GS01 0163
Analysis of Microarray Data

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Lecture 18: A Two-Color Case Study

- Case Study Biology
- Getting Data
- Inferences from GPR Files
- Quality Checks
- Further Analysis
- Adventures with the Gene Expression Omnibus

The Biology

Working with a case study. This follows Chapter 4 of Gentleman et al (2005), “Preprocessing Two-Color Spotted Arrays”, by Y.H. Yang and A.C. Paquet.

The dataset used here is a subset of a larger dataset described in Rodriguez et al (2004), “Differential gene expression by integrin $\beta7+$ and $\beta7-$ memory T helper cells”, BMC Immunology, 5:13.

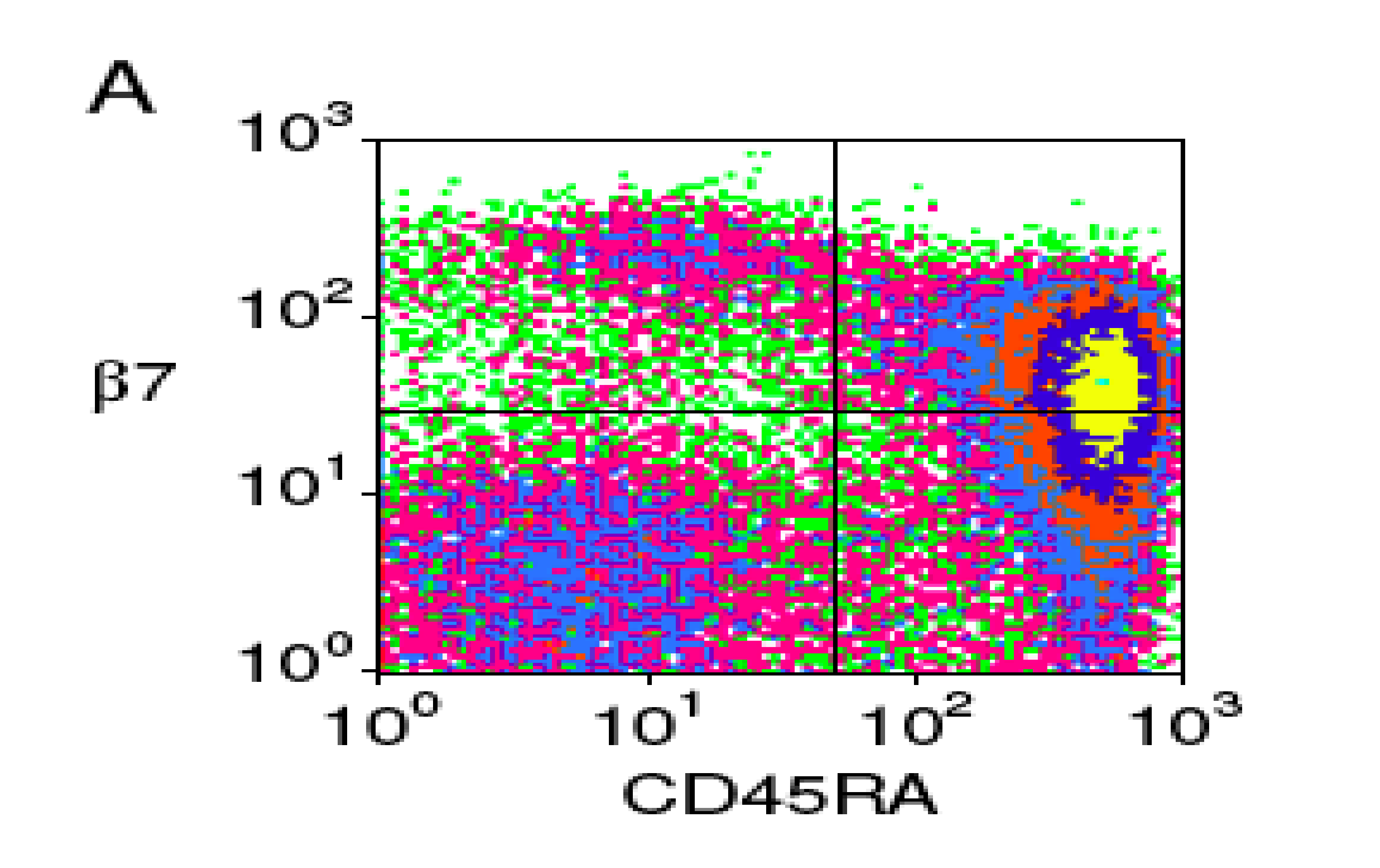
In that paper, they asked whether different types of helper cells were associated with the adhesion or migration of T cells.

How do we Get Cells?

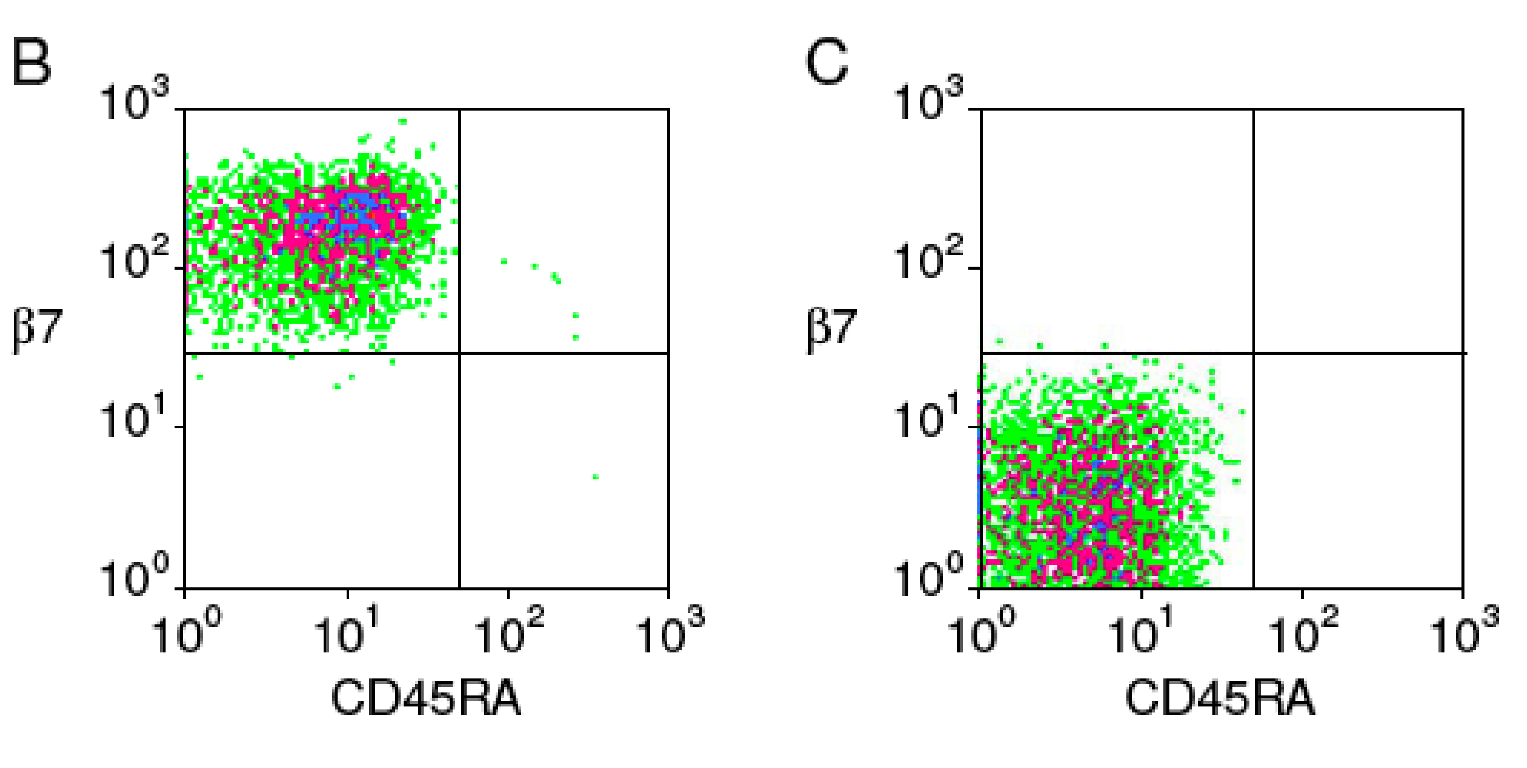
Extract CD4+ T cells, and derive enriched subpopulations that are $\beta 7+$ and $\beta 7-$. Cell subpopulations were obtained using flow cytometry.

Initially, cells are sorted by their levels of $\beta 7$ and CD45RA. High levels of CD45RA are not as interesting here, as their adhesion targets are already known. We want to focus on $\beta 7$ and see if we see separations there.

Cells Before Filtering



Cells After Filtering



After purification, the distributions are separated into our target groups.

Samples are Paired!

Extraction was performed using samples from 9 individuals, so there is a natural data pairing.

Given the pairing, individual arrays were used to contrast the two by hybridizing $\beta 7+$ in one channel and $\beta 7-$ in the other.

In all, 27 arrays were run, including at least 2 for each patient in a dye-swap arrangement.

The actual data is available from the Gene Expression Omnibus (GEO) maintained by the NCBI, with accession number GSE1039. (We will return to this later.)

Stuff Inferrable from GEO

sample, channel 1 (635nm), channel 2 (532nm), Patient ID, Gender (or is ch1 Cy3 and ch2 Cy5?)

GSM16665	−	+	001	F	GPL976	Hs_004_187_2	
GSM16675	+	−	001	F	GPL976	Hs_004_186_2	
GSM16679	−	+	006	F	GPL976	Hs_004_235	
GSM16680	−	+	009	F	GPL976	Hs_004_189_1	
GSM16681	+	−	009	F	GPL976	Hs_004_188	
GSM16685	−	+	001	F	GPL978	6Hs.094	
GSM16686	−	+	001	F	GPL978	6Hs.195.1	**
GSM16687	+	−	003	F	GPL978	6Hs.168	**

and so on. The ones with asterisks are contained in the subset we will look at today.

More on Methods

No data from patients 2 and 5.

The arrays used 70-mer oligos from Operon; there were 23184 spots on the arrays. Two different chip platforms were used when the experiment was run; these are available from GEO as

GPL976 UCSF 4Hs Human v.2 Oligo Array
GPL978 UCSF 6Hs Human v.2 Oligo Array

The RNA was subjected to 2 rounds of amplification using kits from Ambion.

All of the arrays were quantified using Axon's GenePix software, so we have GPR quantification files. The TIFF files are also available for download.

More on Methods, and our Subset

What other information would we like to have?
Run date? (scan date is available; this should be close)
Date of blood draw? (this is given in the TargetBeta7.txt file)
Gene information? (some of this is here)
Patient age? (this was there)

The data used here involves a subset of 6 arrays from this experiment. All 6 were of a single platform type, and had a common layout format.

Why were these 6 chosen?

Getting the Data

Next, we get the 6 GPR files, and some TargetInfo and SpotInfo files

http://www.bioconductor.org/workshops/2005/BioC2005/labs/lab01/Data/integrinbeta7.zip

This zip file includes 6 GPR files, and a text file, TargetBeta7.txt, that contains sample information (e.g., phenoData information). E.g.:

```
FileNames       Subject ID #    Cy3     Cy5
6Hs.195.1.gpr   001             b7 -    b7 +
Hyb buffer      Hyb Temp (deg C)Hyb Time (h)
Ambion Hyb Slide        55      40
Date of Blood Draw      Amplification
2002.10.11              R2 aRNA
```

Using R

The first step is simply to load a whole bunch of packages:

```
> library("marray")
> library("mclust")
> library("convert")
> library("arrayQuality")
> library("colorspace")
> library("grid")
> library("hexbin")
```

Getting the Sample Info

```
> TargetInfo <- read.marrayInfo("Data/TargetBeta7.txt")
> TargetInfo

An object of class "marrayInfo"
@maLabels
[1] "6Hs.195.1.gpr" "6Hs.168.gpr"   "6Hs.166.gpr"
[4] "6Hs.187.1.gpr" "6Hs.194.gpr"   "6Hs.243.1.gpr"

@maInfo
      FileNames Subject ID #  Cy3  Cy5       Hyb buffer
1 6Hs.195.1.gpr            1 b7 - b7 + Ambion Hyb Slide
2   6Hs.168.gpr            3 b7 + b7 - Ambion Hyb Slide
3   6Hs.166.gpr            4 b7 + b7 - Ambion Hyb Slide
4 6Hs.187.1.gpr            6 b7 - b7 + Ambion Hyb Slide
```

```
5   6Hs.194.gpr            8 b7 - b7 + Ambion Hyb Slide
6 6Hs.243.1.gpr           11 b7 + b7 - Ambion Hyb Slide
  Hyb Temp (deg C) Hyb Time (h) Date of Blood Draw
1               55           40         2002.10.11
2               55           40         2003.01.16
3               55           40         2003.01.16
4               55           40         2002.09.16
5               55           40         2002.09.18
6               55           40         2003.01.13
  Amplification  Slide Type Date of Scan
1       R2 aRNA  Aminosilane   2003.07.25
2       R2 aRNA  Aminosilane   2003.08.07
3       R2 aRNA  Aminosilane   2003.08.07
4       R2 aRNA  Aminosilane   2003.07.18
5       R2 aRNA  Aminosilane   2003.07.25
6       R2 aRNA  Aminosilane   2003.08.06
```

```
@maNotes
[1] "Data/TargetBeta7.txt"
```

Getting the Numerical Info

Grab the data from the GPR files:

```
> mraw <- read.GenePix(targets = TargetInfo, path = "Data")

Reading ...  Data/6Hs.195.1.gpr
Reading ...  Data/6Hs.168.gpr
Reading ...  Data/6Hs.166.gpr
Reading ...  Data/6Hs.187.1.gpr
Reading ...  Data/6Hs.194.gpr
Reading ...  Data/6Hs.243.1.gpr
```

Mac errors?

Note: this works on a PC. On a Mac laptop, Keith reported the following error messages:

```
> mraw <- read.GenePix(targets = TargetInfo)
Error in if (skip > 0) readLines(file, skip) :
missing value where TRUE/FALSE needed
In addition: Warning messages:
1: input string 32 is invalid in this locale in:
  grep(pattern, x, ignore.case, extended, value, fixed,
     useBytes)
2: input string 32 is invalid in this locale in:
  grep(pattern, x, ignore.case, extended, value, fixed,
   useBytes)
```

What Can be Inferred?

So, what does our marrayRaw object contain at this point?

We look at the individual slots.

```
> slotNames(mraw)

[1] "maRf"       "maGf"       "maRb"       "maGb"
[5] "maW"        "maLayout"   "maGnames"   "maTargets"
[9] "maNotes"
```

Of these, the first 5 are the basic quantification information, extracted from the GPR files. All of them are 23184 by 6 in size. The others are the associated layout and annotation files. We will extract these to find out a bit more about them.

Summary, Part 1 – Layout

```
> summary(mraw)
Pre-normalization intensity data:
  Object of class marrayRaw.

Number of arrays:  6 arrays.

A) Layout of spots on the array:
Array layout:  Object of class marrayLayout.

Total number of spots: 23184
Dimensions of grid matrix: 12 rows by 4 cols
Dimensions of spot matrices: 23 rows by 21 cols

Currently working with a subset of 23184spots.
```

More Layout

```
Control spots:
There are   5 types of controls :

  Buffer    Empty Negative Positive   probes
       3     1328      225      204    21424

Notes on layout:
```

The layout can be inferred from the gpr files! This is not too suprising, as every row of a GPR file contains entries for grid row, grid col, spot row, and spot col. As a side note, what is the precise order?

Layout Ordering

```
> zedL <- mraw@maLayout
> zedLSC <- maSpotCol(zedL)
> zedLSR <- maSpotRow(zedL)
> zedLGR <- maGridRow(zedL)
> zedLGC <- maGridCol(zedL)
> zedLcoords <- cbind(zedLGR, zedLGC, zedLSR, zedLSC)
> zedLcoords[c(1:2, 20:22), ]
```

```
     zedLGR zedLGC zedLSR zedLSC
[1,]      1      1      1      1
[2,]      1      1      1      2
[3,]      1      1      1     20
[4,]      1      1      1     21
[5,]      1      1      2      1
```

Summary Part 2 – Sample Info

```
B) Samples hybridized to the array:
Object of class marrayInfo.

       maLabels     FileNames SubjectID  Cy3  Cy5
1 6Hs.195.1.gpr 6Hs.195.1.gpr         1 b7 - b7 +
2   6Hs.168.gpr   6Hs.168.gpr         3 b7 + b7 -
..
  Date of Blood Draw  Date of Scan
1          2002.10.11    2003.07.25
2          2003.01.16    2003.08.07
..
```

Since we supplied the `marrayInfo` file in the call to `read.GenePix`, this is imported from there.

Summary Part 3 – Array Summaries

```
C) Summary statistics for log-ratio distribution:
               Min. 1st Qu. Median  Mean 3rd Qu. Max. NA's
6Hs.195.1.gpr -6.13   -1.00  -0.52 -0.50   -0.08 5.95 3415
6Hs.168.gpr   -7.08   -0.80  -0.21 -0.23    0.34 5.19 2839
6Hs.166.gpr   -7.07   -1.25  -0.64 -0.62   -0.02 6.15 3440
6Hs.187.1.gpr -9.81   -0.92  -0.60 -0.55   -0.25 5.00 2942
6Hs.194.gpr   -5.93    0.00   0.44  0.53    0.90 7.74 6090
6Hs.243.1.gpr -6.38   -1.13  -0.69 -0.64   -0.21 7.05 2227
```

Log ratios – what direction is the default? Cy3/Cy5? Cy5/Cy3? (the latter, according to documentation)

Summary Part 4 – Notes

`D) Notes on intensity data:`

`GenePix Data`

Ok, that dealt with most of the microarray structure itself.

What happens if we ask about the gene names? This is what we really want, so that we can understand the biology.

Annotation

```
> mraw@maGnames[1:2,]
An object of class "marrayInfo"
[1] "H200000297" "H200000303"
                   ID
H200000297 H200000297
H200000303 H200000303
                 Name
H200000297 OVGP1 - Oviductal glycoprotein 1, 120kD (mucin 9,
H200000303 TAF1 - TAF1 RNA polymerase II, TATA box binding pr
[1] ""
```

Again, these are read in from the GPR files. The first column here, the maLabels, is the Operon-supplied identifier for that specific oligo, and as such it should be unique.

Getting the Data: TMTOWTDI

So, what if you are working with a Mac?

This `marrayRaw` object and a few other things are available as a package from BioConductor called “beta7”. I had to run a search at the top level of BioConductor to find this; it is part of the “Data” page associated with the monograph. I downloaded the gzipped tar (.tar.gz) file and did an install from local source.

http://www.bioconductor.org/docs/mogr/data

```
> library("beta7")
> data(beta7)
```

loads an marrayRaw object (called beta7) with info on the 6 selected arrays.

How was Data Reported?

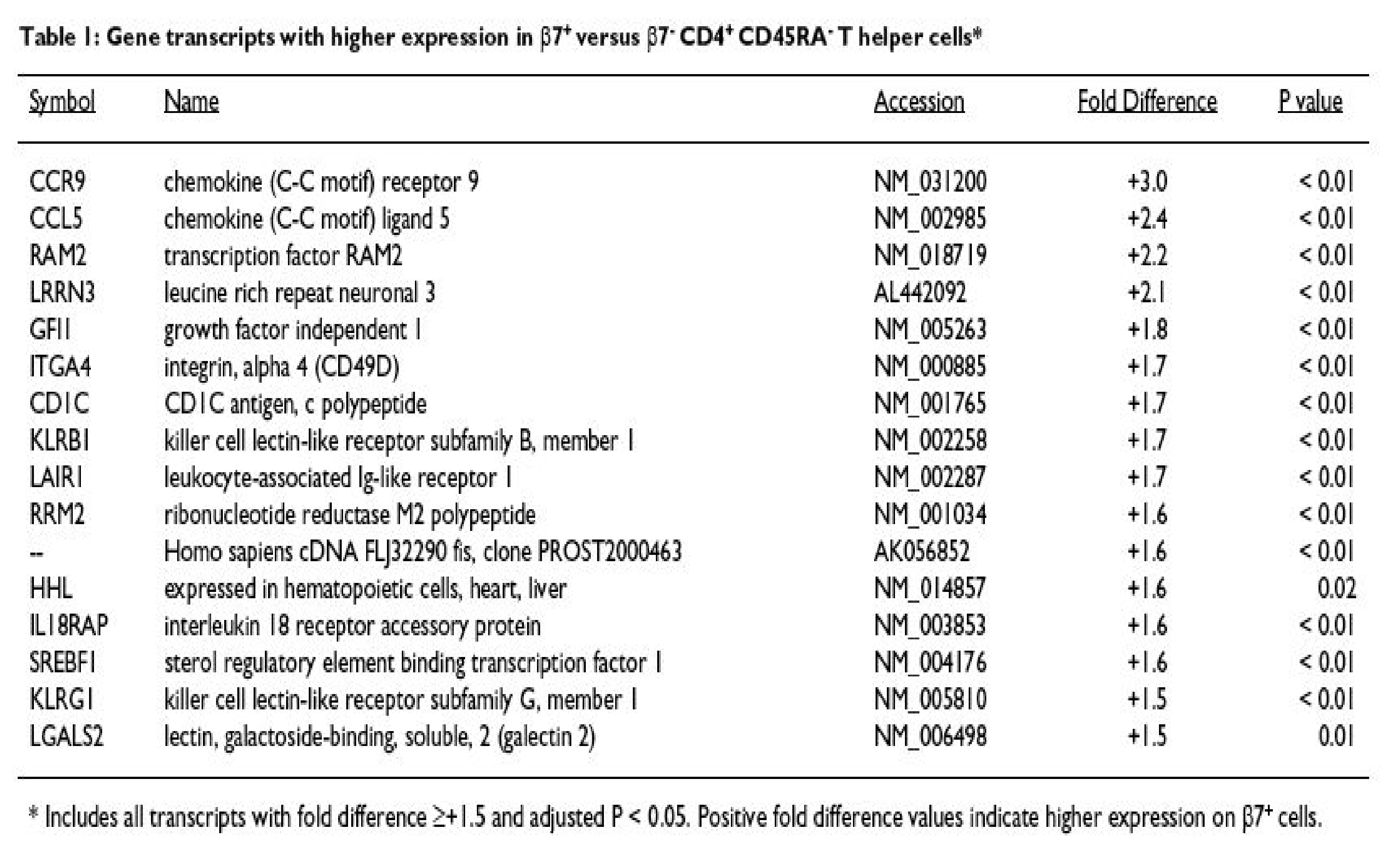


Table 1: Gene transcripts with higher expression in $\beta 7^+$ versus $\beta 7^-$ CD4^+ CD45RA$^-$ T helper cells*

Symbol	Name	Accession	Fold Difference	P value
CCR9	chemokine (C-C motif) receptor 9	NM_031200	+3.0	< 0.01
CCL5	chemokine (C-C motif) ligand 5	NM_002985	+2.4	< 0.01
RAM2	transcription factor RAM2	NM_018719	+2.2	< 0.01
LRRN3	leucine rich repeat neuronal 3	AL442092	+2.1	< 0.01
GFI1	growth factor independent 1	NM_005263	+1.8	< 0.01
ITGA4	integrin, alpha 4 (CD49D)	NM_000885	+1.7	< 0.01
CD1C	CD1C antigen, c polypeptide	NM_001765	+1.7	< 0.01
KLRB1	killer cell lectin-like receptor subfamily B, member 1	NM_002258	+1.7	< 0.01
LAIR1	leukocyte-associated Ig-like receptor 1	NM_002287	+1.7	< 0.01
RRM2	ribonucleotide reductase M2 polypeptide	NM_001034	+1.6	< 0.01
--	Homo sapiens cDNA FLJ32290 fis, clone PROST2000463	AK056852	+1.6	< 0.01
HHL	expressed in hematopoietic cells, heart, liver	NM_014857	+1.6	0.02
IL18RAP	interleukin 18 receptor accessory protein	NM_003853	+1.6	< 0.01
SREBF1	sterol regulatory element binding transcription factor 1	NM_004176	+1.6	< 0.01
KLRG1	killer cell lectin-like receptor subfamily G, member 1	NM_005810	+1.5	< 0.01
LGALS2	lectin, galactoside-binding, soluble, 2 (galectin 2)	NM_006498	+1.5	0.01

* Includes all transcripts with fold difference ≥+1.5 and adjusted $P < 0.05$. Positive fold difference values indicate higher expression on $\beta 7^+$ cells.

There are some unique identifiers here!

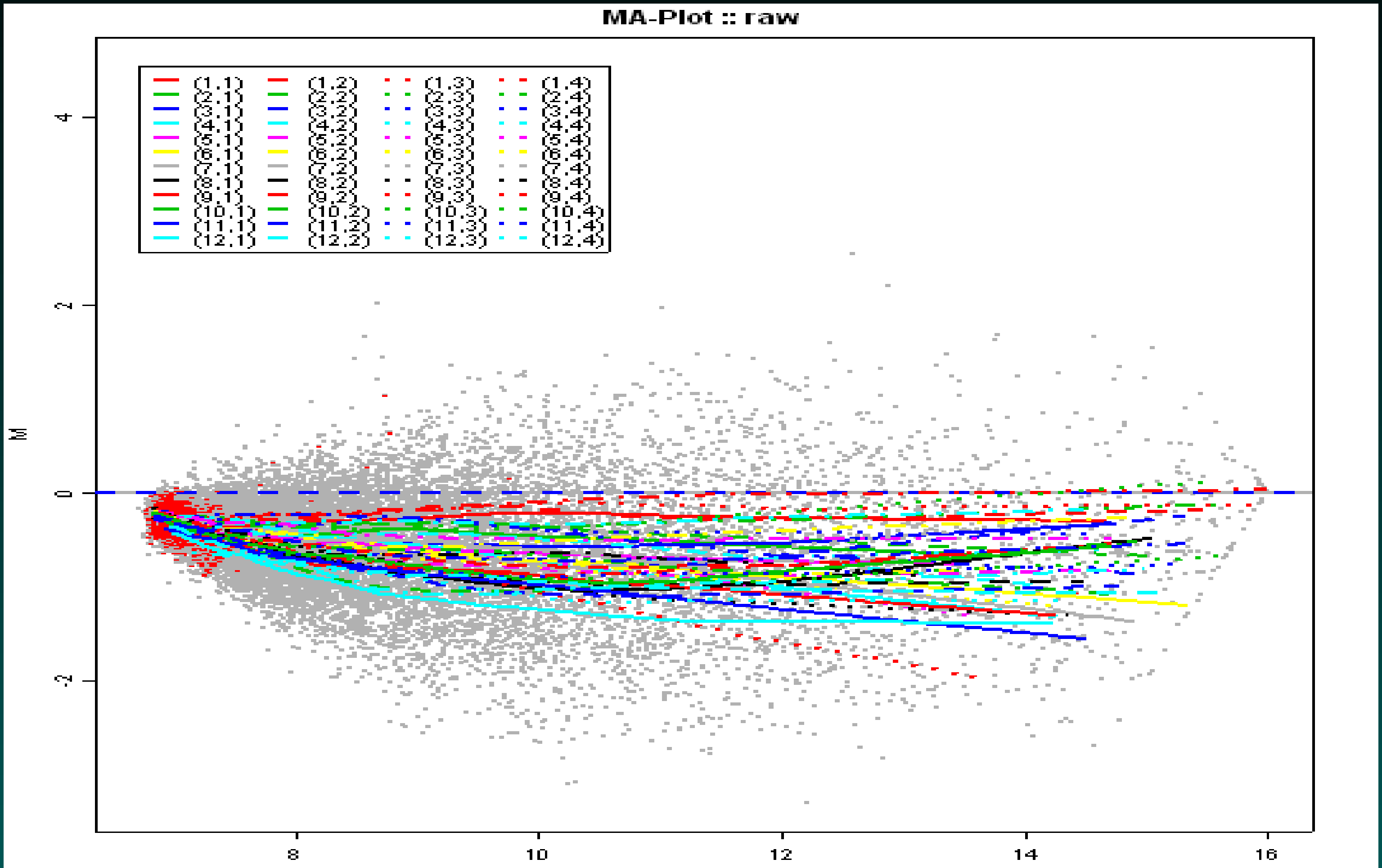
Checking the Data

Ok, now we have the raw data. What do we want to try next? Well, checking array quality would be nice.

```
> maQualityPlots(mraw); # again, works on PC only
save as diagPlot..6Hs.195.1.png
save as diagPlot..6Hs.168.png
save as diagPlot..6Hs.166.png
save as diagPlot..6Hs.187.1.png
save as diagPlot..6Hs.194.png
save as diagPlot..6Hs.243.1.png
```

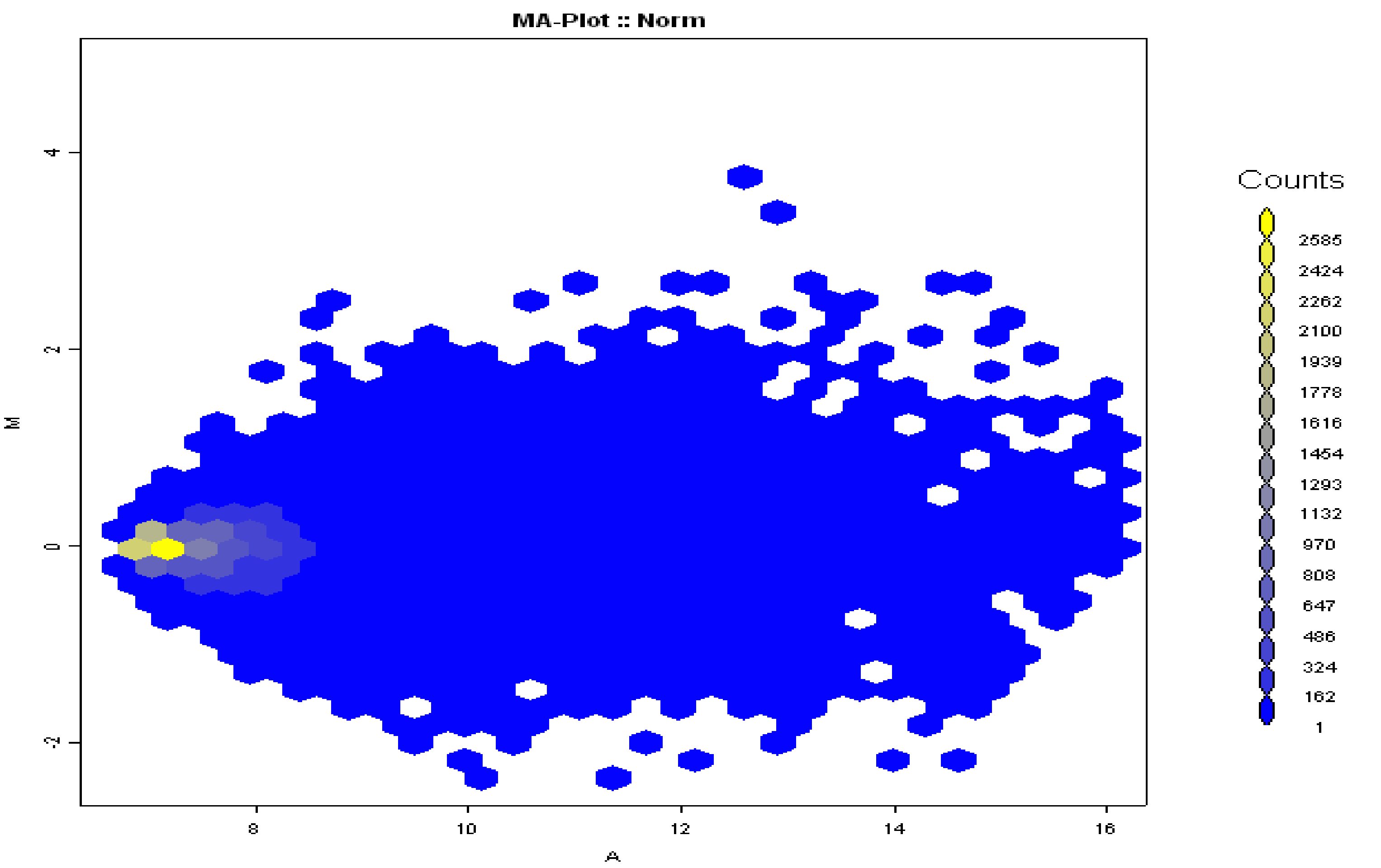
What does this produce? One large png file for each array. This plot has 8 panels...

Panel (a)



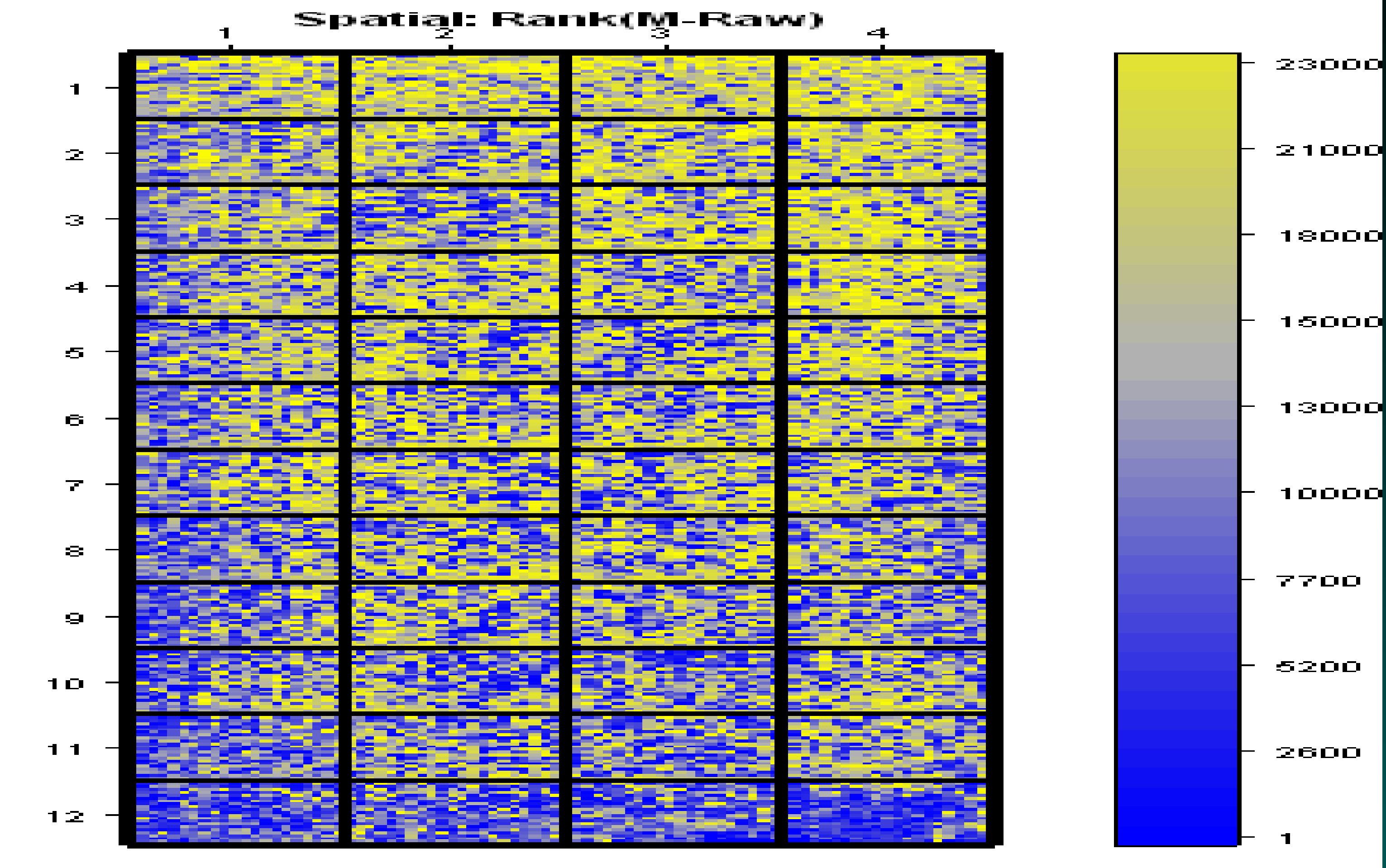
(a) an MA-plot for the raw data, with loess traces for each pin

Panel (b)



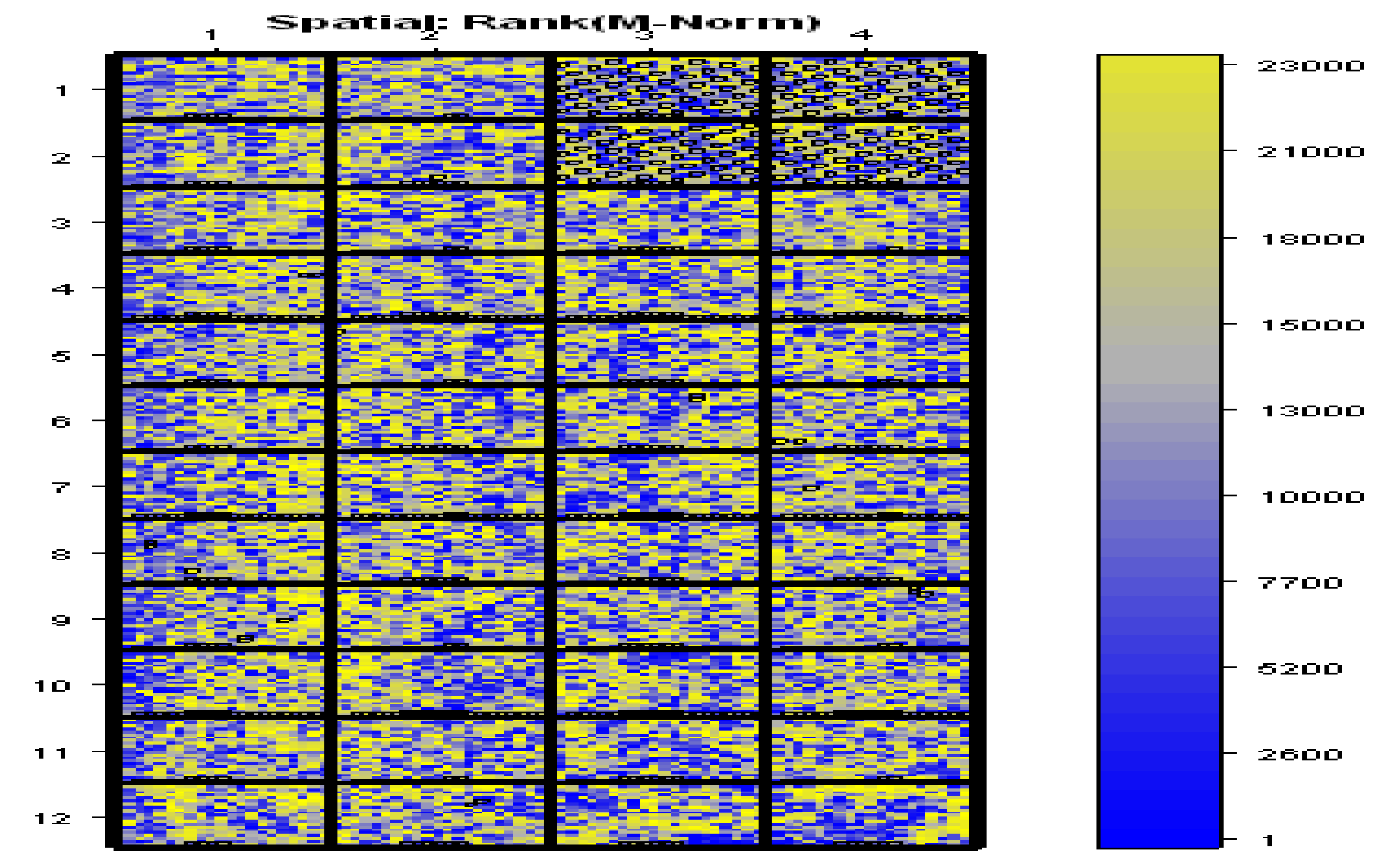
(b) an MA-plot for the data after print-tip loess normalization, displayed using hexbin.

Panel (c)



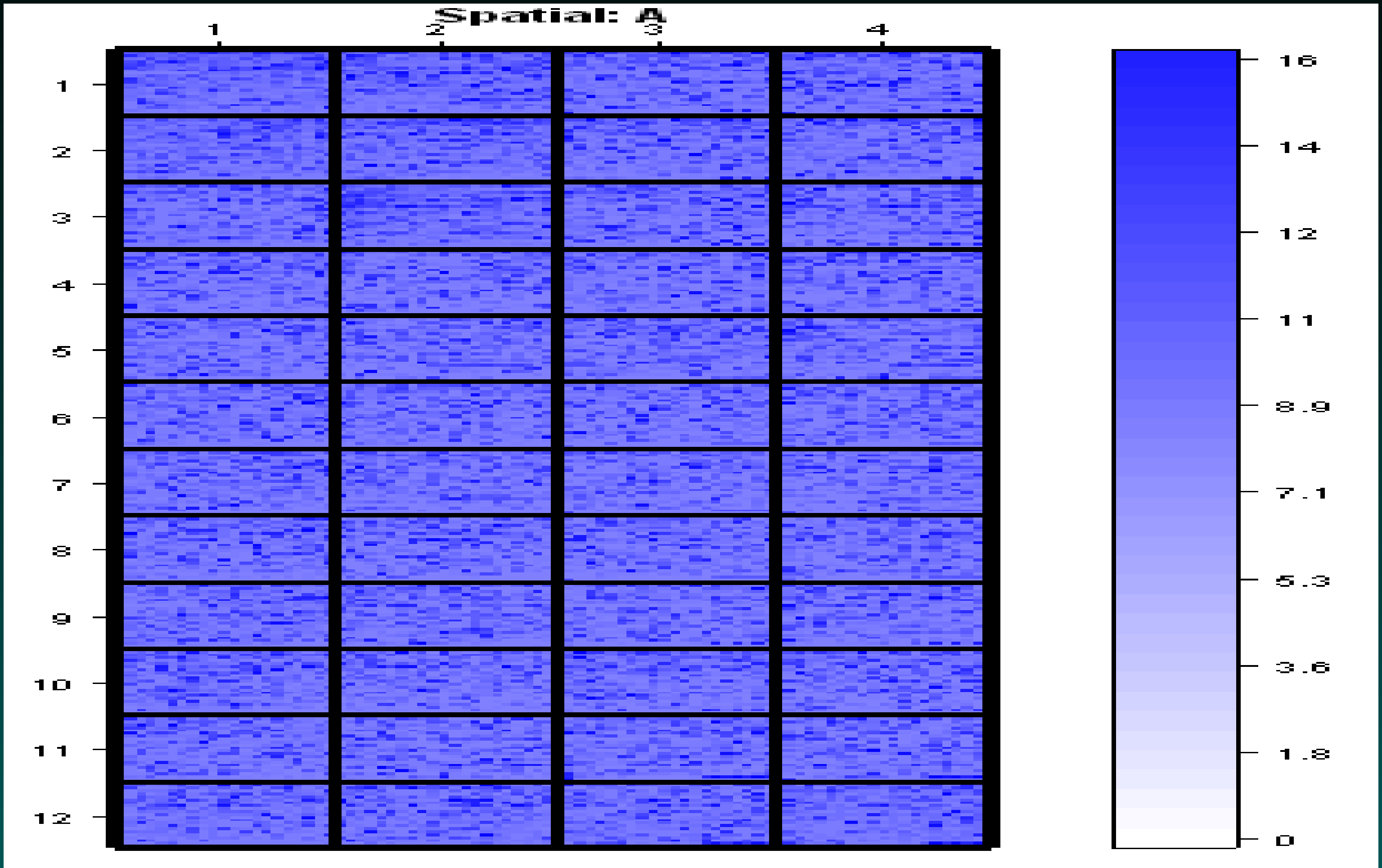
(c) a spatial plot of ranks of the M-Raw differences

Panel (d)



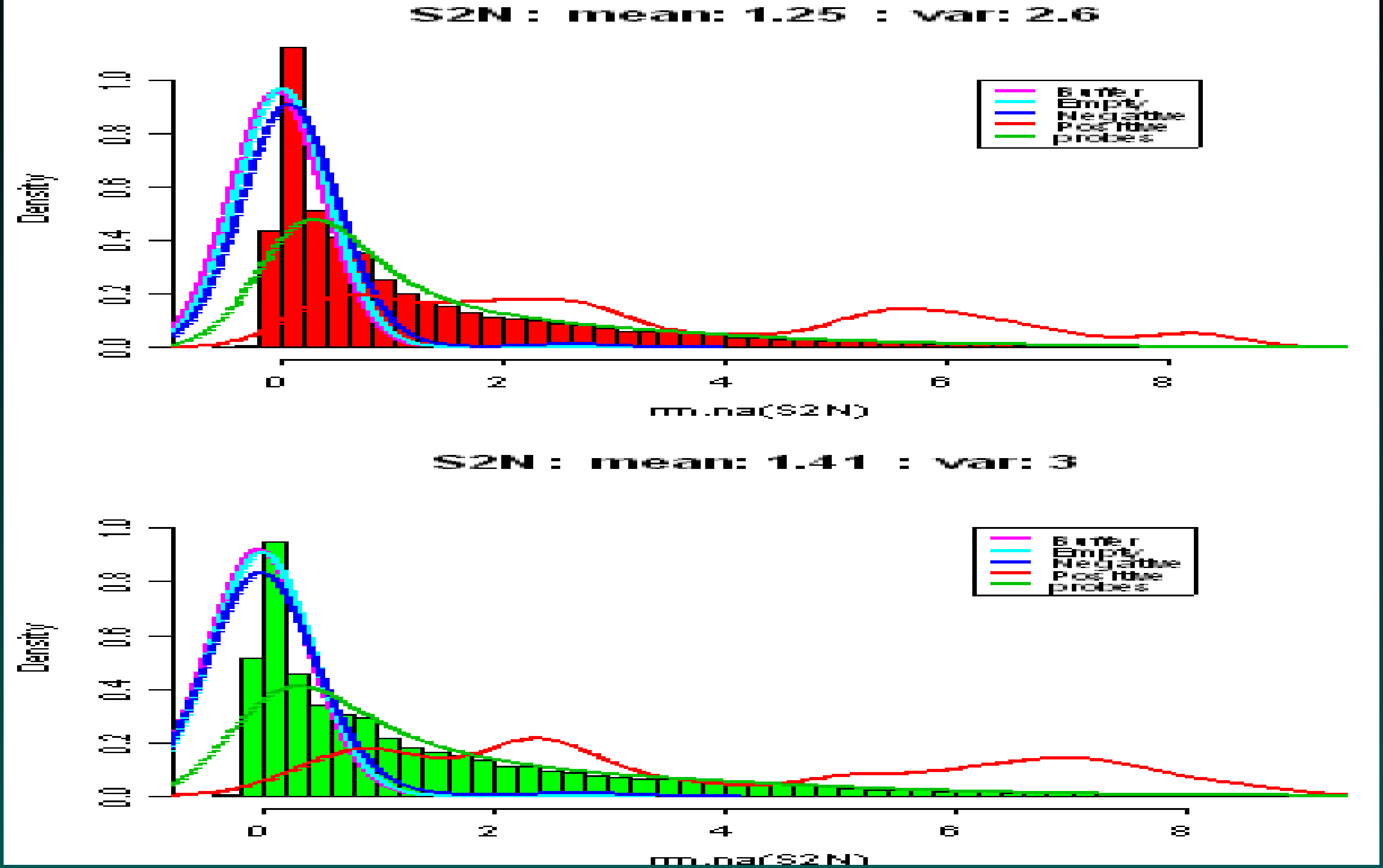
(d) a spatial plot of ranks of the M-Norm differences, with outliers flagged

Panel (e)



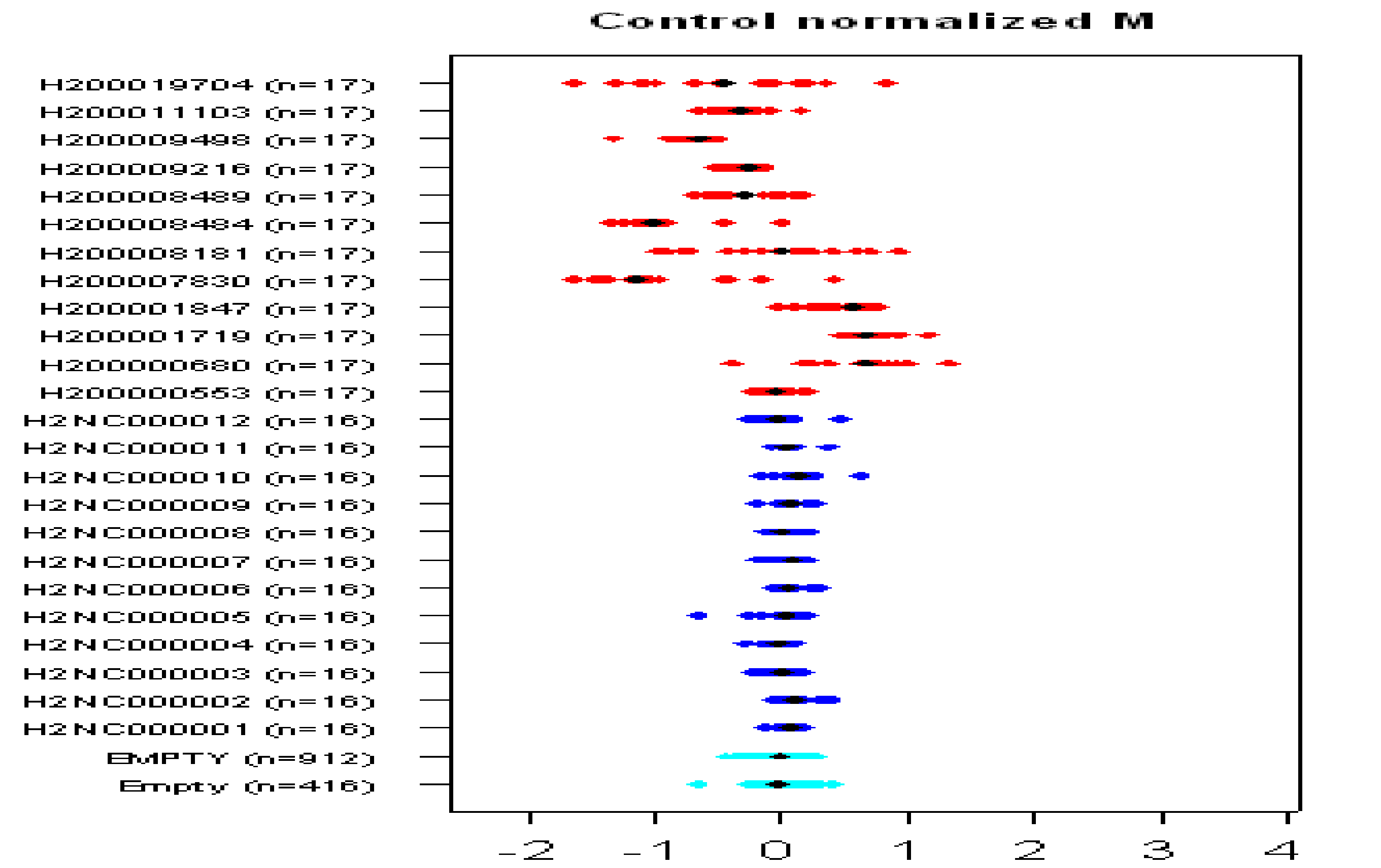
(e) a spatial plot of the A values

Panel (f)



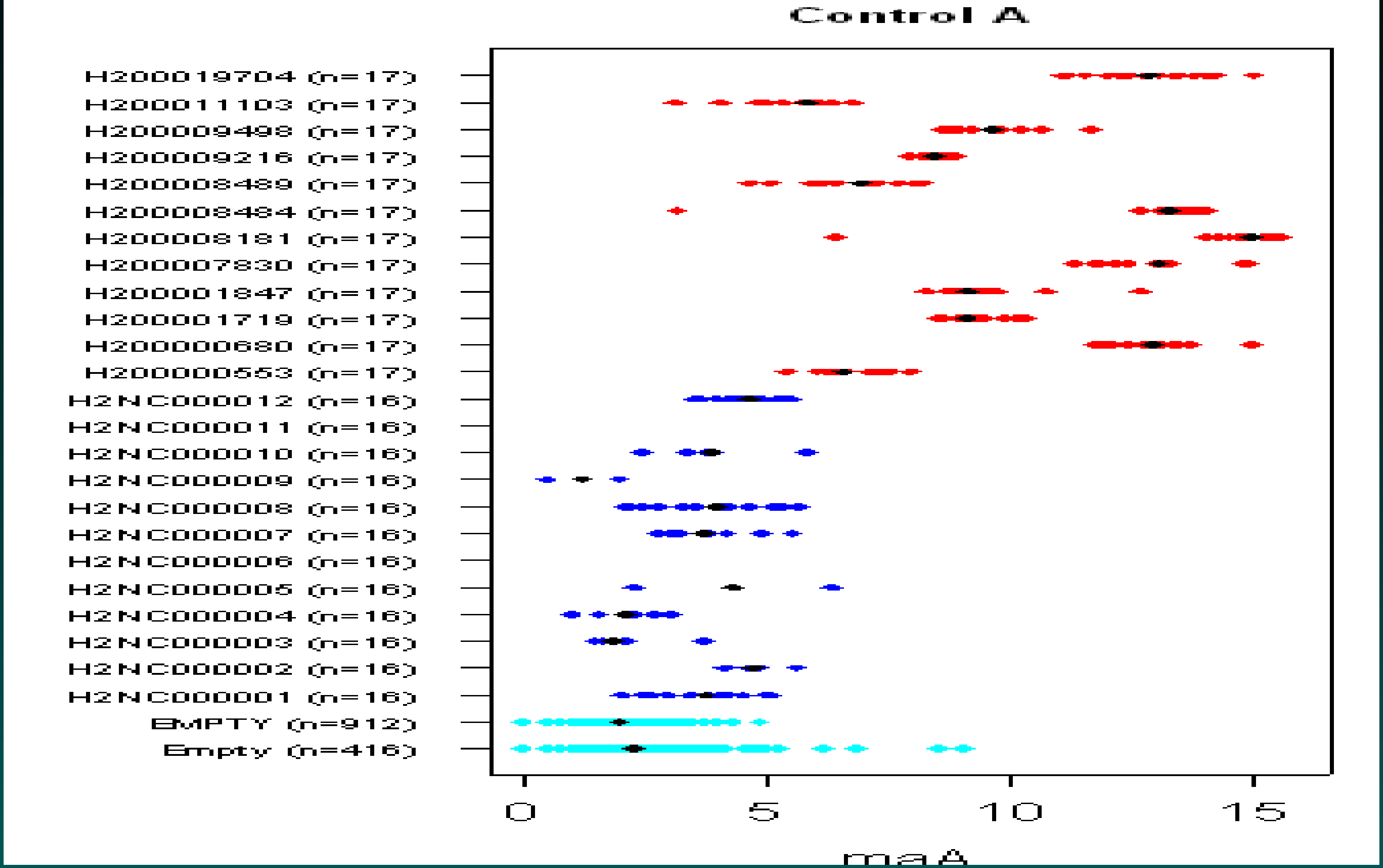
(f) signal to noise distribution plots for each channel (presumably assessed on the raw data)

Panel (g)



(g) M distributions for replicated controls using the normalized values

Panel (h)



(h) A distributions for replicated controls using the normalized values

What next?

Ok, given that the arrays look ok, we would like to do some numerical contrasts. What needs to be done before we do this?

What next?

Ok, given that the arrays look ok, we would like to do some numerical contrasts. What needs to be done before we do this?

Go from an marrayRaw object to an marrayNorm object.

```
> normdata <- maNorm(mraw)
```

By default, this will invoke print-tip loess as the processing method.

Exporting the Data

```
> write.marray(normdata)
```

```
NULL
```

This will create a file “maRawResults.xls”, even though the normalized data was used. This will give grid R,C, spot R,C, the spot ID, the gene name, and the associated log ratio values. It presumes that we know which direction the ratios are taken in (in, fact, Cy5/Cy3).

Using the Data Further

```
> library("convert")
> mdata <- as(normdata, "exprSet")
```

This would seem to coerce our marrayNorm object into an exprSet, which we can then act upon to get more information. This is partially correct.

The gene names are not retained or passed, so keeping track of the annotation must be done by index value or attached separately.

How was the Data Analyzed?

According to the methods, they worked just with the foreground measurements; no background was subtracted.

Print-tip loess was used to normalize the array data, and log ratios were computed.

Differentially expressed genes were estimated using a linear model (and the limma package). The model:

$$Y_{ij} = \mu + A_i + \epsilon_{ij}$$

The individual (b7+/b7-) log ratio values for each array are expressed in terms of an overall level, a patient effect, and a chip effect. The patient effect lets them deal with replicates intelligently.

More Analysis

For each gene, a “moderated t-test” was performed using an empirical Bayes approach, pooling information about the variance to make the results more stable.

The genes had to be significant at a 0.01 level after a Bonferroni correction, and the mean fold change had to be more than 1.5.

What Other Info was Provided?

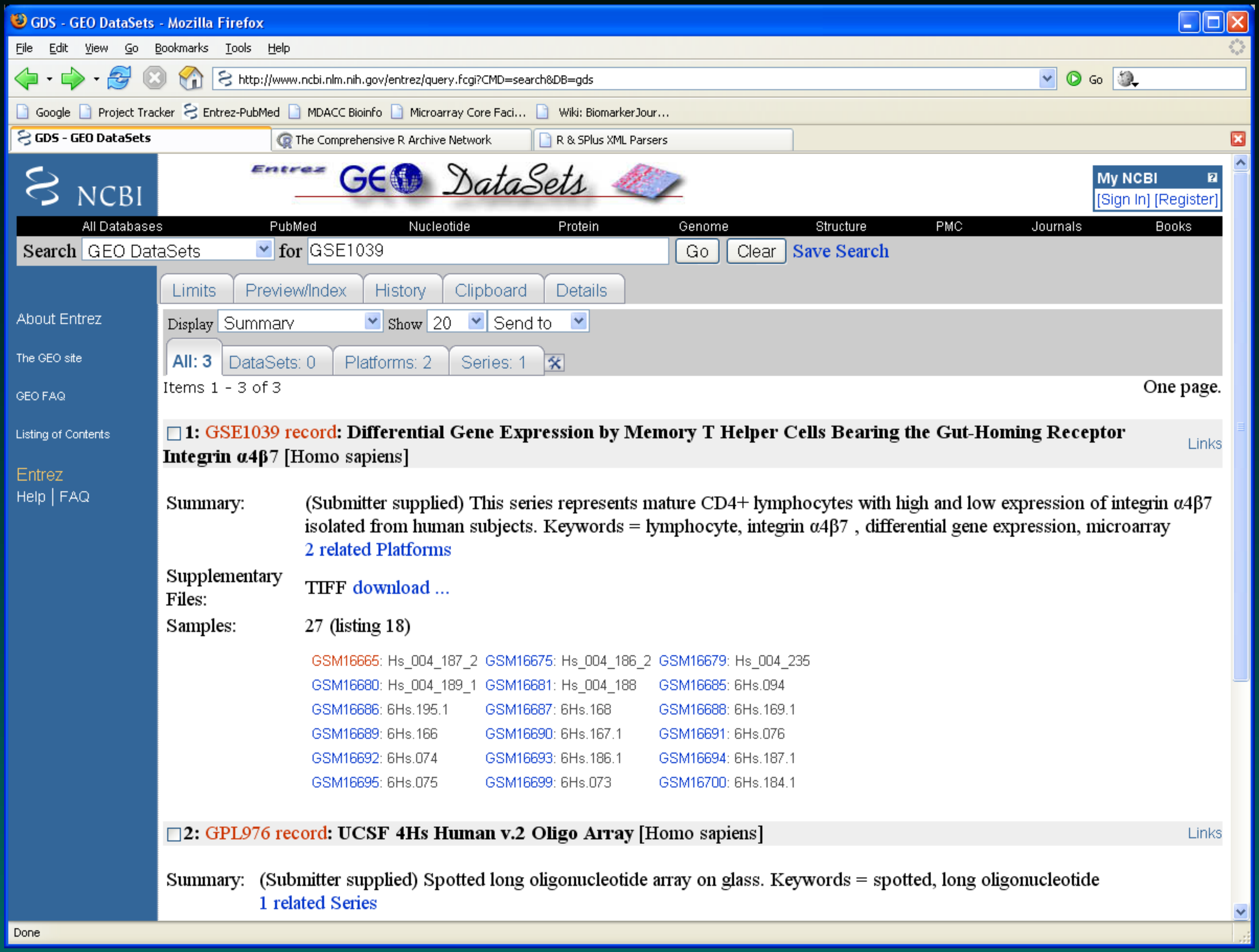
Together with the paper, and the data posted to GEO (the layouts of the arrays used, the gpr files, and more information about what the genes are), there was also a supplementary information file giving a MIAME-compliant list of information.

This list was important, as it specified which samples were labeled with Cy5, and which with Cy3. What is recorded in GEO is simply "Channel 1" and "Channel 2".

Adventures with the Gene Expression Omnibus

I went back to GEO to find the full data set, to see how easy (ha!) it would be to get the whole thing into R. Maybe by Thursday I will have figured this all out. In the mean time, you get to share my confusion/frustration....

Searching for the Data Set


GDS - GEO DataSets - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=gds
Go
Google Project Tracker Entrez-PubMed MDACC Bioinfo Microarray Core Faci... Wiki: BiomarkerJour...
GDS - GEO DataSets
The Comprehensive R Archive Network
R & SPlus XML Parsers
NCBI
Entrez GEO DataSets
My NCBI
[Sign In] [Register]
All Databases PubMed Nucleotide Protein Genome Structure PMC Journals Books
Search GEO DataSets for GSE1039 Go Clear Save Search
Limits Preview/Index History Clipboard Details
Display Summary Show 20 Send to
All: 3 DataSets: 0 Platforms: 2 Series: 1
About Entrez
The GEO site
GEO FAQ
Listing of Contents
Entrez
Help | FAQ
Links
Done

Items 1 - 3 of 3 One page.

□1: GSE1039 record: **Differential Gene Expression by Memory T Helper Cells Bearing the Gut-Homing Receptor Integrin α4β7** [Homo sapiens]

Summary: (Submitter supplied) This series represents mature CD4+ lymphocytes with high and low expression of integrin α4β7 isolated from human subjects. Keywords = lymphocyte, integrin α4β7 , differential gene expression, microarray
2 related Platforms

Supplementary Files: TIFF download ...

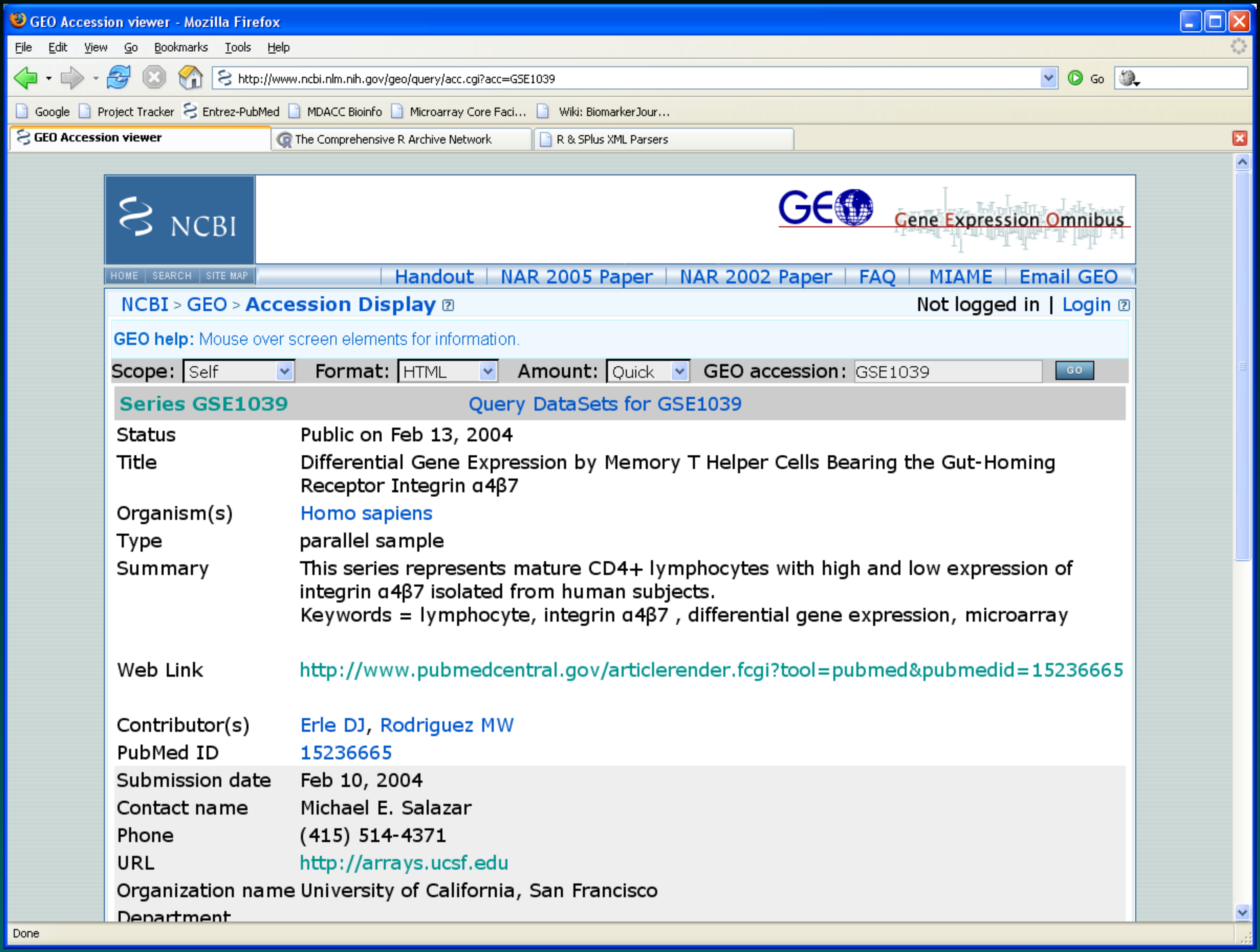
Samples: 27 (listing 18)

GSM16665: Hs_004_187_2	GSM16675: Hs_004_186_2	GSM16679: Hs_004_235
GSM16680: Hs_004_189_1	GSM16681: Hs_004_188	GSM16685: 6Hs.094
GSM16686: 6Hs.195.1	GSM16687: 6Hs.168	GSM16688: 6Hs.169.1
GSM16689: 6Hs.166	GSM16690: 6Hs.167.1	GSM16691: 6Hs.076
GSM16692: 6Hs.074	GSM16693: 6Hs.186.1	GSM16694: 6Hs.187.1
GSM16695: 6Hs.075	GSM16699: 6Hs.073	GSM16700: 6Hs.184.1

□2: GPL976 record: **UCSF 4Hs Human v.2 Oligo Array** [Homo sapiens]

Summary: (Submitter supplied) Spotted long oligonucleotide array on glass. Keywords = spotted, long oligonucleotide
1 related Series

The Source Page for GSE1039


GEO Accession viewer - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE1039
Go
Google
Project Tracker
Entrez-PubMed
MDACC Bioinfo
Microarray Core Faci...
Wiki: BiomarkerJour...
GEO Accession viewer
The Comprehensive R Archive Network
R & SPlus XML Parsers
NCBI
GEO Gene Expression Omnibus
HOME SEARCH SITE MAP
Handout | NAR 2005 Paper | NAR 2002 Paper | FAQ | MIAME | Email GEO
NCBI > GEO > Accession Display
Not logged in | Login
GO
Done

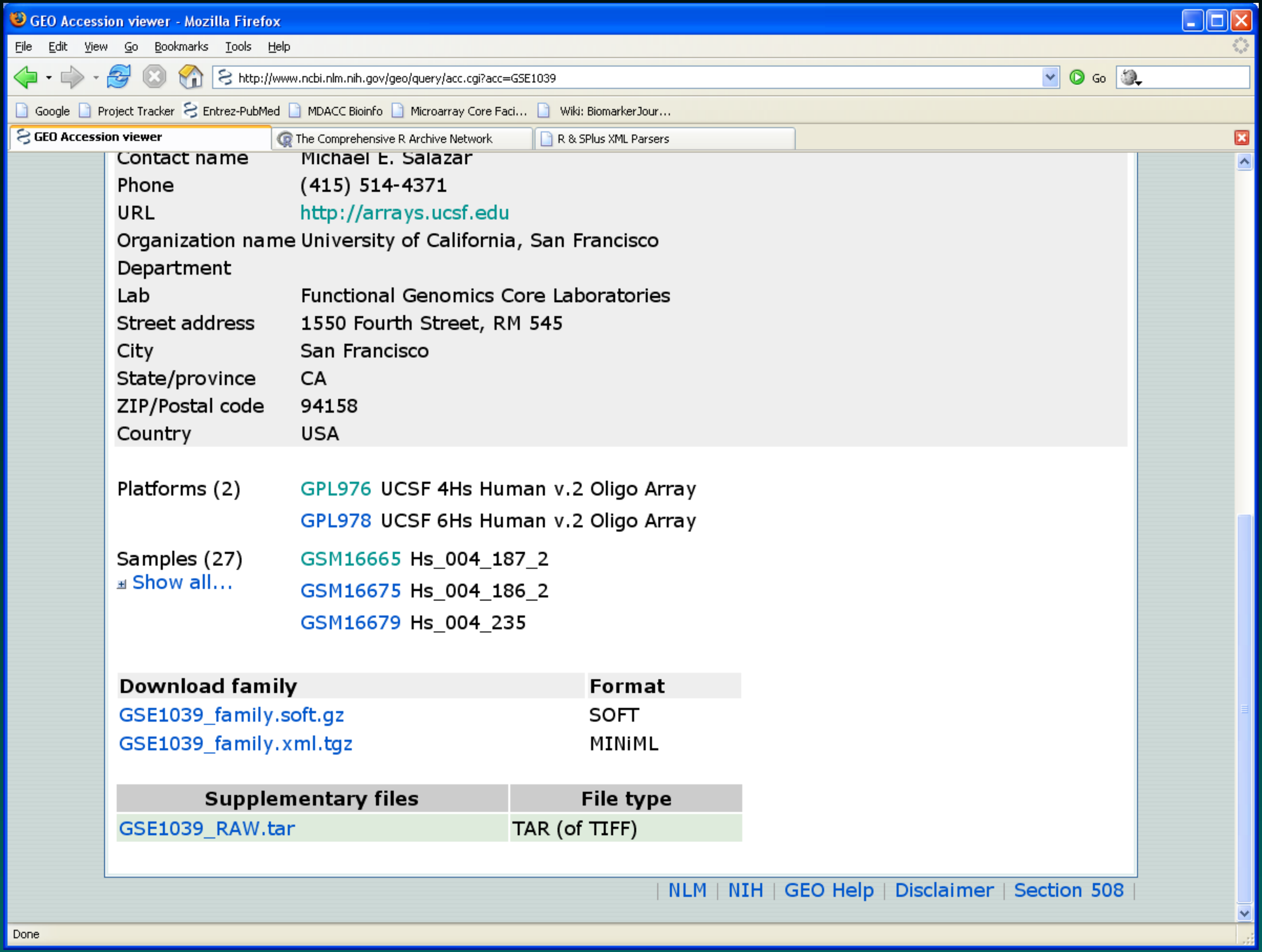
GEO help: Mouse over screen elements for information.

Scope: Self Format: HTML Amount: Quick GEO accession: GSE1039

Series GSE1039 Query DataSets for GSE1039

Status	Public on Feb 13, 2004
Title	Differential Gene Expression by Memory T Helper Cells Bearing the Gut-Homing Receptor Integrin α4β7
Organism(s)	Homo sapiens
Type	parallel sample
Summary	This series represents mature CD4+ lymphocytes with high and low expression of integrin α4β7 isolated from human subjects. Keywords = lymphocyte, integrin α4β7 , differential gene expression, microarray
Web Link	http://www.pubmedcentral.gov/articlerender.fcgi?tool=pubmed&pubmedid=15236665
Contributor(s)	Erle DJ, Rodriguez MW
PubMed ID	15236665
Submission date	Feb 10, 2004
Contact name	Michael E. Salazar
Phone	(415) 514-4371
URL	http://arrays.ucsf.edu
Organization name	University of California, San Francisco
Department	

Scroll Down to FInd the Link



GEO Accession viewer - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE1039
Go
Google
Project Tracker
Entrez-PubMed
MDACC Bioinfo
Microarray Core Faci...
Wiki: BiomarkerJour...
GEO Accession viewer
The Comprehensive R Archive Network
R & SPlus XML Parsers

Contact name	Michael E. Salazar
Phone	(415) 514-4371
URL	http://arrays.ucsf.edu
Organization name	University of California, San Francisco
Department	
Lab	Functional Genomics Core Laboratories
Street address	1550 Fourth Street, RM 545
City	San Francisco
State/province	CA
ZIP/Postal code	94158
Country	USA
Platforms (2)	GPL976 UCSF 4Hs Human v.2 Oligo Array GPL978 UCSF 6Hs Human v.2 Oligo Array
Samples (27) ⊞ Show all...	GSM16665 Hs_004_187_2 GSM16675 Hs_004_186_2 GSM16679 Hs_004_235

Download family	Format
GSE1039_family.soft.gz	SOFT
GSE1039_family.xml.tgz	MINiML

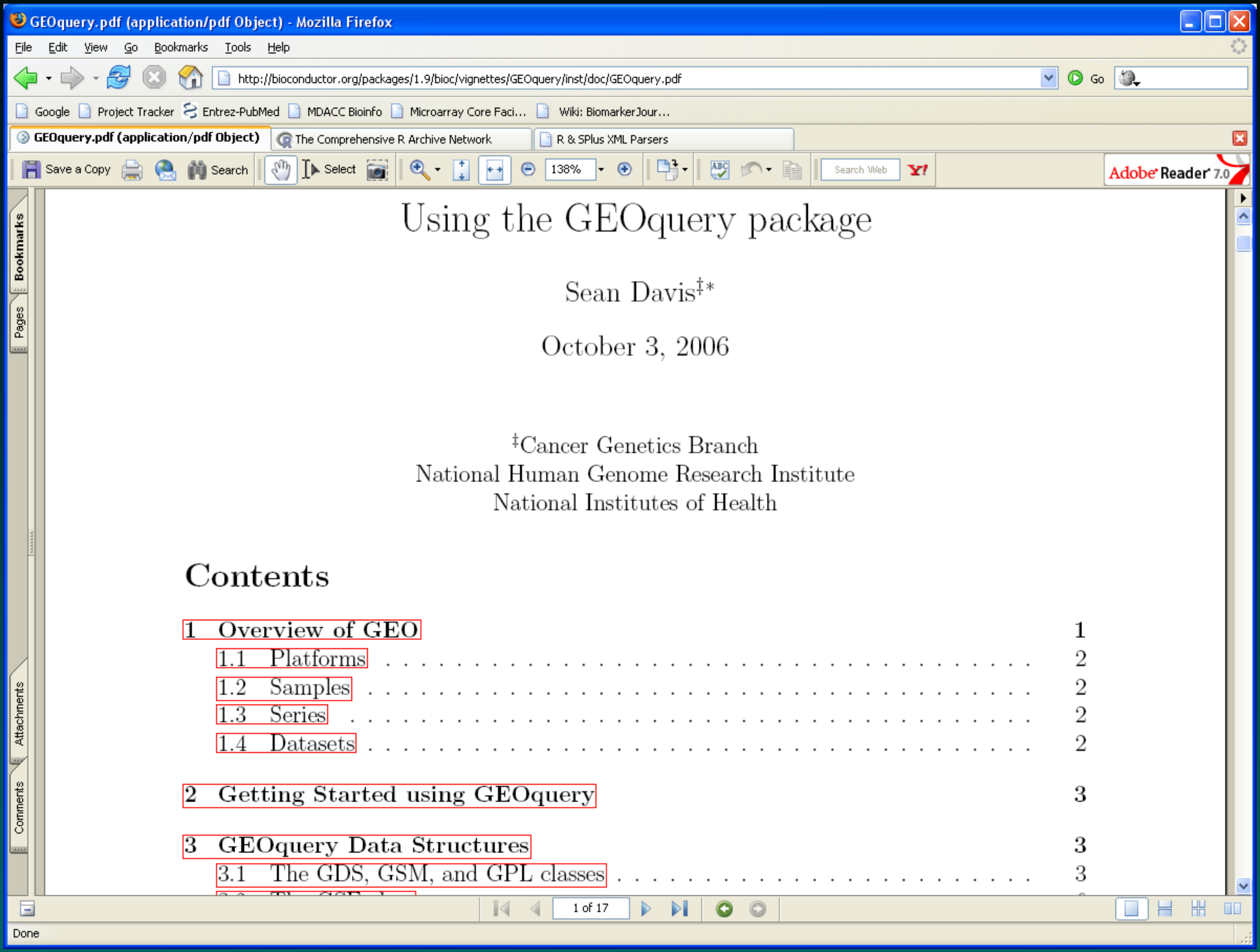
Supplementary files	File type
GSE1039_RAW.tar	TAR (of TIFF)

File Formats

The data set is available in two different file formats:

1. SOFT format
   - One big file that clunps everything together, with special characters separating the pieces
   - A google search for “BioConductor SOFT” uncovers the `GEOquery` package to handle SOFT files
2. MINiML format
   - A “tarball” of files, with the documentation in one XML file and everfything else in tab-separated-values format with no headers
   - Another google search turns up the `XML` package for R

GEOquery


GEOquery.pdf (application/pdf Object) - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://bioconductor.org/packages/1.9/bioc/vignettes/GEOquery/inst/doc/GEOquery.pdf
Go
Google
Project Tracker
Entrez-PubMed
MDACC Bioinfo
Microarray Core Faci...
Wiki: BiomarkerJour...
GEOquery.pdf (application/pdf Object)
The Comprehensive R Archive Network
R & SPlus XML Parsers
Save a Copy
Search
Select
138%
Search Web
Adobe Reader 7.0
Bookmarks
Pages
Attachments
Comments
1 of 17
Done

Using the GEOquery package

Sean Davis[‡][*]

October 3, 2006

[‡]Cancer Genetics Branch
National Human Genome Research Institute
National Institutes of Health

Contents

GEOquery

So, I installed the `GEOquery` package, and then loaded it.

```
> require(GEOquery)
```

```
[1] TRUE
```

If you just pass the “GSE1039” identifier into the `getGEO` function, it will download the file from the NCBI and start processing it. Since I had already downloaded it myself, I used the `filename` parameter to the function.

```
> gse1039 <- getGEO(filename = "GSE1039_family.soft")
```

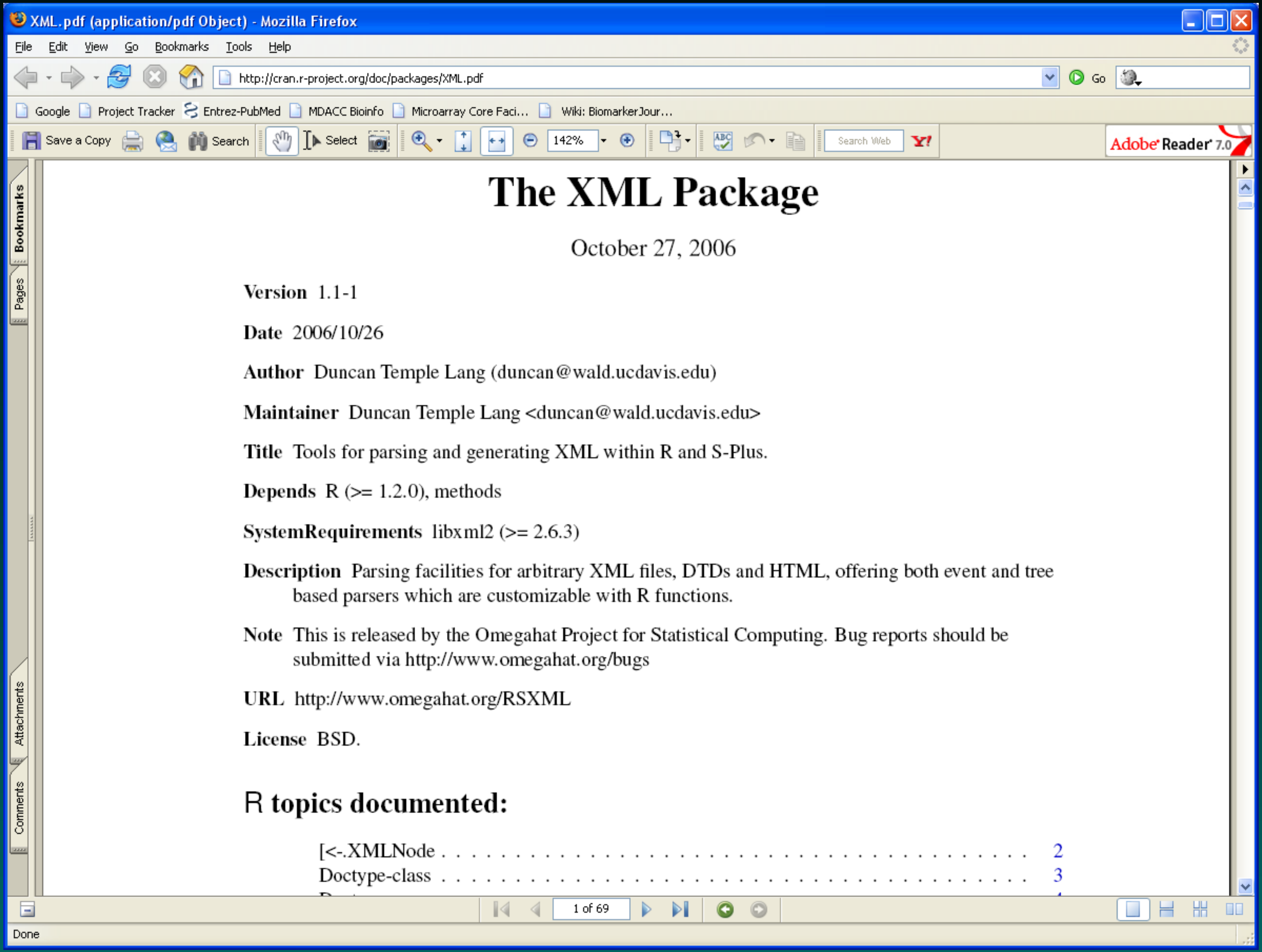
Then I waited. And waited. After more than an hour-and-half, I finally saw some results:

```
Parsing....
^PLATFORM = GPL976
^SAMPLE = GSM16675
^SAMPLE = GSM16679
^SAMPLE = GSM16680
^SAMPLE = GSM16681
^SAMPLE = GSM16685
^SAMPLE = GSM16686
^SAMPLE = GSM16687
^SAMPLE = GSM16688
^SAMPLE = GSM16689
^SAMPLE = GSM16690
^SAMPLE = GSM16691
^SAMPLE = GSM16692
^SAMPLE = GSM16693
^SAMPLE = GSM16694
```

```
^SAMPLE = GSM16695
^SAMPLE = GSM16699
^SAMPLE = GSM16700
^SAMPLE = GSM16704
^SAMPLE = GSM16705
^SAMPLE = GSM16706
^SAMPLE = GSM16719
^SAMPLE = GSM16720
^SAMPLE = GSM16724
^SAMPLE = GSM16725
^SAMPLE = GSM16726
^SAMPLE = GSM16727
```

While this was going on, I decided to check out the XML approach ...

XML



The XML Package

October 27, 2006

Version 1.1-1

Date 2006/10/26

Author Duncan Temple Lang (duncan@wald.ucdavis.edu)

Maintainer Duncan Temple Lang <duncan@wald.ucdavis.edu>

Title Tools for parsing and generating XML within R and S-Plus.

Depends R (>= 1.2.0), methods

SystemRequirements libxml2 (>= 2.6.3)

Description Parsing facilities for arbitrary XML files, DTDs and HTML, offering both event and tree based parsers which are customizable with R functions.

Note This is released by the Omegahat Project for Statistical Computing. Bug reports should be submitted via http://www.omegahat.org/bugs

URL http://www.omegahat.org/RSXML

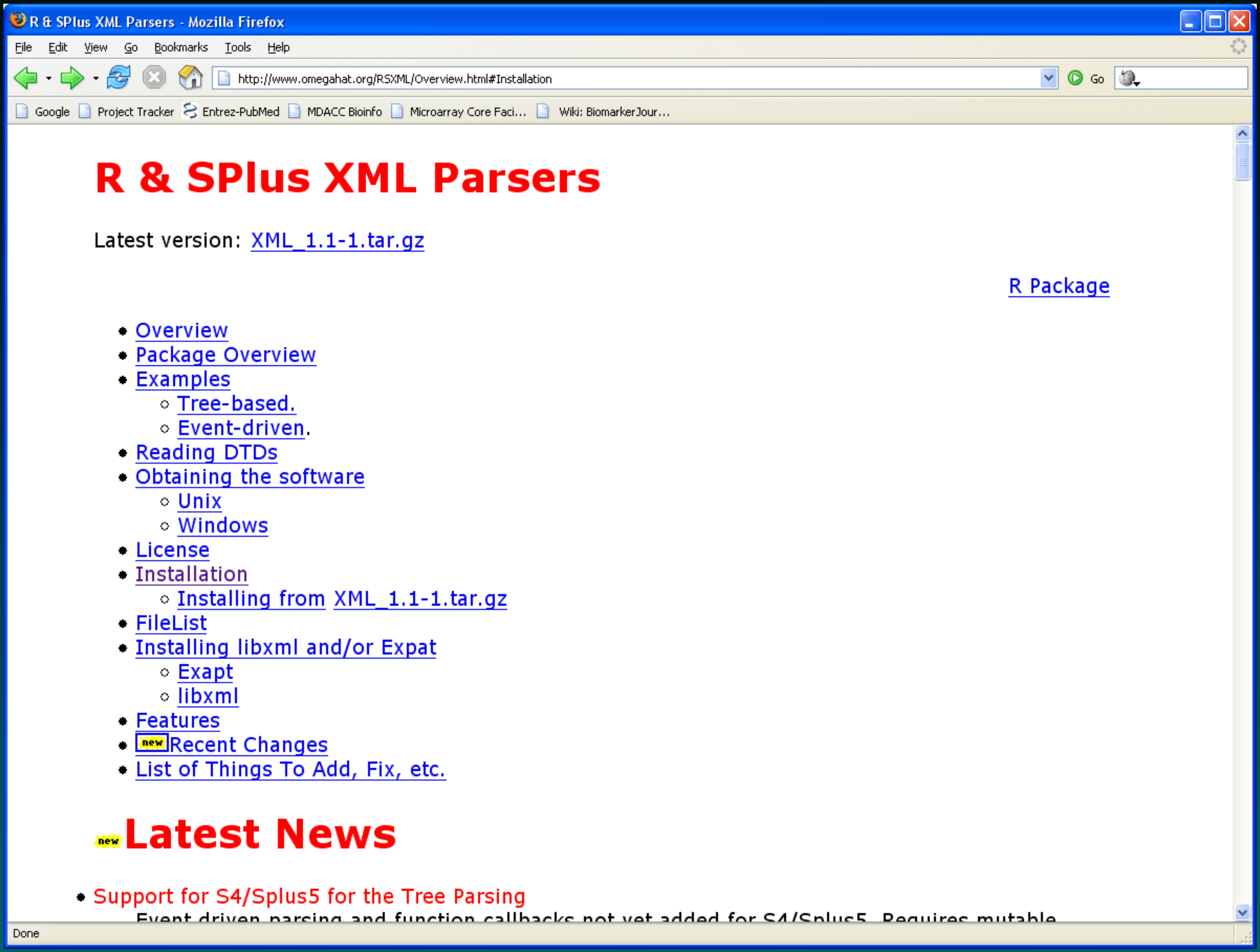
License BSD.

R topics documented:

Adventures with XML, Part 1

After installing the XML package from CRAN, I foolishly went ahead and tried to use it. Of course, this naive attempt failed. Unlike most packages, this one is decidely not self-contained. It required a separate set of files to parse XML files, which I learned by going back to the original web site form the developer of the package.

R XML Home Page


R & SPlus XML Parsers - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://www.omegahat.org/RSXML/Overview.html#Installation
Go
Google
Project Tracker
Entrez-PubMed
MDACC Bioinfo
Microarray Core Faci...
Wiki: BiomarkerJour...
Done

R & SPlus XML Parsers

Latest version: XML_1.1-1.tar.gz

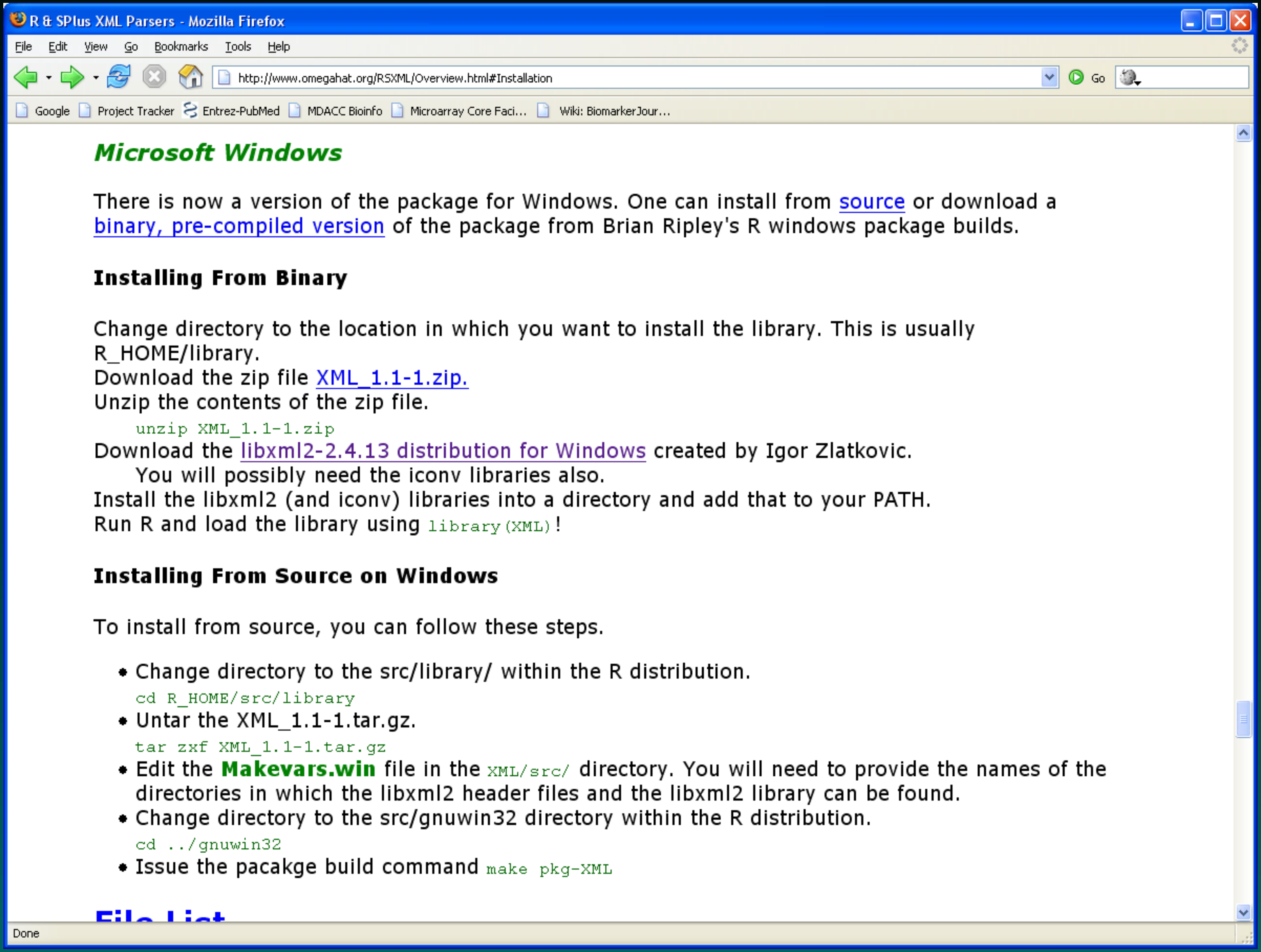
R Package

- Overview
- Package Overview
- Examples
  - Tree-based.
  - Event-driven.
- Reading DTDs
- Obtaining the software
  - Unix
  - Windows
- License
- Installation
  - Installing from XML_1.1-1.tar.gz
- FileList
- Installing libxml and/or Expat
  - Exapt
  - libxml
- Features
- new Recent Changes
- List of Things To Add, Fix, etc.

new Latest News

- Support for S4/Splus5 for the Tree Parsing
  Event driven parsing and function callbacks not yet added for S4/Splus5. Requires mutable

The XML Installation Instructions


R & SPlus XML Parsers - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://www.omegahat.org/RSXML/Overview.html#Installation
Go
Google Project Tracker Entrez-PubMed MDACC Bioinfo Microarray Core Faci... Wiki: BiomarkerJour...
Done

Microsoft Windows

There is now a version of the package for Windows. One can install from source or download a binary, pre-compiled version of the package from Brian Ripley's R windows package builds.

Installing From Binary

Change directory to the location in which you want to install the library. This is usually R_HOME/library.
Download the zip file XML_1.1-1.zip.
Unzip the contents of the zip file.

```
unzip XML_1.1-1.zip
```

Download the libxml2-2.4.13 distribution for Windows created by Igor Zlatkovic.
You will possibly need the iconv libraries also.
Install the libxml2 (and iconv) libraries into a directory and add that to your PATH.
Run R and load the library using `library(XML)`!

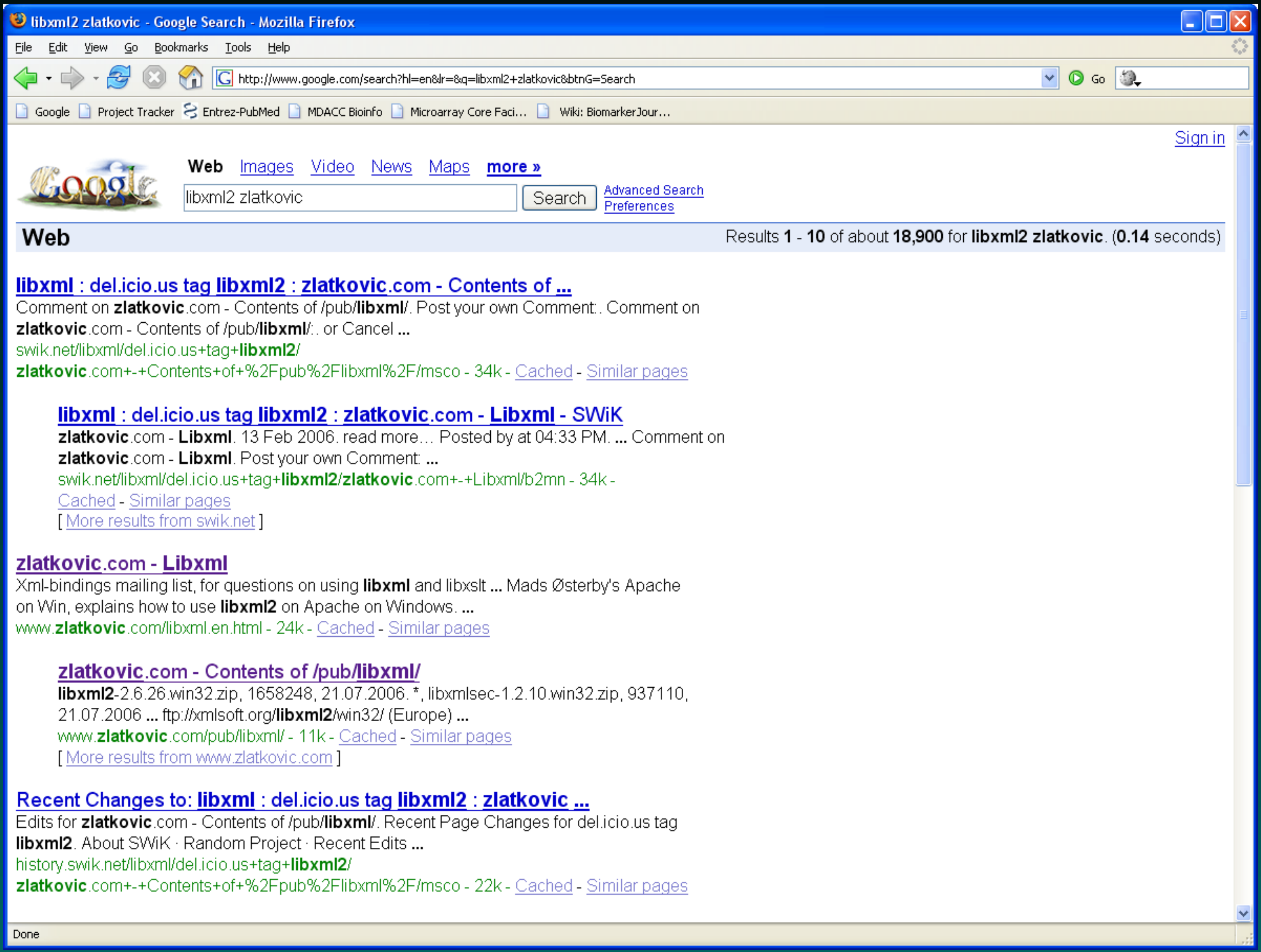
Installing From Source on Windows

To install from source, you can follow these steps.

- Change directory to the src/library/ within the R distribution.
  ```
  cd R_HOME/src/library
  ```
- Untar the XML_1.1-1.tar.gz.
  ```
  tar zxf XML_1.1-1.tar.gz
  ```
- Edit the **Makevars.win** file in the `XML/src/` directory. You will need to provide the names of the directories in which the libxml2 header files and the libxml2 library can be found.
- Change directory to the src/gnuwin32 directory within the R distribution.
  ```
  cd ../gnuwin32
  ```
- Issue the pacakge build command `make pkg-XML`

File List

The Link to libxml2 Was Broken...


libxml2 zlatkovic - Google Search - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://www.google.com/search?hl=en&lr=&q=libxml2+zlatkovic&btnG=Search
Go
Google Project Tracker Entrez-PubMed MDACC Bioinfo Microarray Core Faci... Wiki: BiomarkerJour...
Sign in
Web Images Video News Maps more »
libxml2 zlatkovic
Search
Advanced Search
Preferences
Done

Web Results **1** - **10** of about **18,900** for **libxml2 zlatkovic**. (**0.14** seconds)

libxml : del.icio.us tag libxml2 : zlatkovic.com - Contents of ...
Comment on **zlatkovic**.com - Contents of /pub/**libxml**/. Post your own Comment:. Comment on **zlatkovic**.com - Contents of /pub/**libxml**/:. or Cancel **...**
swik.net/libxml/del.icio.us+tag+**libxml2**/
zlatkovic.com+-+Contents+of+%2Fpub%2Flibxml%2F/msco - 34k - Cached - Similar pages

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zlatkovic.com - **Libxml**. 13 Feb 2006. read more… Posted by at 04:33 PM. **...** Comment on **zlatkovic**.com - **Libxml**. Post your own Comment: **...**
swik.net/libxml/del.icio.us+tag+**libxml2**/**zlatkovic**.com+-+Libxml/b2mn - 34k - Cached - Similar pages
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zlatkovic.com - Libxml
Xml-bindings mailing list, for questions on using **libxml** and libxslt **...** Mads Østerby's Apache on Win, explains how to use **libxml2** on Apache on Windows. **...**
www.**zlatkovic**.com/libxml.en.html - 24k - Cached - Similar pages

zlatkovic.com - Contents of /pub/libxml/
libxml2-2.6.26.win32.zip, 1658248, 21.07.2006. *, libxmlsec-1.2.10.win32.zip, 937110, 21.07.2006 **...** ftp://xmlsoft.org/**libxml2**/win32/ (Europe) **...**
www.**zlatkovic**.com/pub/libxml/ - 11k - Cached - Similar pages
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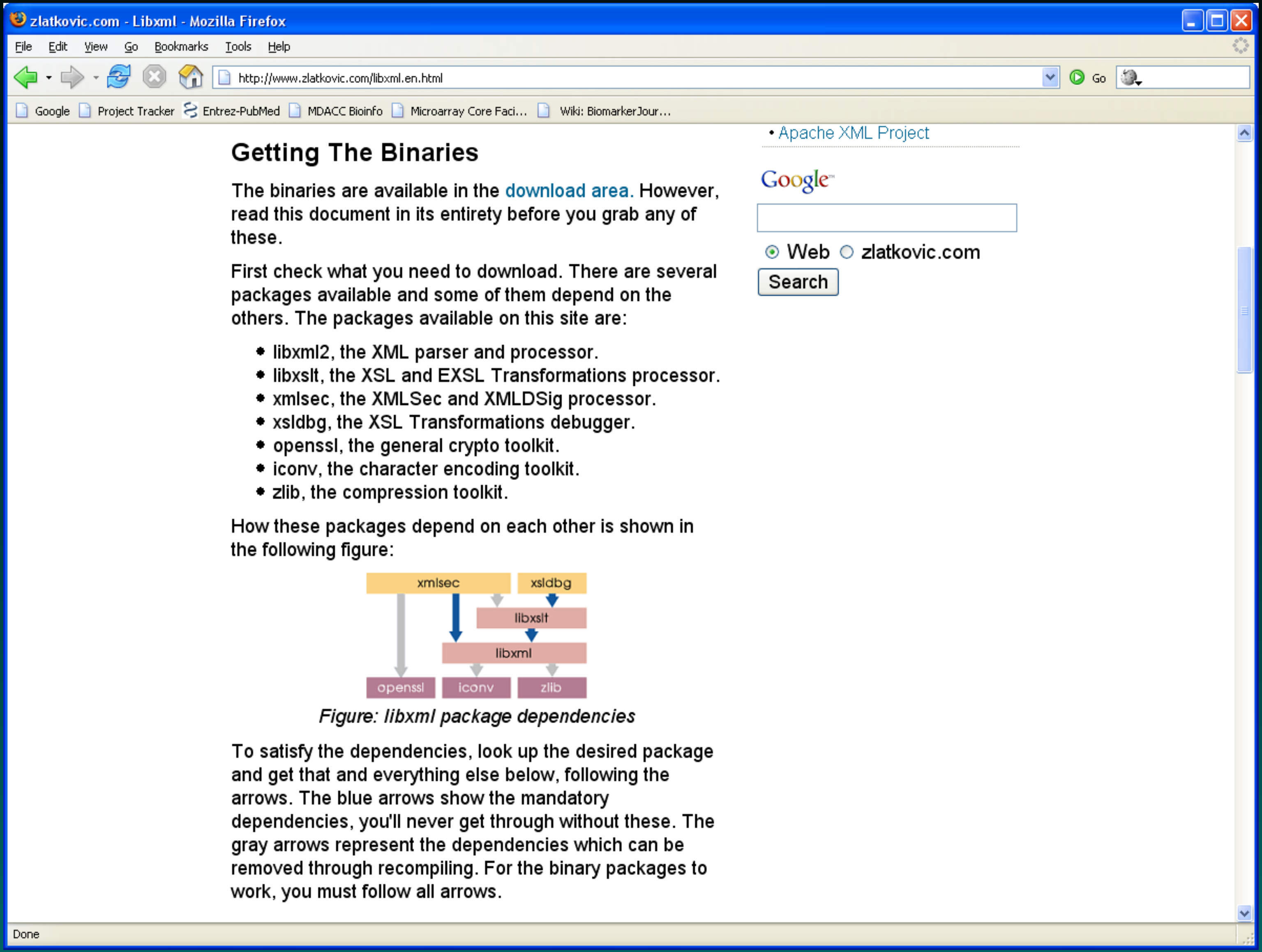
Edits for **zlatkovic**.com - Contents of /pub/**libxml**/. Recent Page Changes for del.icio.us tag **libxml2**. About SWiK · Random Project · Recent Edits **...**
history.swik.net/libxml/del.icio.us+tag+**libxml2**/
zlatkovic.com+-+Contents+of+%2Fpub%2Flibxml%2F/msco - 22k - Cached - Similar pages

This Time, We Read the Instructions First...



zlatkovic.com - Libxml - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://www.zlatkovic.com/libxml.en.html
Go
Google Project Tracker Entrez-PubMed MDACC Bioinfo Microarray Core Faci... Wiki: BiomarkerJour...
• Apache XML Project
Google
◉ Web ○ zlatkovic.com
Search
Done

Getting The Binaries

The binaries are available in the download area. However, read this document in its entirety before you grab any of these.

First check what you need to download. There are several packages available and some of them depend on the others. The packages available on this site are:

- libxml2, the XML parser and processor.
- libxslt, the XSL and EXSL Transformations processor.
- xmlsec, the XMLSec and XMLDSig processor.
- xsldbg, the XSL Transformations debugger.
- openssl, the general crypto toolkit.
- iconv, the character encoding toolkit.
- zlib, the compression toolkit.

How these packages depend on each other is shown in the following figure:

Figure: libxml package dependencies

To satisfy the dependencies, look up the desired package and get that and everything else below, following the arrows. The blue arrows show the mandatory dependencies, you'll never get through without these. The gray arrows represent the dependencies which can be removed through recompiling. For the binary packages to work, you must follow all arrows.

Adventures with XML, Part 2

```
> require(XML)

[1] TRUE

> mytree <- xmlTreeParse("GSE1039/GSE1039_family.xml")

GSE1039/GSE1039_family.xml:242: parser error : Input is not
 proper  UTF-8, indicate encoding !
Bytes: 0xB5 0x6D 0x20 0x6F
        <Description>the X-coordinate in ţm of the center of
                                         ^
```

UTF-8 Encoding Ain't What it Used To Be

After more trials and tribulations, we learned that the XML file lied when it claimed to be "UTF-8" encoded. It included some characters for the Greek letter "mu" and some superscripts (as in R^2) that were not encoded propoerly, breaking the XML parser. So, I fired up my trusty copy of emacs and did a global "search-and-replace" to remove the offending characters. Then we get

```
> mytree <- xmlTreeParse("GSE1039/GSE1039_family2.xml")
```

with no error messages. But I know have an object that represents a parse tree, and not enough time or energy to figure out how to use it....