

Introduction to *R* and *Bioconductor*

Martin Morgan (martin.morgan@roswellpark.org)
Roswell Park Cancer Institute
Buffalo, NY, USA

11 July, 2016

R: Statistical Computing Environment

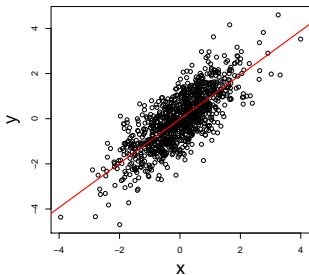
```
> 1 + 2                                # calculator
[1] 3

> x <- rnorm(1000)                       # vectors, statistical
> y <- x + rnorm(1000, sd=.8)            # vectorized calculation
> df <- data.frame(x=x, y=y)            # object construction
> fit <- lm(y ~ x, df)                   # linear model, formula
> class(fit)                             # discovery
[1] "lm"

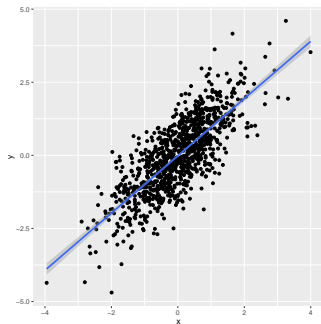
> head(methods(class="lm"), 3)
[1] "add1.lm"  "alias.lm" "anova.lm"
```

R: Statistical Computing Environment

```
> plot(y ~ x, df, cex.lab=2)  
> abline(fit, col="red",  
+       lwd=2)
```



```
> library(ggplot2)  
> ggplot(df, aes(x, y)) +  
+   geom_point() +  
+   stat_smooth(method="lm")
```



R: Statistical Computing Environment

- ▶ Vectors – logical, integer, numeric, character, ...
 - ▶ `list()` – contains other vectors (recursive)
 - ▶ `factor()`, `NA` – statistical concepts
 - ▶ Can be *named* – `c(Portugal=1, France=0)`
- ▶ `matrix()`, `array()` – a vector with a 'dim' attribute.
- ▶ `data.frame()` – like spreadsheets; list of equal length vectors.
 - ▶ Homogenous types within a column, heterogenous types across columns.
 - ▶ An example of an *R class*.
- ▶ Other classes – more complicated arrangement of vectors.
 - ▶ Examples: the value returned by `lm()`; the *DNAStrngSet* class used to hold DNA sequences.
 - ▶ function, 'generic', and 'method'
- ▶ Packages – base, recommended, contributed.

R: programming concepts

- ▶ Functions – built-in (e.g., `rnorm()`); user-defined
- ▶ Subsetting – logical, numeric, character; `df[df$x > 0,]`
- ▶ Iteration – over vector elements, `lapply()`, `mapply()`, `apply()`, ...; e.g., `lapply(df, mean)`

R: help!

- ▶ `?data.frame, ?"plot<tab>"`
- ▶ `methods(class=class(fit)), methods(anova)`
- ▶ `help(package="Biostrings")`
- ▶ `vignette(package="GenomicRanges")`
- ▶ [StackOverflow](#); R-help mailing list

“Hey, can you help me with this? I tried...”

Bioconductor

Analysis & comprehension of high-throughput genomic data

- ▶ 15 years old; 1211 packages; widely used
- ▶ Sequencing (RNAseq, ChIPseq, variants, copy number, ...), microarrays, flow cytometry, proteomics, ...
- ▶ <http://bioconductor.org>,
<https://support.bioconductor.org>

Themes

- ▶ Interoperable – classes to work with genome-scale data, shared (where possible!) across packages
- ▶ Usable – package vignettes, man pages, examples, ...
- ▶ Reproducible – ‘release’ and ‘devel’ versions, updated every 6 months

Bioconductor: GenomicRanges

```
> gr = exons(TxDb.Hsapiens.UCSC.hg19.knownGene); gr
```

```
GRanges with 289969 ranges and 1 metadata column:
```

	seqnames	ranges	strand	exon_id
	<Rle>	<IRanges>	<Rle>	<integer>
[1]	chr1	[11874, 12227]	+	1
[2]	chr1	[12595, 12721]	+	2
[3]	chr1	[12613, 12721]	+	3
...
[289967]	chrY	[59358329, 59359508]	-	277748
[289968]	chrY	[59360007, 59360115]	-	277749
[289969]	chrY	[59360501, 59360854]	-	277750

```
---  
seqinfo: 93 sequences (1 circular) from hg19 genome
```

GRanges

```
length(gr); gr[1:5]  
seqnames(gr)  
start(gr)  
end(gr)  
width(gr)  
strand(gr)
```

DataFrame

```
mcols(gr)  
gr$exon_id
```

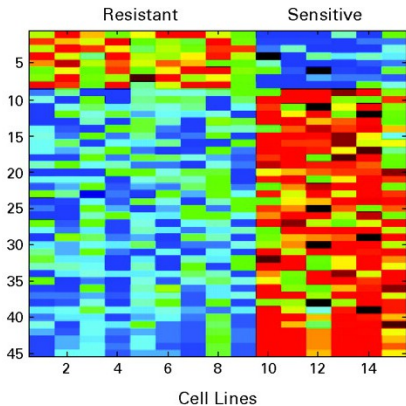
Seqinfo

```
seqlevels(gr)  
seqlengths(gr)  
genome(gr)
```

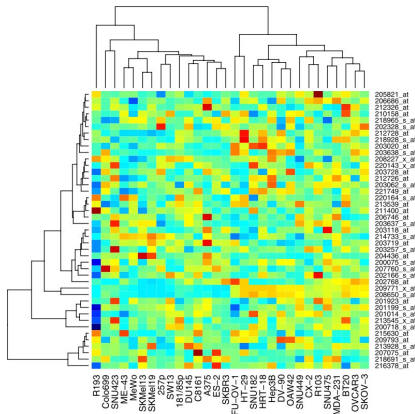
- ▶ Data: aligned reads, called peaks, SNP locations, CNVs, ...
- ▶ Annotation: gene models, variants, regulatory regions, ...
- ▶ `findOverlaps()`, `nearest()`, and many other useful range-based operations.

Bioconductor: SummarizedExperiment motivation

Cisplatin-resistant non-small-cell lung cancer gene sets



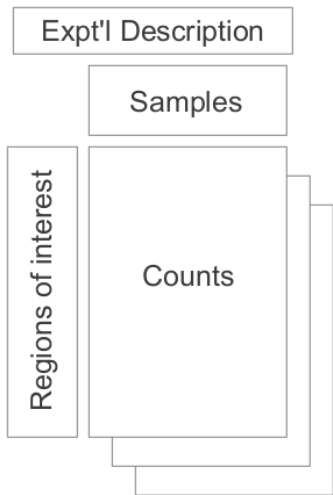
Hsu *et al.* 2007 J Clin Oncol 25:
4350-4357 (retracted)



Baggerly & Coombes 2009 Ann
Appl Stat 3: 1309-1334

Coordinated, programmatic manipulation of feature, sample, and assay data

Bioconductor: SummarizedExperiment



Regions of interest \times samples

- ▶ `assay()` – matrix, e.g., counts of reads overlapping regions of interest.
- ▶ `rowData()` – regions of interest as GRanges or GRangesList
- ▶ `colData()` – DataFrame describing samples.

```
> se[, se$Treatment == "Control"] # Control samples only
```

Bioconductor: a fun demo of *GRanges* interoperability

GenomicFeatures And 'annotation' packages to represent gene models as *GRanges*.

GenomicAlignments To input aligned reads as *GRanges*.

Gviz For visualization.

shiny For interactivity.

Bioconductor: Resources

<http://bioconductor.org>

- ▶ Packages – biocViews, landing pages (e.g., *AnnotationHub*)
- ▶ Course & conference material; work flows; publications
- ▶ Developer resources

<https://support.bioconductor.org>

- ▶ Question & answer forum for users; usually fast, expert, friendly responses
- ▶ Contributed tutorials, news

Citations

- ▶ Huber et al. (2015) Orchestrating high-throughput genomic analysis with *Bioconductor*. *Nature Methods* 12:115-121.
- ▶ Lawrence et al. (2013) Software for Computing and Annotating Genomic Ranges. *PLoS Comput Biol* 9(8): e1003118.

Acknowledgments

- ▶ Core: Valerie Obenchain, Hervé Pagès, (Dan Tenenbaum), Lori Shepherd, Marcel Ramos, Yubo Cheng.
- ▶ The research reported in this presentation was supported by the National Cancer Institute and the National Human Genome Research Institute of the National Institutes of Health under Award numbers U24CA180996 and U41HG004059. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the National Science Foundation.

<https://bioconductor.org>,

<https://support.bioconductor.org>