# R / Bioconductor for Analysis and Comprehension of High-Throughput Sequence Data

Martin T. Morgan (mtmorgan@fhcrc.org)
Fred Hutchinson Cancer Research Center
Seattle, WA, USA

3 February 2014

# Overview

1. Introduction to *R* and *Bioconductor*
2. Sequencing work flows
3. Successful computational biology software
4. Exemplars: algorithms into actions
5. Challenges & opportunities

# Introduction: *Bioconductor*

Analysis and comprehension of high-throughput genomic data

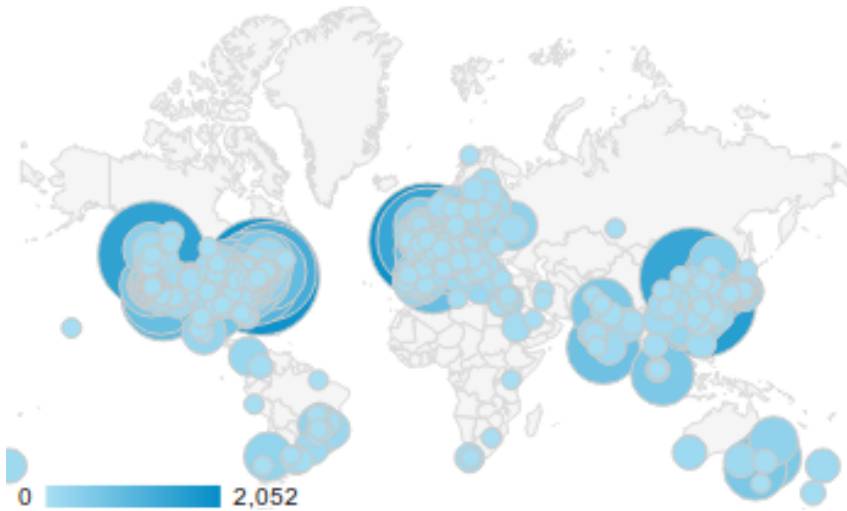
- `http://bioconductor.org`
- $>$ 11 years old, 749 packages

Themes

- Rigorous statistics
- Reproducible work flows
- Integrative analysis

# Introduction: *Bioconductor*

- 1341 PubMed full-text citations in trailing 12 months
- 28,000 web visits / month; 75,000 unique IP downloads / year
- Annual conferences; courses; active mailing list; ...




**Bioconductor Conference**, July 30 - Aug 1, Boston, USA

# Introduction: What is *Bioconductor* good for?

- Microarrays: expression, copy number, SNPs, methylation, ...
- Sequencing: RNA-seq, ChIP-seq, called variants, ...
  - Especially *after* assembly / alignment
- Annotation: genes, pathways, gene models (exons, transcripts, etc.), ...
- Flow cytometry, proteomics, image analysis, high-throughput screens, ...

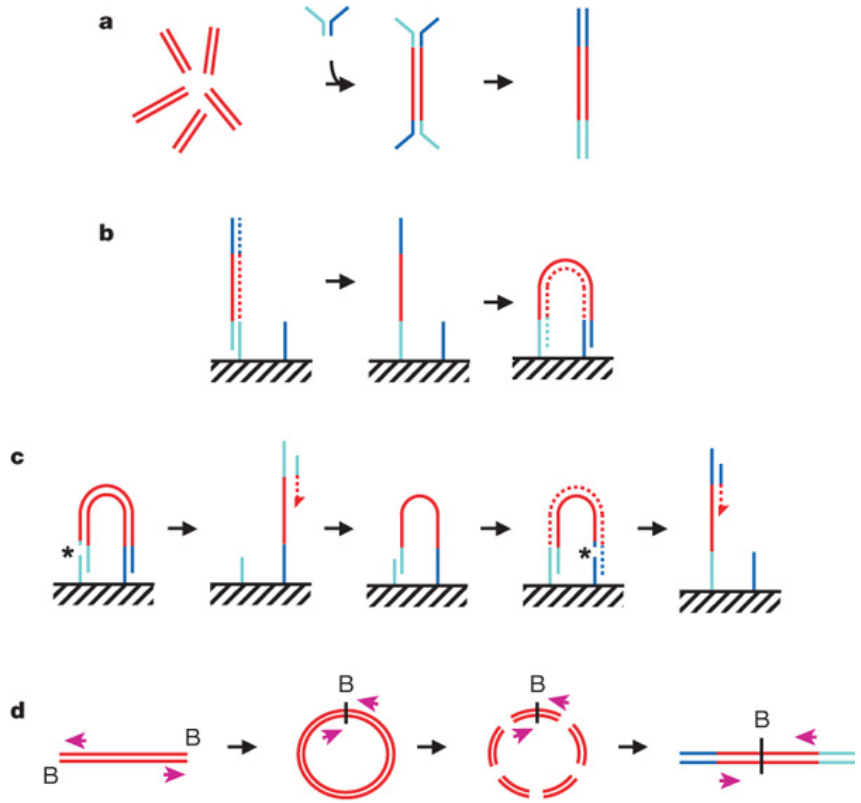
# Introduction: *R*

- http://r-project.org
- Open-source, statistical programming language; widely used in academia, finance, pharma, ...
- Core language and base packages
- Interactive sessions, scripts
- $>$ 5000 contributed packages

```
## Two 'vectors'
x <- rnorm(1000)
y <- x + rnorm(1000, sd=.5)
## Integrated container
df <- data.frame(X=x, Y=y)
## Visualize
plot(Y ~ X, df)
## Regression; 'object'
fit <- lm(Y ~ X, df)
## Methods on the object
abline(fit) # regression line
anova(fit)  # ANOVA table
```

# Sequencing: Work flows

1. Experimental design
2. 'Wet lab' sample prep
3. Sequencing
   - 100's of millions of reads
   - 30-150 nucleotides
   - Single and paired-end
   - Bar codes, lanes & flow cells
4. Alignment
5. Analysis: DNA, RNA, epigenetics, integrative, microbiome, ...




Bentley et al., 2008, Nature 456: 53-9

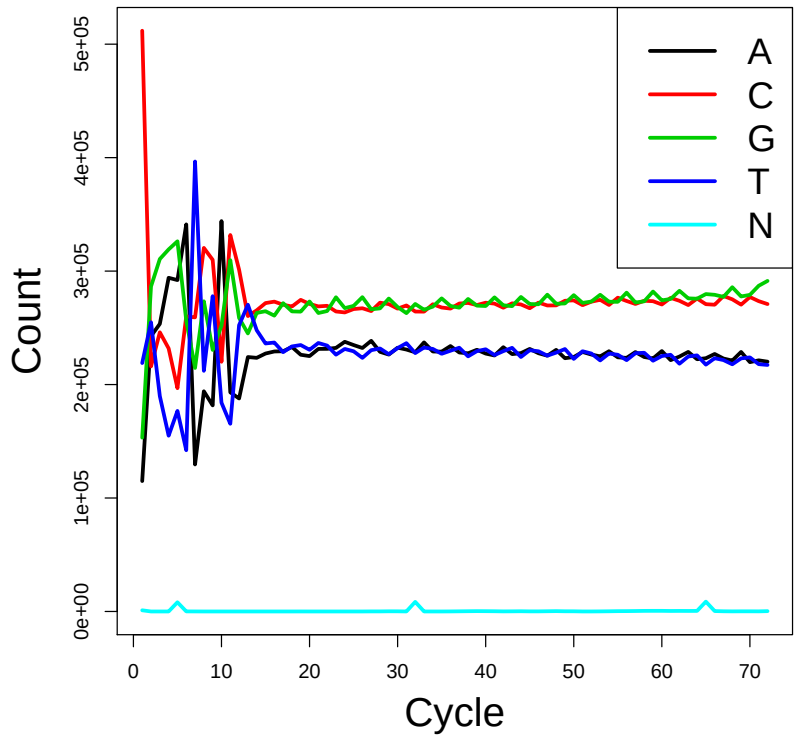
```
@ERR127302.1703 HWI-EAS350_0441:1:1:1460:19184#0/1
CCTGAGTGAAGCTGATCTTGATCTACGAAGAGAGATAGATCTTGATCGTCGAGGAGATGCTGACCTTGACCT
+
HHGHHGHHHHHHHHDGG<GDGGE@GDGGD<?B8??ADAD<BE@EE8EGDGA3CB85*,77@>>CE?=896=:
@ERR127302.1704 HWI-EAS350_0441:1:1:1460:16861#0/1
GCGGTATGCTGGAAGGTGCTCGAATGGAGAGCGCCAGCGCCCCGGCGCTGAGCCGCAGCCTCAGGTCCGCCC
+
DE?DD>ED4>EEE>DE8EEEDE8B?EB<@3;BA79?,881B?@73;1?##########################
@ERR127302.1705 HWI-EAS350_0441:1:1:1460:13054#0/1
AAAACACCCTGCAATCTTTCAGACAGGATGTTGACAATGCGTCTCTGGCACGTCTTGACCTTGAACGCAAAG
+
EEDEE>AD>BBGGB8E8EEEGBGGGGBGGGGG3G>E3*?BE??BBC8GB8??:??GGDGDDD>D>B<GDDC8
@ERR127302.1706 HWI-EAS350_0441:1:1:1460:14924#0/1
CACCCAGTGGGGTGGAGTCGGAGCCACTGGTCCTGCTGCTGGCTGCCTCTCTGCTCCACCTTGTGACCCAGG
+
HHHHHGEEGEEADDGDBG>GGD8EG,<6<?AGGADFEHHC@>D@<@G@>AB@B?8AA>CE@D8@B=?CC>AG
@ERR127302.1707 HWI-EAS350_0441:1:1:1461:6983#0/1
CGACGCTGACACCGGAACGGCAGCAGCAGCAGGACGATTAAGACAAGGAGGATGGCTCCACAGACGCTCATG
+
GEEGEGE@GGGGGGEGGGGGBB>G3?33?8*;;79?<9@?DD8@DDEE888;-BB?.A##############
@ERR127302.1708 HWI-EAS350_0441:1:1:1461:10827#0/1
AAAGAAGGTCCTTGCAATAGACTGCCTCTGCTTGAGAACTTATGATGTAATTATTGCATGCTGCTAATATAC
+
GGGGGDDEBFGGGGGBE,DAGDDGGGEEEG<EEFDECFFEEEDE@<>ACEBEFDEEFE<EDC@E<EECCBEB
@ERR127302.1709 HWI-EAS350_0441:1:1:1461:7837#0/1
CAGCCACAGAACCACGGCACGGAAGACATGAGGCAGCATGCTCACGAGAGAGGTGAGGGTCTCCCCTCCAGG
+
HHGHHHH>DH:@.7@49;88G8>G>DDG@D>D@G@GE>@DDBDDG<A82?#######################
```

# Sequencing: The *ShortRead* package

```
## Use the 'ShortRead' package
library(ShortRead)
## Create an object to represent a sample from a file
sampler <- FastqSampler("ERR127302_1.fastq.gz")
## Apply a method to yield a random sample
fq <- yield(sampler)
## Access sequences of sampled reads using `sread()`
## Summarize nucleotide use by cycle
## 'abc' is a nucleotide x cycle matrix of counts
abc <- alphabetByCycle(sread(fq))
## Subset of interesting nucleotides
abc <- abc[c("A", "C", "G", "T", "N"),]
```

# Sequencing: The *ShortRead* package

```
## Create a plot from a
## matrix
matplot(t(abc), type="l",
  lty=1, lwd=3,
  xlab="Cycle",
  ylab="Count",
  cex.lab=2)
## Add a legend
legend("topright",
  legend=rownames(abc),
  lty=1, lwd=3, col=1:5,
  cex=1.8)
```



# Sequencing: Essential packages and classes

- *Biostrings* and *DNAStringSet*
- *GenomicRanges* and *GRanges*
- *GenomicFeatures* and *TranscriptDb*
- *VariantAnnotation* and *VCF*
- Input and output: *rtracklayer* (WIG, BED, etc.), *Rsamtools* (BAM), *ShortRead* (FASTQ) file input

# Principles: Some key points

- *R* is a high-level programming language, so lots can be accomplished with just a little code
- Packages such as *ShortRead* provide a great way to benefit from the expertise of others (and to contribute your own expertise back to the community!)
  - The path from 'user' to 'developer' is not that long, and has been taken by many!
- Objects and methods such as *data.frame*, *ShortReadQ* and `alphabetByCycle()`) help to manage complicated data
  - Reducing possibility for clerical and other mistakes
  - Facilitating inter-operability between different parts of an analysis
- Scripts make work flows reproducible
- Visualizing data is an important part of exploratory analysis

# Principles: Successful computational biology software

1. Extensive: software, annotation, integration
   - 750 inter-operable *Bioconductor* packages
2. Statistical: volume, technology, experimental design
   - *R* a 'natural' for statistical analysis
3. Reproducible: long-term, multi-participant science
   - Objects, scripts, vignettes, packages, . . . encourage reproducible research
4. Leading edge: novel, technology-driven
   - Packages and user community closely track leading edge science
5. Accessible: affordable, transparent, usable
   - *Bioconductor* is free and open, with extensive documentation and an active and supportive user community

Case study: differential expression of known genes; see also reproducible research lecture.

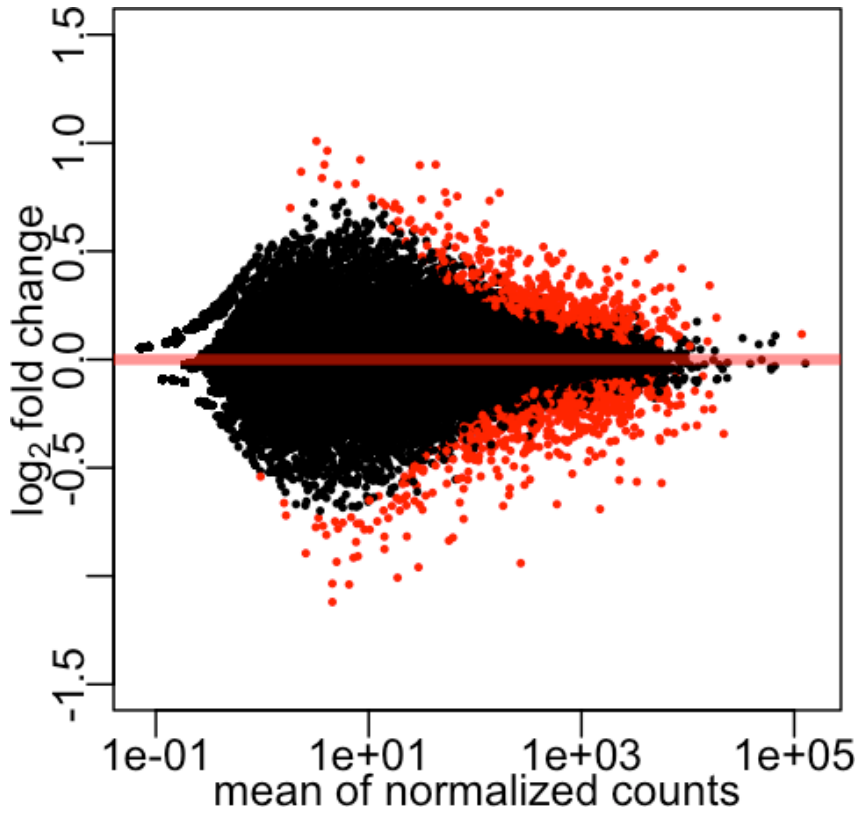
# Exemplars: Algorithms to action

1. Batch effects
2. Methylation
3. RNA-seq Differential Representation
4. Visualization

# Exemplar: Differential Representation

Haglund et al., 2012 J Clin Endocrin Metab

- Scientific finding: identify genes whose expression is regulated by estrogen receptors in parathyroid adenoma cells
- Statistical challenges: between-sample normalization; appropriate statistical model; efficient estimation; . . .



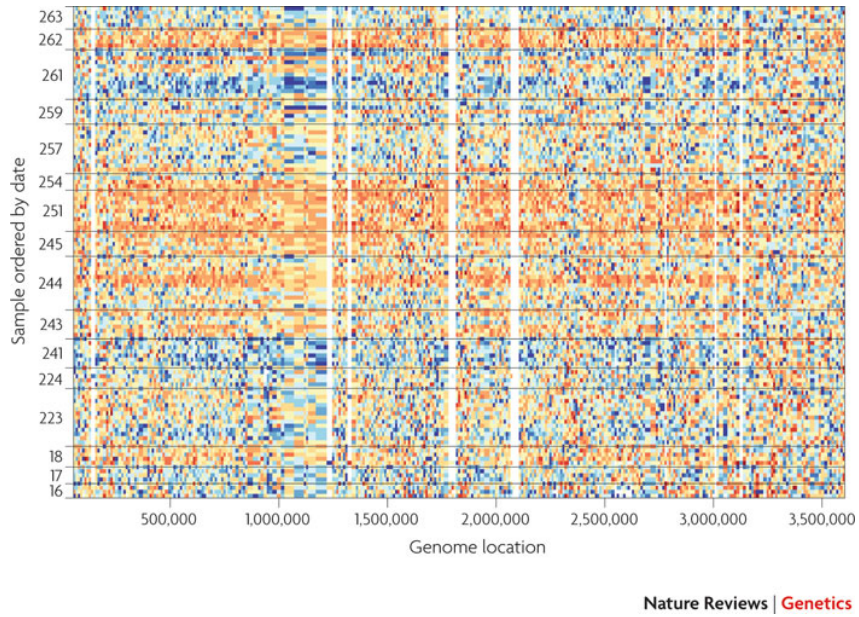

*Bioconductor* support: *DESeq2*, *edgeR*, many statistical 'lessons learned' from microarrays; extensive integration with down-stream tools

# Exemplar: Batch Effects

Leek et al., 2010, Nature Reviews Genetics 11, 733-739, Leek & Story PLoS Genet 3(9): e161

- Scientific finding: pervasive batch effects
- Statistical insights: surrogate variable analysis: identify and build surrogate variables; remove known batch effects
- Benefits: reduce dependence, stabilize error rate estimates, and improve reproducibility

*Bioconductor* support: *sva*




HapMap samples from one facility, ordered by date of processing. From

# Exemplar: Batch Effects

Leek et al., 2010, Nature Reviews Genetics 11, 733-739, Leek & Story PLoS Genet 3(9): e161

- Scientific finding: pervasive batch effects
- Statistical insights: surrogate variable analysis: identify and build surrogate variables; remove known batch effects
- Benefits: reduce dependence, stabilize error rate estimates, and improve reproducibility

*Bioconductor* support: *sva*

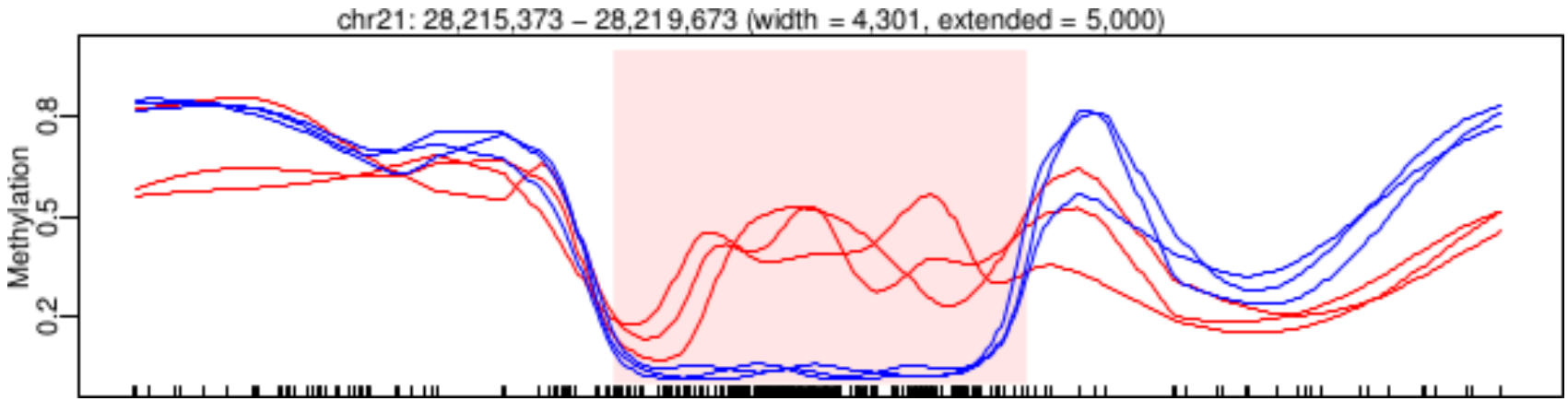
1. Remove signal due to variable(s) of interest
2. Identify subset of genes driving orthogonal signatures of EH
3. Build a surrogate variable based on full EH signature of that subset
4. Include significant surrogate variables as covariates

EH: expression heterogeneity

# Exemplar: Methylation

Hansen et al., 2011, Nature Genetics 43, 768-775

- Scientific finding: stochastic methylation variation of cancer-specific de-methylated regions (DMR), distinguishing cancer from normal tissue, in several cancers.
- Statistical challenges: smoothing, non-specific filtering, $t$ statistics, find DMRs

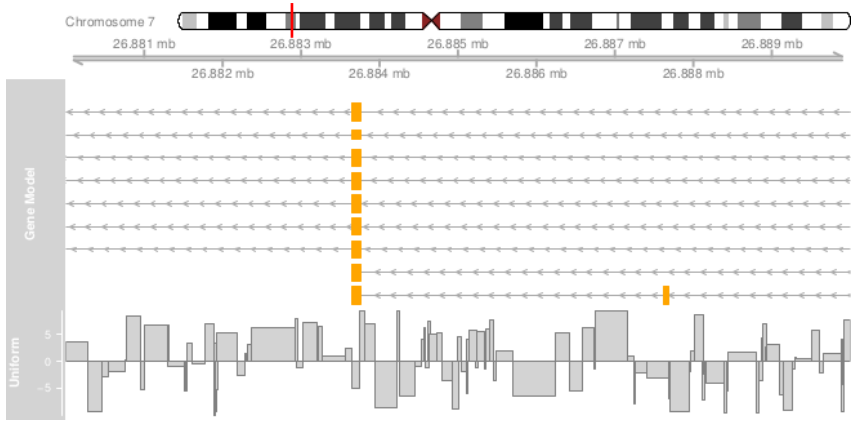



*Bioconductor* support: whole-genome (*bsseq*) or reduced representation (*MethylSeekR*) bisulfite sequencing; Illumina 450k arrays (*minfi*)

# Exemplar: Visualization

*Gviz*

- Track-like visualizations
- Data panels
- Fully integrated with *Bioconductor* sequence representations




*ggbio*
*epivizr*

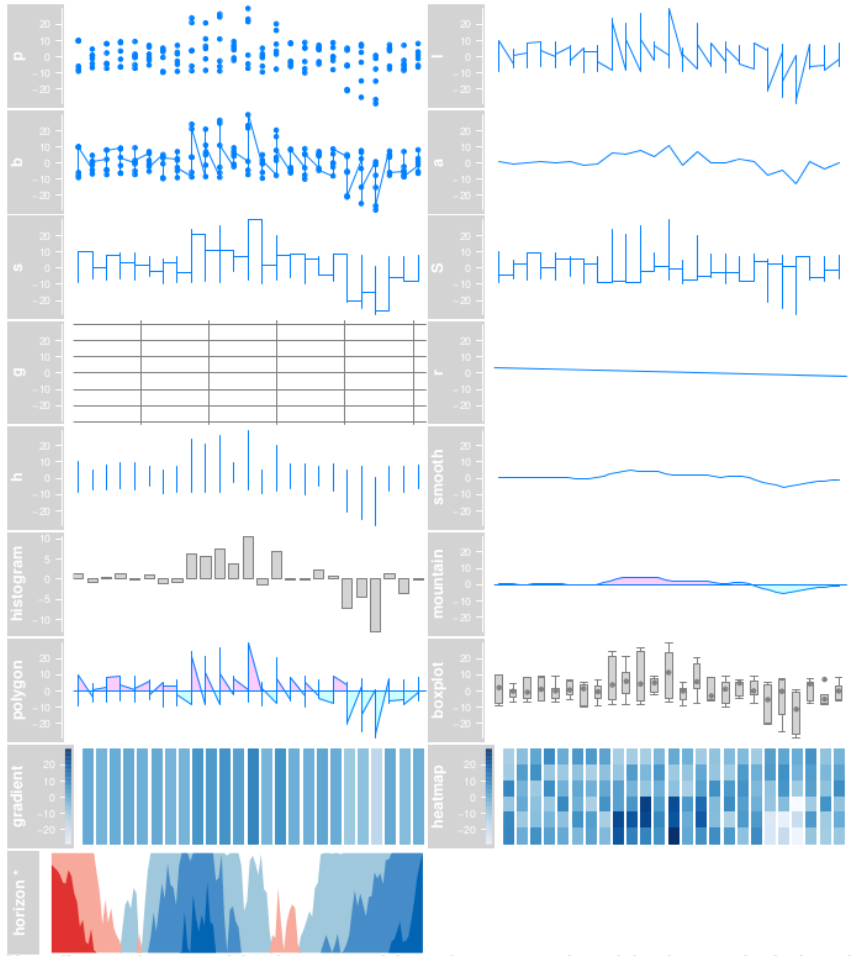
# Exemplar: Visualization

*Gviz*

- Track-like visualizations
- Data panels
- Fully integrated with *Bioconductor* sequence representations

*ggbio*

*epivizr*

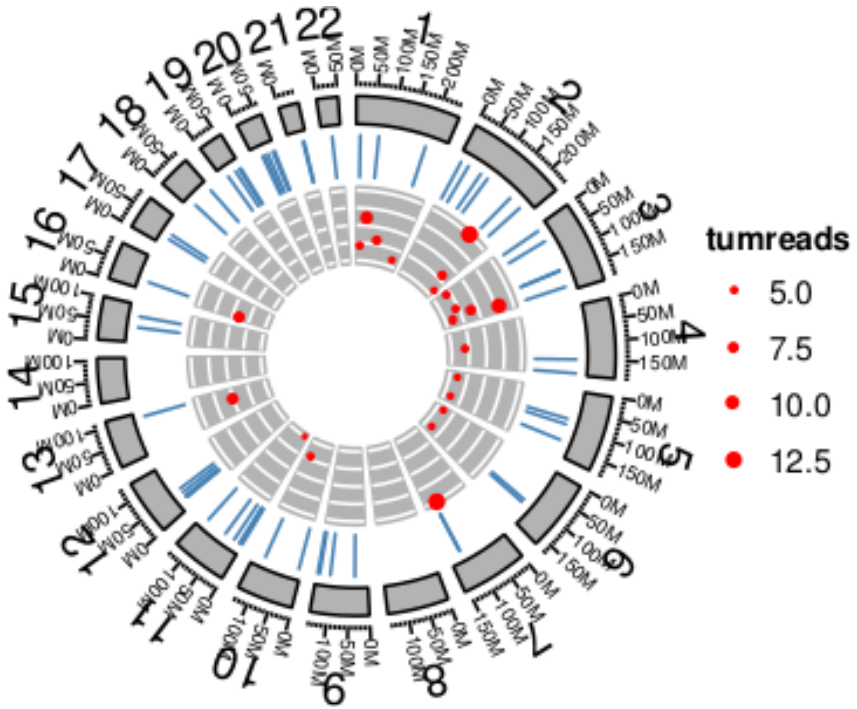


# Exemplar: Visualization

*Gviz*
*ggbio*

- Comprehensive visualizations
- `autoplot` file and data types
- Fully integrated with *Bioconductor* sequence representations

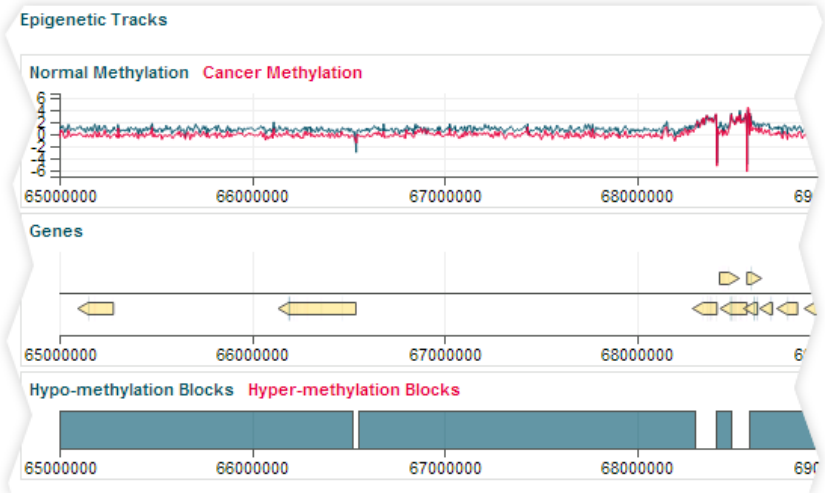
*epivizr*



# Exemplar: Visualization

*Gviz*
*ggbio*
*epivizr*

- Genome browser with socket communication to *R*
- Fully integrated with *Bioconductor* sequence representations



# Challenges & Opportunities

- Big data – transparent management within *R*, facile use of established resources
- Developer and user training

Resources

- `http://r-project.org`, *An Introduction to R* manual; Dalgaard, *Introductory Statistics with R*; *R* for Dummies
- `http://bioconductor.org/`
- `http://rstudio.org`
- StackOverflow, *Bioconductor* mailing list

# Acknowledgements


- *Bioconductor* team: Marc Carlson, Valerie Obenchain, Hervé Pagès, Paul Shannon, Dan Tenenbaum
- Technical advisory council: Vincent Carey, Wolfgang Huber, Robert Gentleman, Rafael Irizzary, Sean Davis, Kasper Hansen
- Scientific advisory board: Simon Tavaré, Vivian Bonazzi, Vincent Carey, Wolfgang Huber, Robert Gentleman, Rafael Irizzary, Paul Flicek, Simon Urbanek.
- NIH / NHGRI U41HG0004059
- The *Bioconductor* community
- . . . and the organizers of this course!