Bioconductor

Course in Practical Microarray Analysis
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Slides ©2002 Sandrine Dudoit, Robert Gentleman.
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Statistical computing

Everywhere …

- for statistical design and analysis:
  - technology development and validation, data pre-processing, estimation, testing, clustering, prediction, etc.
- for integration with biological information resources (in house and external databases)
  - gene annotation (Unigene, LocusLink)
  - graphical (pathways, chromosome maps)
  - patient data, tissue banks

Outline

- Overview of Bioconductor packages
  - **Biobase**
  - **annotate**
  - **genefilter**
  - **marrayClasses**, …**Input**, …**Norm**, …**Plots**
  - **Affy**

- Dynamic statistical reports using Sweave:

  'reproducible analyses'

Bioconductor

- Bioconductor is an open source project to design and provide high quality software and documentation for bioinformatics.
- Current focus: microarrays and gene (transcript) annotation
- Most of the early developments are in the form of R packages.
- Open to (your?) contributions
- Software and documentation are available from www.bioconductor.org.

Bioconductor packages

- General infrastructure
  - Biobase
  - annotate, AnnBuilder
  - tkWidgets
- Pre-processing for Affymetrix data
  - affy.
- Pre-processing for cDNA data
  - marrayClasses, marrayInput, marrayNorm, marrayPlots.
- Differential expression
  - edd, genefilter, multtest, ROC.
- etc.

Bioconductor training

- Extensive documentation and training materials for self-instruction and short courses
  - all available on WWW.
- R help system
  - interactive with browser or printable manuals;
  - detailed description of functions and examples;
  - E.g. `help(maNorm)`, `? marrayLayout.`
- R demo system
  - User-friendly interface for running demonstrations of R scripts.
  - E.g. `demo(marrayPlots).`

Biobase

contains class definitions and infrastructure

classes:

- phenoData: sample covariate data (e.g. cell treatment, tissue origin, diagnosis)
- miame (minimal information about μarray experiments)
- exprSet: matrix of expression data, phenoData, miame, and other quantities of interest.
- aggregate: an infrastructure to put an aggregation procedure (cross-validation, bootstrap) on top of any analysis

exprSet

- objects of type exprSet allow subsetting w.r.t. genes (probes) and w.r.t. samples.
- Expression values, gene and patient annotation are kept consistent under the subsetting

⇒ a frequent source of confusion or even 'bugs' is eliminated!

genefilter: separation of tasks

Task	Programming pendant
Define the filter criterion	A function that takes the data for one gene
Apply it to the data and obtain a selection	A logical vector
Apply the selection to the data	A new exprSet with the subset of interesting genes

genefilter: supplied filters

- `kOverA` - k samples with expression values larger than A.
- `gapFilter` - samples need to have a large IQR or a gap (jump).
- `ttest` - select genes according to t-test p-values using a covariate.
- `Anova` - select genes according to an Anova p-value.
- `coxfilter` - use Cox model p-values.

genefilter: example

Two filters: gene should be above “100” for 5 times and have a Cox-PH-model p-value <0.01

```
kF <- kOverA(5, 100)
cF <- coxfilter(survtime, cens, p=0.01)
```

Assemble them in a filtering function

```
ff <- filterfun(kF, cF)
```

Apply the filter

```
sel <- genefilter(exprs(DATA), ff)
```

Select the relevant subset of the data

```
mySub <- DATA[sel,]
```

annotate

Goal: associate experimental data with available meta data, e.g. gene annotation, literature.

Tasks:

associate vendor identifiers (Affy, RZPD, …) to other identifiers

associate transcripts with biological data such as chromosomal position of the gene

associate genes with published data (PubMed).

produce nice-to-read tabular summaries of analyses.

PubMed
www.ncbi.nlm.nih.gov

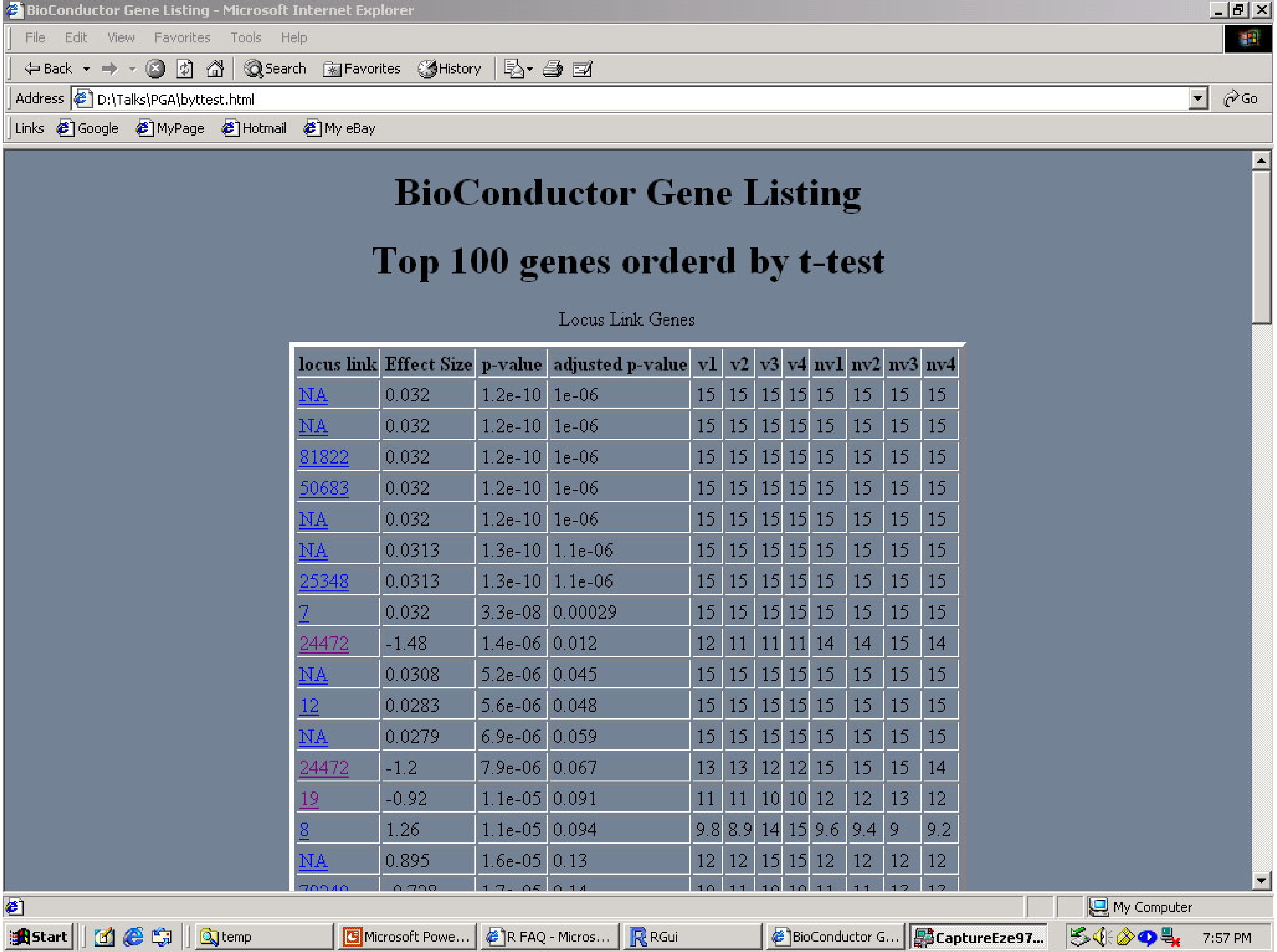
- For any gene there is often a large amount of data available from PubMed.
- We have provided the following tools for interacting with PubMed.
  - **`pubMedAbst`**: defines a class structure for PubMed abstracts in R.
  - **`pubmed:`** the basic engine for talking to PubMed.
- WARNING: be careful you can query them too much and be banned!

PubMed: high level tools

- `pm.getabst`: obtain (download) the specified PubMed abstracts (stored in XML).
- `pm.titles`: select the titles from a set of PubMed abstracts.
- `pm.abstGrep`: regular expression matching on the abstracts.

Data rendering

- A simple interface, `ll.htmlpage`, can be used to generate a webpage for your own use or to send to other scientists involved in the project.


BioConductor Gene Listing - Microsoft Internet Explorer
File Edit View Favorites Tools Help
Back
Search
Favorites
History
Address D:\Talks\PGA\byttest.html
Go
Links Google MyPage Hotmail My eBay
My Computer
Start
temp
Microsoft Powe...
R FAQ - Micros...
RGui
BioConductor G...
CaptureEze97...
7:57 PM

BioConductor Gene Listing

Top 100 genes orderd by t-test

Locus Link Genes

locus link	Effect Size	p-value	adjusted p-value	v1	v2	v3	v4	nv1	nv2	nv3	nv4
NA	0.032	1.2e-10	1e-06	15	15	15	15	15	15	15	15
NA	0.032	1.2e-10	1e-06	15	15	15	15	15	15	15	15
81822	0.032	1.2e-10	1e-06	15	15	15	15	15	15	15	15
50683	0.032	1.2e-10	1e-06	15	15	15	15	15	15	15	15
NA	0.032	1.2e-10	1e-06	15	15	15	15	15	15	15	15
NA	0.0313	1.3e-10	1.1e-06	15	15	15	15	15	15	15	15
25348	0.0313	1.3e-10	1.1e-06	15	15	15	15	15	15	15	15
7	0.032	3.3e-08	0.00029	15	15	15	15	15	15	15	15
24472	-1.48	1.4e-06	0.012	12	11	11	11	14	14	15	14
NA	0.0308	5.2e-06	0.045	15	15	15	15	15	15	15	15
12	0.0283	5.6e-06	0.048	15	15	15	15	15	15	15	15
NA	0.0279	6.9e-06	0.059	15	15	15	15	15	15	15	15
24472	-1.2	7.9e-06	0.067	13	13	12	12	15	15	15	14
19	-0.92	1.1e-05	0.091	11	11	10	10	12	12	13	12
8	1.26	1.1e-05	0.094	9.8	8.9	14	15	9.6	9.4	9	9.2
NA	0.895	1.6e-05	0.13	12	12	15	15	12	12	12	12
70240	-0.728	1.7e-05	0.14	10	11	10	10	11	11	12	12

Data packages

The Bioconductor project is starting to develop and deploy packages that contain only data.

Available: Affymetrix hu6800, hgu95a, hgu133a, mgu74a, rgu34a, KEGG, GO

These packages contain many different mappings between relevant data, e.g.

KEGG:	EnzymeID – GO Category
hgu95a:	Affy Probe set ID - EnzymeID

Update: simply by R function update.packages()

dataset: hgu95a

maps to LocusLink, GenBank, gene symbol, gene Name.

chromosomal location, orientation.

maps to KEGG pathways, to enzymes.

data packages will be updated and expanded regularly as new or updated data become available.

Diagnostic plots and normalization for cDNA microarrays

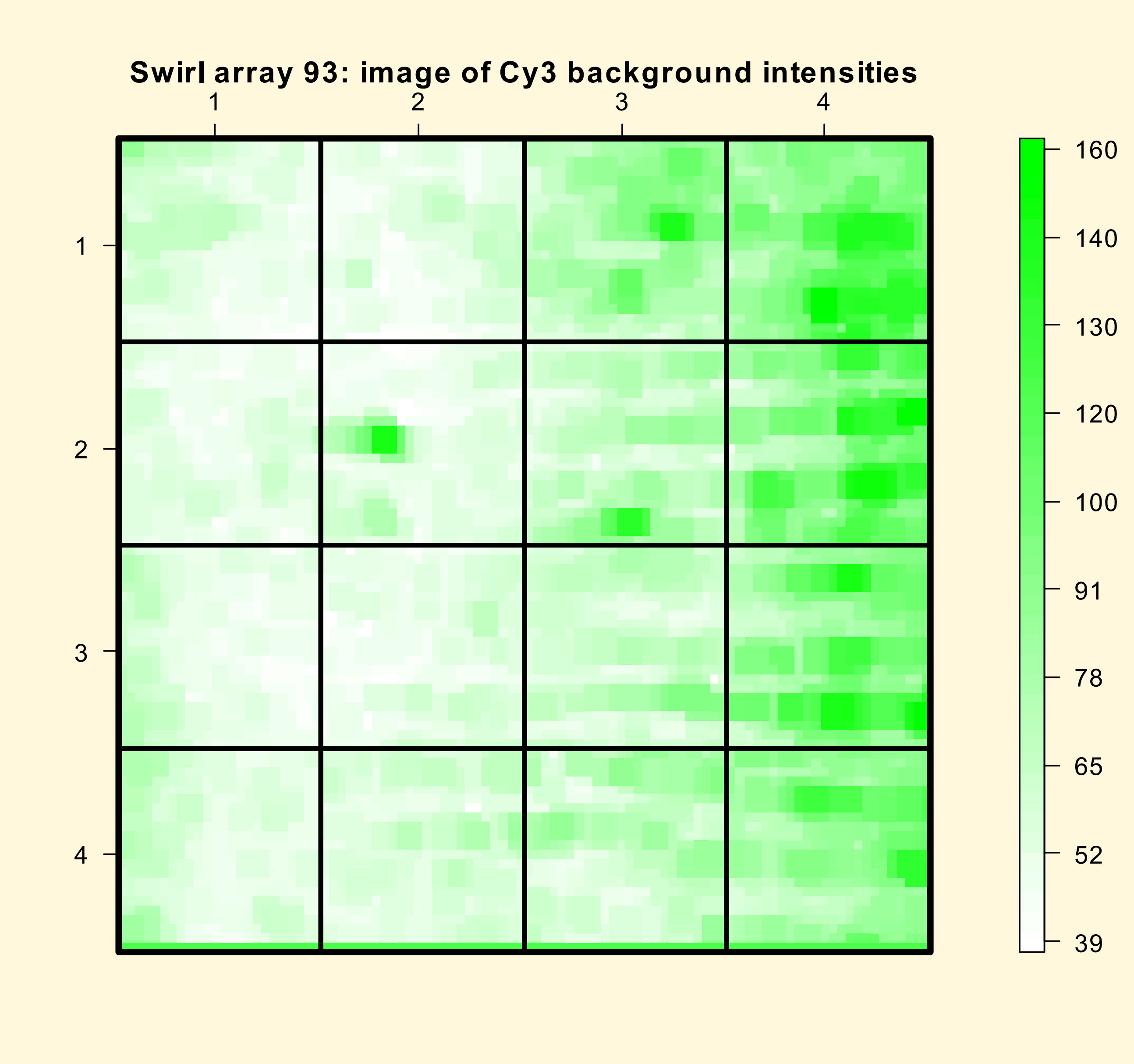
(S Dudoit, Y Yang, T Speed, et al)

- `marrayClasses`:
  - class definitions for microarray data objects and basic methods
- `marrayInput`:
  - reading in intensity data and textual data describing probes and targets;
  - automatic generation of microarray data objects;
  - widgets for point & click interface.
- `marrayPlots`: diagnostic plots.
- `marrayNorm`: robust adaptive location and scale normalization procedures.

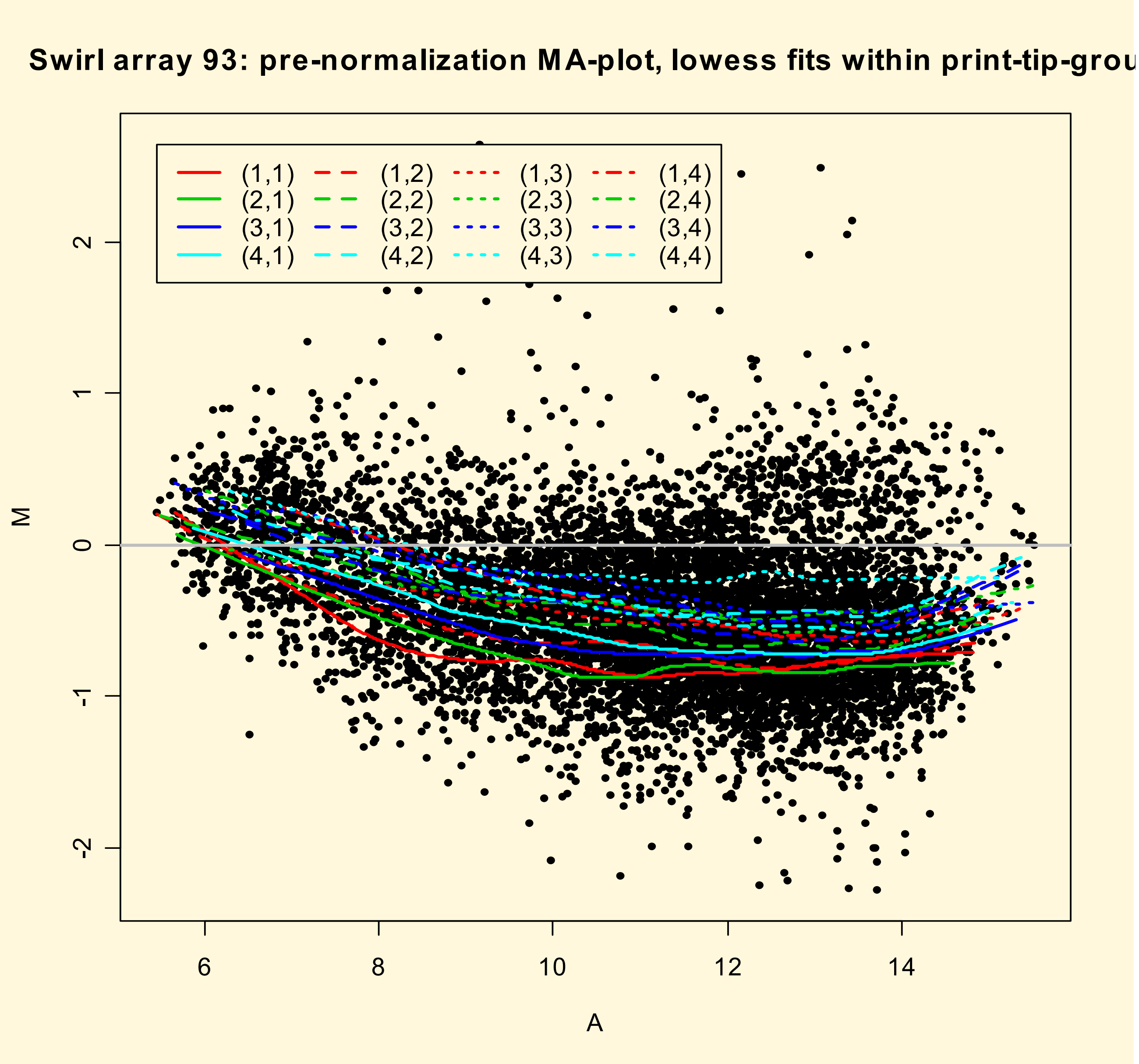
`marrayPlots` package

- `maImage`: 2D spatial images of microarray spot statistics.
- `maBoxplot`: boxplots of microarray spot statistics, stratified by layout parameters.
- `maPlot`: scatter-plots of microarray spot statistics, with fitted curves and text highlighted, e.g., MA-plots with loess fits by sector.
- See `demo(marrayPlots)`.

demo(marrayPlots)



demo(marrayPlots)



`marrayNorm` package

robust adaptive location and scale normalization for a batch of arrays

- intensity or A-dependent location normalization (`maNormLoess`);
- 2D spatial location normalization (`maNorm2D`);
- median location normalization (`maNormMed`);
- scale normalization using MAD (`maNormMAD`);
- composite normalization.

marrayInput package

- Start from
  - image quantitation data, i.e., output files from image analysis software, e.g., .gpr for GenePix or .spot for Spot.
  - Textual description of probe sequences and target samples, e.g., gal files, god lists.

- read.marrayLayout, read.marrayInfo, and read.marrayRaw: read microarray data into R and create microarray objects of class marrayLayout, marrayInfo, and marrayRaw, resp.

Multiple hypothesis testing

- Bioconductor R `multtest` package
- Multiple testing procedures for controlling
  - FWER: Bonferroni, Holm (1979), Hochberg (1986), Westfall & Young (1993) maxT and minP.
  - FDR: Benjamini & Hochberg (1995), Benjamini & Yekutieli (2001).
- Tests based on *t*- or *F*-statistics for one- and two-factor designs.
- Permutation procedures for estimating adjusted p-values.
- Documentation: tutorial on multiple testing.

Sweave

- The Sweave framework allows dynamic generation of statistical documents intermixing documentation text, code and code output (textual and graphical).
- Fritz Leisch's `Sweave` function from R `tools` package.
- See `? Sweave` and manual http://www.ci.tuwien.ac.at/~leisch/Sweave

Sweave input

Source: a text file which consists of a sequence of documentation and code segments ('chunks')

- Documentation chunks
  - start with @
  - can be text in a markup language like LaTeX.
- Code chunks
  - start with <<*name*>>=
  - can be R or S-Plus code.
- File extension: `.rnw`, `.Rnw`, `.snw`, `.Snw`.

Sweave output

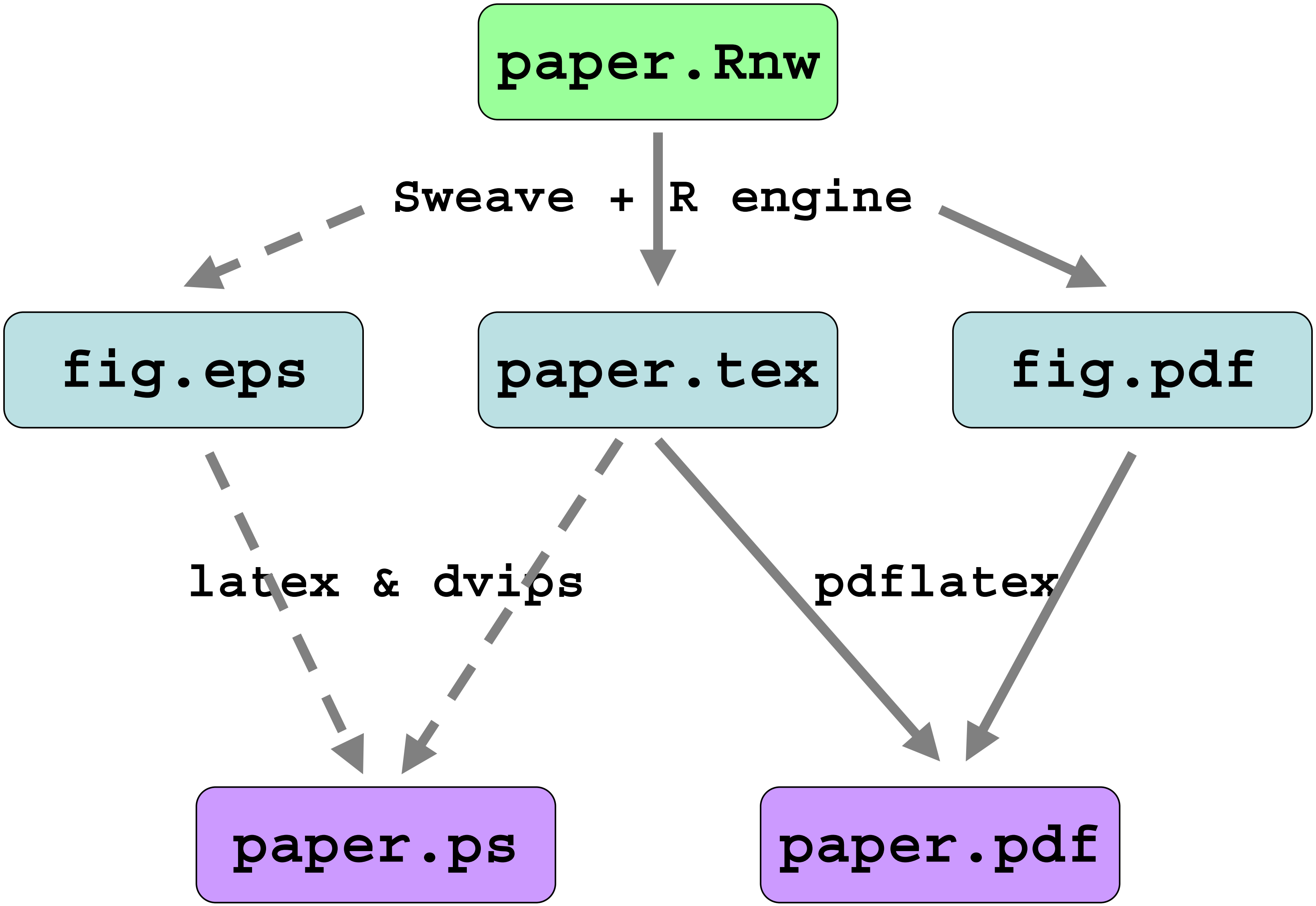
After running `Sweave` and Latex, obtain a single document, e.g. `pdf` file containing

- the documentation text
- the R code
- the code output: text and graphs.

The document can be automatically regenerated whenever the data, code or text change.

Ideal medium for the communication of data analyses that want to be reproducible by other researchers: they can read the document and at the same time have the code chunks executed by their computer!

Sweave



paper.Rnw

Sweave + R engine

fig.eps

paper.tex

fig.pdf

latex & dvips

pdflatex

paper.ps

paper.pdf