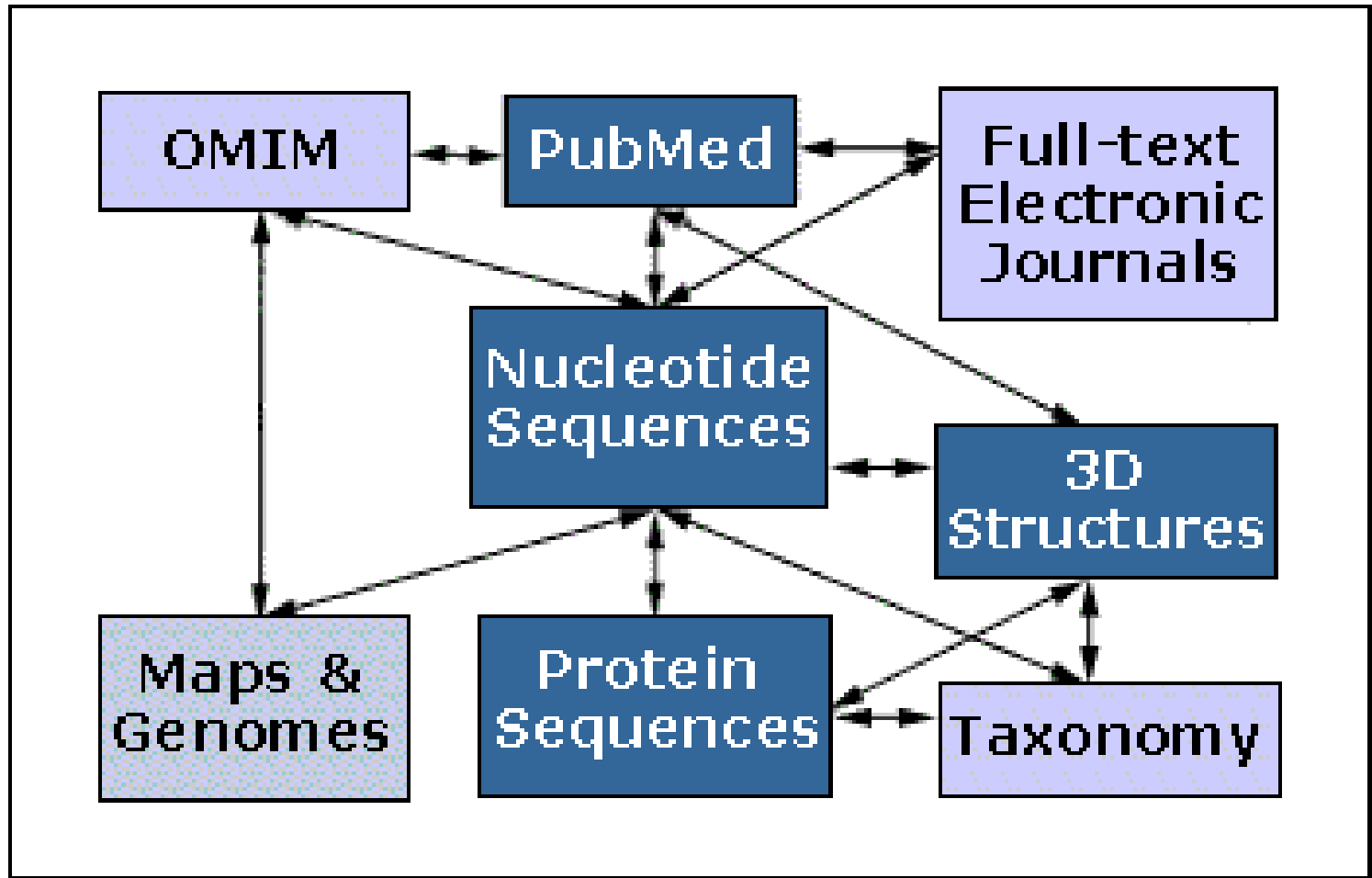
`annotate`: matching IDs

Important tasks

- Associate manufacturers probe identifiers (e.g. Affymetrix IDs) to other available identifiers (e.g. gene symbol, PubMed PMID, LocusLink LocusID, GenBank accession number).

- Associate probes with biological data such as chromosomal position, pathways.

- Associate probes with published literature data via PubMed.

`annotate`: matching IDs

Affymetrix identifier HGU95A chips	"`36823_at`"
LocusLink, LocusID	"`10900`"
GenBank accession #	"`AF055026`"
Gene symbol	"`RPIP8`"
PubMed, PMID	"`9523700`" "`9110174`" "`8619474`"
Chromosomal location	"`17`", "`17q21.31`"

Annotation data packages

- The Bioconductor project has started to deploy packages that contain only data. E.g. `hgu95av2` package for Affymetrix HGU95A GeneChips series, also, `hgu133a`, `hu6800`, `mgu74a`, `rgu34a`.

- These data packages are built using `AnnBuilder`.

- These packages contain many different mappings to interesting data.

- They are available from the Bioconductor website and also using `update.packages2`.

Meta-data packages

- Maps to GenBank accession number, LocusLink LocusID, gene symbol, gene name, UniGene cluster.
- Maps to chromosomal location: chromosome, cytoband, physical distance (bp), orientation.
- Maps to KEGG pathways, enzymes, Gene Ontology Consortium (GO).
- Maps to PubMed PMID.
- These packages will be updated and expanded regularly as new or updated data become available.

`hu95av2` data package (1.2.6)

A data package containing annotation data for hgu95av2 ®

hgu95av2	Genomic Annotation data package built with AnnBuilder
hgu95av2ACCNUM	An annotation data file for ACCNUM in the hgu95av2 package
hgu95av2CHR	An annotation data file for CHR in the hgu95av2 package
hgu95av2CHRLOC	An annotation data file for CHRLOC in the hgu95av2 package
hgu95av2CHRORI	An annotation data file for CHRORI in the hgu95av2 package
hgu95av2ENZYME	An annotation data file for ENZYME in the hgu95av2 package
hgu95av2ENZYME2PROBE	An annotation data file for ENZYME2PROBE in the hgu95av2 package
hgu95av2GENENAME	An annotation data file for GENENAME in the hgu95av2 package
hgu95av2GO	An annotation data file for GO in the hgu95av2 package
hgu95av2GO2ALLPROBES	An annotation data file for GO2ALLPROBES in the hgu95av2 package
hgu95av2GO2PROBE	An annotation data file for GO2PROBE in the hgu95av2 package
hgu95av2GRIF	An annotation data file for GRIF in the hgu95av2 package
hgu95av2LOCUSID	An annotation data file for LOCUSID in the hgu95av2 package
hgu95av2MAP	An annotation data file for MAP in the hgu95av2 package
hgu95av2PATH	An annotation data file for PATH in the hgu95av2 package
hgu95av2PATH2PROBE	An annotation data file for PATH2PROBE in the hgu95av2 package
hgu95av2PMID	An annotation data file for PMID in the hgu95av2 package
hgu95av2PMID2PROBE	An annotation data file for PMID2PROBE in the hgu95av2 package
hgu95av2QC	Quality control information for hgu95av2
hgu95av2SUMFUNC	An annotation data file for SUMFUNC in the hgu95av2 package
hgu95av2SYMBOL	An annotation data file for SYMBOL in the hgu95av2 package
hgu95av2UNIGENE	An annotation data file for UNIGENE in the hgu95av2 package

`annotate`: matching IDs

- Much of what `annotate` does relies on matching symbols.
- This is basically the role of a hash table in most programming languages.
- In R, we rely on environments (they are similar to hash tables).
- The annotation data packages provide R environment objects containing key and value pairs for the mappings between two sets of probe identifiers.
- Keys can be accessed using the R `ls` function.
- Matching values in different environments can be accessed using the `get` or `multiget` functions.

`annotate`: matching IDs

E.g. **`hgu95av2`** package.

- To load package **`library(hgu95av2)`**

- For info on the package and list of mappings available

  `? hgu95av2`

  `hgu95av2()`

- For info on a particular mapping

  `? hgu95av2PMID`

annotate: matching IDs

```
> library(hgu95av2)
> get("36823_at", env = hgu95av2ACCNUM)
[1] "AF055026"
> get("36823_at", env = hgu95av2LOCUSID)
[1] "10900"
> get("36823_at", env = hgu95av2SYMBOL)
[1] "RPIP8"
> get("36823_at", env = hgu95av2GENENAME)
[1] "RaP2 interacting protein 8"
> get("36823_at", env = hgu95av2SUMFUNC)
[1] "Rap2 interacting protein 8; interacts
  with the activated form of Rap2|Proteome"
> get("36823_at", env = hgu95av2UNIGENE)
[1] "Hs.6755"
```

annotate: matching IDs

```
> get("36823_at", env = hgu95av2CHR)
[1] "17"
> get("36823_at", env = hgu95av2CHRLOC)
      17
42396727
> get("36823_at", env = hgu95av2CHRORI)
 17

"+"
> get("36823_at", env = hgu95av2MAP)
[1] "17q21.31"
> get("36823_at", env = hgu95av2PMID)
[1] "9523700" "9110174" "8619474"
> get("36823_at", env = hgu95av2GO)
           P                  P
"GO:0005083" "GO:0007264"
```

`annotate`: database searches and report generation

- Provide tools for searching and processing information from various biological databases.

- Provide tools for regular expression searching of PubMed abstracts.

- Provide nice HTML reports of analyses, with links to biological databases.

annotate: **WWW queries**

- Functions for querying WWW databases from R rely on the **browseURL** function

```
browseURL("www.r-project.org")
```

`annotate`: **GenBank query**
www.ncbi.nlm.nih.gov/Genbank/index.html

- Given a vector of GenBank accession numbers or NCBI UIDs, the **genbank** function
  - opens a browser at the URLs for the corresponding GenBank queries;
  - returns an **XMLdoc** object with the same data.

```
genbank("AF055026",disp="browser")
```
http://www.ncbi.nih.gov/entrez/query.fcgi?tool=bioconductor&cmd=Search&db=Nucleotide&term=AF055026

```
genbank("3005754",disp="data",
    type="uid")
```

`annotate`: LocusLink query
www.ncbi.nlm.nih.gov/LocusLink/

- **`locuslinkByID`**: given one or more LocusIDs, the browser is opened at the URL corresponding to the first gene.

  `locuslinkByID("10900")`

  http://www.ncbi.nih.gov/LocusLink/LocRpt.cgi?l=10900

- **`locuslinkQuery`**: given a search string, the results of the LocusLink query are displayed in the browser.

  `locuslinkQuery("Rap2")`

  http://www.ncbi.nih.gov/LocusLink/list.cgi?Q=Rap2&ORG=Hs&V=0

`annotate`: **PubMed query**
www.ncbi.nlm.nih.gov

- For any gene there is often a large amount of data available from PubMed.

- The `annotate` package provides the following tools for interacting with PubMed
  - `pubMedAbst`: a class structure for PubMed abstracts in R.
  - `pubmed`: the basic engine for talking to PubMed.

- WARNING: be careful you can query them too much and be banned!

annotate: pubMedAbst class

Class structure for storing and processing
 PubMed abstracts in R

- **authors**
- **pmid**
- **abstText**
- **articleTitle**
- **journal**
- **pubDate**
- **abstUrl**

`annotate`: high level tools for PubMed query

- **`pm.getabst`**: download the specified PubMed abstracts (stored in XML) and create a list of **`pubMedAbst`** objects.

- **`pm.titles`**: extract the titles from a list of PubMed abstracts.

- **`pm.abstGrep`**: regular expression matching on the abstracts.

annotate: PubMed example

```
pmid <-get("36823_at", env=hgu95av2PMID)
pubmed(pmid, disp="browser")
```

http://www.ncbi.nih.gov/entrez/query.fcgi?tool=bioconductor&cmd=Retrieve&db=PubMed&list_uids=9523700%2c9110174%2c8619474

```
absts <- pm.getabst("36823_at",
  base="hgu95av2")
pm.titles(absts)
pm.abstGrep("GTP",absts[[1]])
```

annotate: PubMed example


```
[1] "W Yu"        "B Andersson"   "KC Worley"     "DM Muzny"
[5] "Y Ding"      "W Liu"         "JY Ricafrente" "MA Wentland"
[9] "G Lennon"    "RA Gibbs"

Slot "abstText":
        A total of 100 kb of DNA derived from 69 individual human brain cDNA c...

Slot "articleTitle":
        Large-scale concatenation cDNA sequencing....

Slot "journal":
[1] "Genome Res"

Slot "pubDate":
[1] "Apr 1997"

Slot "abstUrl":
[1] "No URL Provided"


$"36823_at"[[3]]
An object of class pubMedAbs
Slot "pmid":
[1] "8619474"

Slot "authors":
[1] "B Andersson"   "MA Wentland"   "JY Ricafrente" "W Liu"
[5] "RA Gibbs"

Slot "abstText":
        The efficiency of shotgun DNA sequencing depends to a great extent on ...

Slot "articleTitle":
        A "double adaptor" method for improved shotgun library construction....

Slot "journal":
[1] "Anal Biochem"

Slot "pubDate":
[1] "Apr 1996"

Slot "abstUrl":
[1] "No URL Provided"


> pm.titles(absts)
[[1]]
[1] "Identification of a specific effector of the small GTP-binding protein Rap2."
[2] "Large-scale concatenation cDNA sequencing."
[3] "A \"double adaptor\" method for improved shotgun library construction."

> pm.abstGrep("GTP",absts[[1]])
[1]  TRUE FALSE FALSE
>
```

`annotate`: data rendering

- A simple interface, `ll.htmlpage`, can be used to generate an HTML report of your results.

- The page consists of a table with one row per gene, with links to LocusLink.

- Entries can include various gene identifiers and statistics.

ll.htmlpage function from **annotate** package

genelist.html

BioConductor Gene Listing

Golub et al. data, genes with permutation maxT adjusted p-value < 0.01

Locus Link Genes

LocusID	Gene name	Chromosome	ALL mean	AML mean	t-statistic	raw p-value	adj p-value
7791	X95735_at	7	-0.295	1.59	-10.6	2e-05	2e-05
1471	M27891_at	20	-0.81	2.08	-9.78	2e-05	2e-05
2184	M55150_at	15	0.488	1.24	-8.03	2e-05	0.00014
4067	M16038_at	8	-0.284	1.1	-7.98	2e-05	0.00016
334	L09209_s_at	11	-0.162	1.36	-7.97	2e-05	2e-04
6929	M31523_at	19	0.855	-0.391	7.55	2e-05	5e-04
5928	X74262_at	1	0.869	-0.565	7.42	2e-05	0.00078
7155	Z15115_at	3	1.94	0.945	7.35	2e-05	0.001
26999	L47738_at	5	0.734	-0.779	7.31	2e-05	0.00114
4602	U22376_cds2_s_at	6	1.86	0.294	7.28	2e-05	0.00116
65108	HG1612-HT1612_at	1	1.91	0.888	7.11	2e-05	0.0017
34	M91432_at	1	0.431	-0.771	7.08	2e-05	0.0018
5925	L41870_at	13	-0.438	-1.3	7.08	2e-05	0.0018
546	U72936_s_at	NA	-0.097	-1.07	7.07	2e-05	0.0018
7430	X51521_at	6	1.92	1.07	7.06	2e-05	0.00186
4056	U50136_rna1_at	5	0.71	1.51	-6.97	2e-05	0.00232
54741	Y12670_at	1	-0.167	0.892	-6.96	2e-05	0.00238
7203	X74801_at	1	0.611	-0.183	6.95	2e-05	0.00238
3576	Y00787_s_at	4	-0.371	2.32	-6.87	2e-05	0.00288
6709	J05243_at	9	0.413	-0.982	6.86	2e-05	0.00288
1725	U26266_s_at	19	-0.209	-1.16	6.85	4e-05	0.00294
3205	U82759_at	7	-0.64	0.504	-6.82	2e-05	0.00306
945	M23197_at	19	-0.881	0.354	-6.79	2e-05	0.0033
1509	M63138_at	11	1.21	2.12	-6.77	2e-05	0.00344
6955	M12959_s_at	14	1.13	0.132	6.76	2e-05	0.00352
967	X62654_rna1_at	12	0.0513	1.33	-6.76	2e-05	0.00352
5341	X07743_at	2	-0.959	0.535	-6.74	2e-05	0.00378
140465	M31211_s_at	12	0.108	-0.953	6.71	2e-05	0.00404
7336	U62136_at	8	-0.163	-0.92	6.68	2e-05	0.00428
3660	X15949_at	4	-0.541	-1.33	6.61	2e-05	0.00492
8655	U32944_at	14	0.026	0.358	6.61	2e-05	0.00492

annotate: chromLoc class

Location information for <u>one gene</u>

- **chrom**: chromosome name.

- **position**: starting position of the gene in bp.

- **strand**: chromosome strand +/-.

annotate: **chromLocation class**
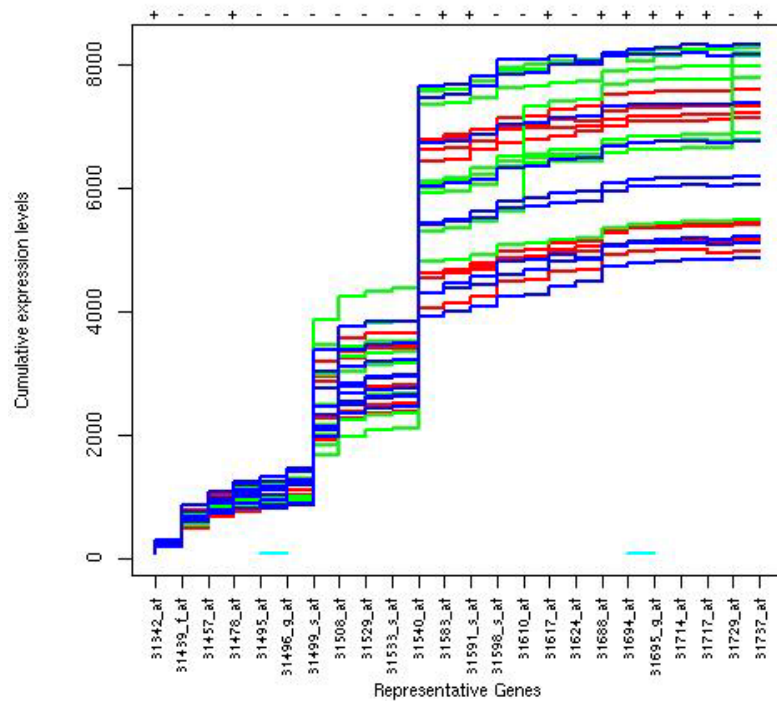
Location information for a <u>set of genes</u>

- **organism:** organism that the genes correspond to.
- **dataSource:** source of the gene location data.
- chromLocs: location of the genes on the chromso
- probesToChrom:mapping from probes to chromosome
- chromInfo:information about names, lengths etc
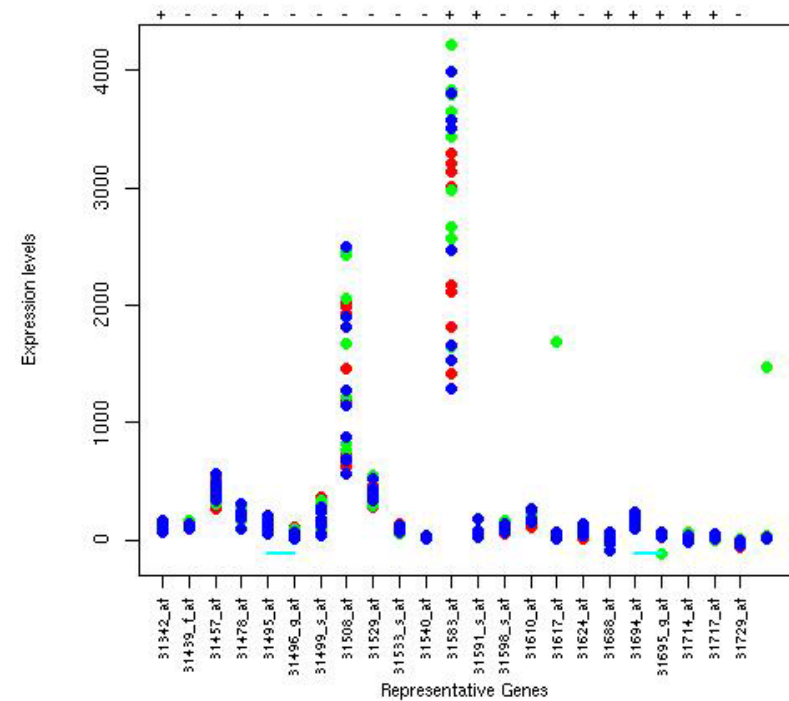- geneSymbols:mapping from probes to symbols

geneplotter: cPlot
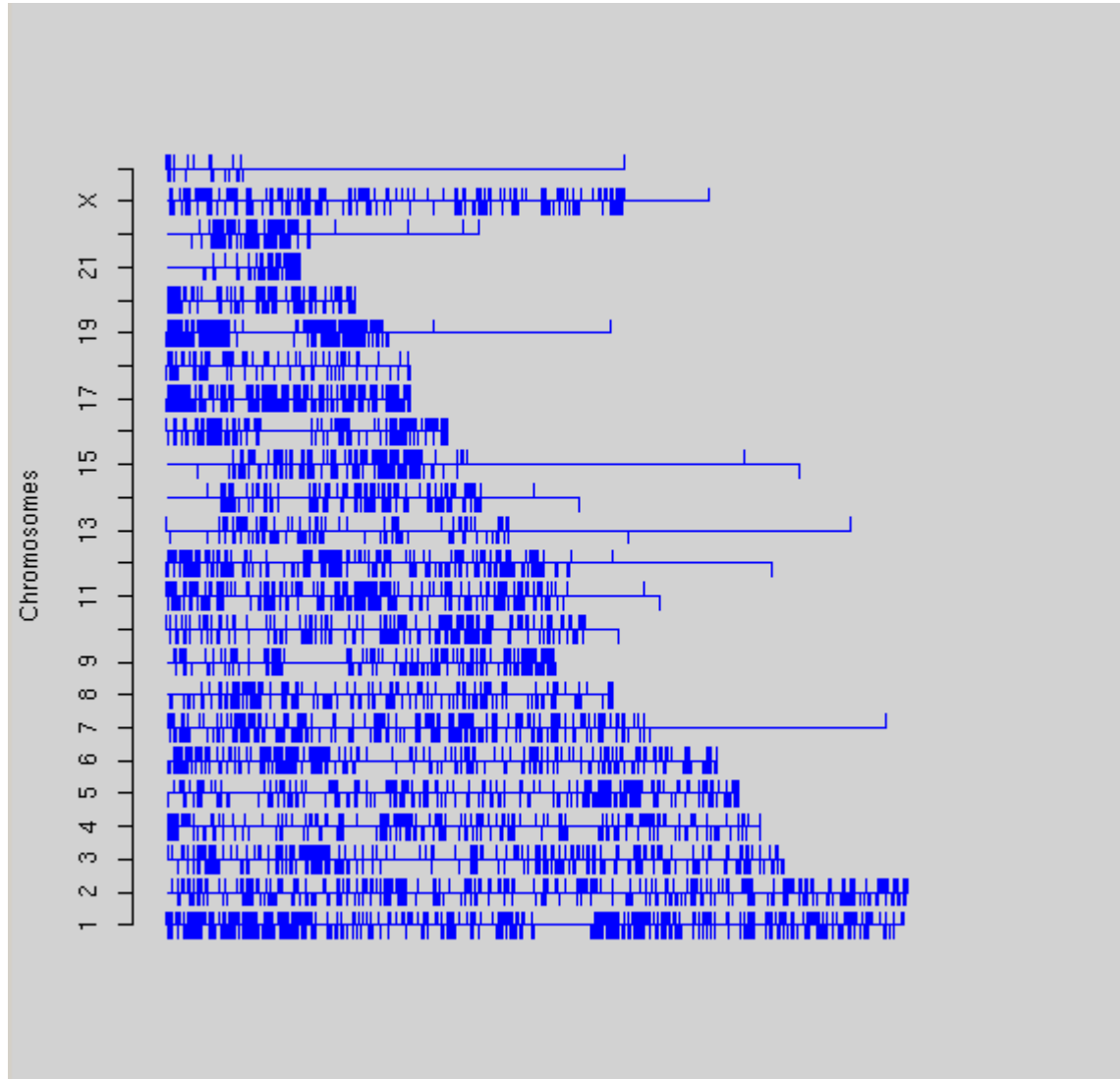
geneplotter: alongChrom

geneplotter: alongChrom

Expression levels by genes in chromosome 1
scaling method: none

Gene filtering

- A very common task in microarray data analysis is gene-by-gene selection.
- Filter genes based on
  - data quality criteria, e.g. absolute intensity or variance;
  - subject matter knowledge;
  - their ability to differentiate cases from controls;
  - their spatial or temporal expression pattern.
- Depending on the experimental design, some highly specialized filters may be required and applied sequentially.

Gene filtering

- *Cohort/Clinical trial.* Filter genes based on association with survival, e.g. using a Cox model.

- *Factorial experiment.* Filter genes based on interaction between two treatments, e.g. using ANOVA.

- *Time-course experiment*. Filter genes based on periodicity of expression pattern, e.g. using Fourier transform.

`genefilter` package

- The `genefilter` package provides tools to sequentially apply filters to the rows (genes) of a matrix.

- There are two main functions, `filterfun` and `genefilter`, for assembling and applying the filters, respectively.

- Any number of functions for specific filtering tasks can be defined and supplied to `filterfun`.

  E.g. Cox model p-values, coefficient of variation.

genefilter: separation of tasks

1. Select/define functions for specific filtering tasks.

2. Assemble the filters using the **filterfun** function.

3. Apply the filters using the **genefilter** function → a logical vector, **TRUE** indicates genes that are retained.

4. Apply that vector to the **exprSet** to obtain a microarray object for the subset of interesting genes.

genefilter: supplied filters

Filters supplied in the package

- **kOverA** – select genes for which k samples have expression measures larger than A.

- **gapFilter** – select genes with a large IQR or gap (jump) in expression measures across samples.

- **ttest** – select genes according to t-test nominal p-values.

- **Anova** – select genes according to ANOVA nominal p-values.

- **coxfilter** – select genes according to Cox model nominal p-values.

`genefilter`: **writing filters**

- It is very simple to write your own filters.

- You can use the supplied filtering functions as templates.

- The basic idea is to rely on lexical scope to provide values (bindings) for the variables that are needed to do the filtering.

genefilter: **How to?**

1. First, build the filters

   ```
   f1 <- anyNA
   f2 <- kOverA(5, 100)
   ```

2. Next, assemble them in a filtering function

   ```
   ff <- filterfun(f1,f2)
   ```

3. Finally, apply the filter

   ```
   wh <- genefilter(exprs(golubTest),
   ff)
   ```

4. Use **wh** to obtain the relevant subset of the data

   ```
   mySub <- golubTest[wh,]
   ```

Differential gene expression

- Identify genes whose expression levels are associated with a response or covariate of interest
  - clinical outcome such as survival, response to treatment, tumor class;
  - covariate such as treatment, dose, time.
- Estimation: estimate effects of interest and variability of these estimates.
  E.g. slope, interaction, or difference in means in a linear model.
- Testing: assess the statistical significance of the observed associations.

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