

Bioconductor for high-throughput genetic data

分析处理高通量基因数据：*Bioconductor*

Martin Morgan

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29 May 2016

Bioconductor

Statistical analysis and comprehension
of high-throughput genomic data
统计学方法分析理解高通量基因组数据

- Sequence (RNA-seq, ChIP-seq, Variants, ...)
- Microarrays (expression, methylation, SNP, copy number, ...)
- Flow cytometry
- Proteomics
- Image analysis
- ...

<https://bioconductor.org/>

<https://support.bioconductor.org>

The screenshot shows the Bioconductor website homepage. The browser address bar displays "bioconductor.org". The page features a teal header with the Bioconductor logo and navigation links: Home, Install, Help, Developers, and About. A search bar is located in the top right corner. The main content area is divided into several sections:

- BioC 2016**: Announces the annual conference "BioC 2016: Where Software and Biology Connect" at Stanford University on June 24-26.
- About Bioconductor**: Describes the tools for high-throughput genomic data analysis, noting its use of R and open development. It mentions two releases per year, 1211 software packages, and an active user community. It also lists availability as an AMI and Docker images.
- News**: Lists recent updates, including Bioconductor 3.3 availability, the F1000 Research Channel launch, and information on abstracts, literature, newsletters, and support.
- Install**: Provides links for getting started with Bioconductor, including installation, exploring packages, getting support, the latest newsletter, following on Twitter, and installing R.
- Learn**: Lists master Bioconductor tools such as courses, support sites, package vignettes, literature citations, common work flows, FAQs, and community resources.
- Use**: Offers resources for creating bioinformatic solutions with Bioconductor, including software, annotation, experiment packages, Amazon Machine Image, latest release announcements, and support sites.
- Develop**: Encourages contributions to Bioconductor, listing resources like 'devel' packages, software, annotation, and experiment packages, package guidelines, new package submission, developer resources, and build reports.

At the bottom, there are links for Support and Events, and a Twitter feed for @Bioconductor.

Bioconductor

1211 Software Packages 1211个软件包

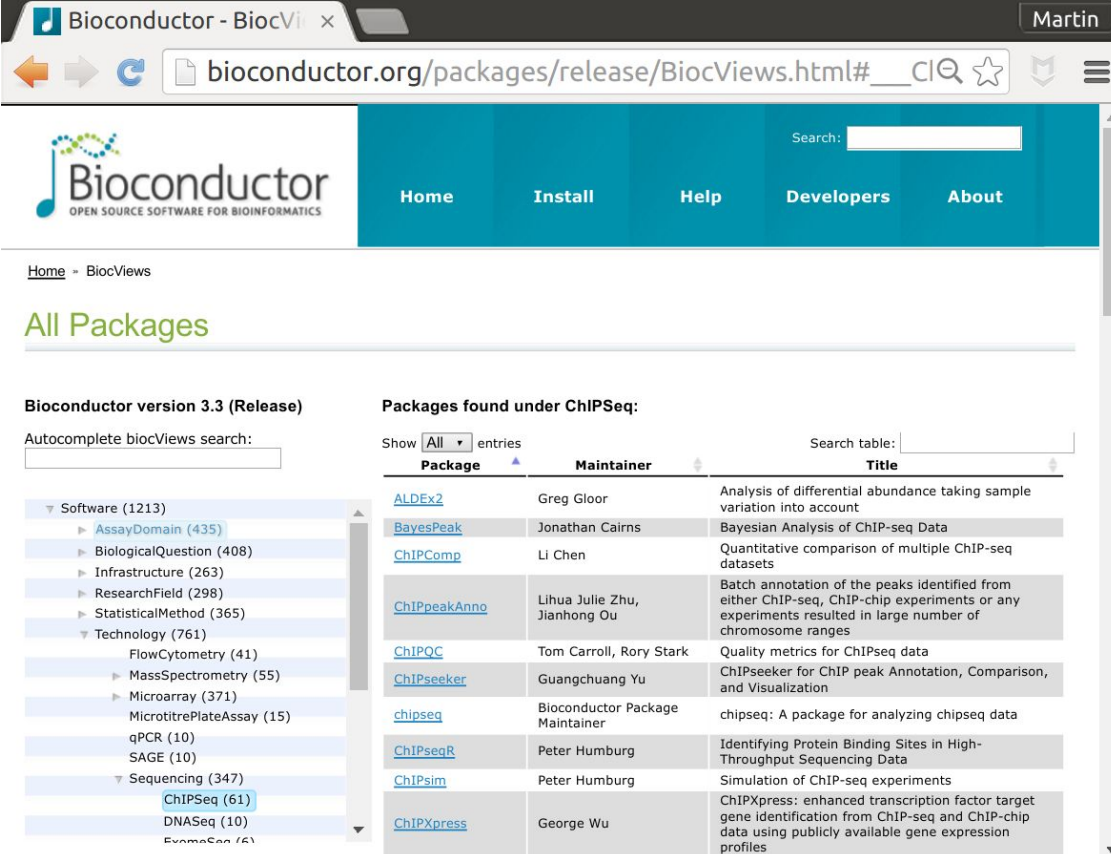
- All packages
<https://bioconductor.org/packages>
- Example package: [GenomicRanges](#)

Features 特征

- Vignettes and support site for learning
- Stable 'release' branch for users; reproducible research
- 'Devel' branch for new features & packages
- Classes for inter-operability between packages

source(["https://bioconductor.org/biocLite.R"](https://bioconductor.org/biocLite.R))

biocLite("GenomicRanges")



Bioconductor - BiocViews x Martin

bioconductor.org/packages/release/BiocViews.html#___CIQ ☆

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OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

Home Install Help Developers About

Search:

Home - BiocViews

All Packages

Bioconductor version 3.3 (Release)

Autocomplete biocViews search:

- ▼ Software (1213)
 - ▶ AssayDomain (435)
 - ▶ BiologicalQuestion (408)
 - ▶ Infrastructure (263)
 - ▶ ResearchField (298)
 - ▶ StatisticalMethod (365)
 - ▼ Technology (761)
 - FlowCytometry (41)
 - ▶ MassSpectrometry (55)
 - ▶ Microarray (371)
 - MicrotitrePlateAssay (15)
 - qPCR (10)
 - SAGE (10)
 - ▼ Sequencing (347)
 - ChIPSeq (61)
 - DNaseSeq (10)
 - ExprnSeq (6)

Packages found under ChIPSeq:

Show entries Search table:

Package	Maintainer	Title
ALDEx2	Greg Gloor	Analysis of differential abundance taking sample variation into account
BayesPeak	Jonathan Cairns	Bayesian Analysis of ChIP-seq Data
ChIPComp	Li Chen	Quantitative comparison of multiple ChIP-seq datasets
ChIPpeakAnno	Lihua Julie Zhu, Jianhong Ou	Batch annotation of the peaks identified from either ChIP-seq, ChIP-chip experiments or any experiments resulted in large number of chromosome ranges
ChIPQC	Tom Carroll, Rory Stark	Quality metrics for ChIPseq data
ChIPseeker	Guangchuang Yu	ChIPseeker for ChIP peak Annotation, Comparison, and Visualization
chipseq	Bioconductor Package Maintainer	chipseq: A package for analyzing chipseq data
ChIPseqR	Peter Humburg	Identifying Protein Binding Sites in High-Throughput Sequencing Data
ChIPsim	Peter Humburg	Simulation of ChIP-seq experiments
ChIPXpress	George Wu	ChIPXpress: enhanced transcription factor target gene identification from ChIP-seq and ChIP-chip data using publicly available gene expression profiles

Bioconductor

1211 Software Packages 1211个软件包

- All packages
<https://bioconductor.org/packages>
- Example package: [GenomicRanges](#)

Features 特征

- Vignettes and support site for learning
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- 'Devel' branch for new features & packages
- Classes for inter-operability between packages

```
source("https://bioconductor.org/biocLite.R")
```

```
biocLite("GenomicRanges")
```

The screenshot shows the Bioconductor website for the GenomicRanges package. The page includes a navigation bar with links for Home, Install, Help, Developers, and About. The main content area displays the package name 'GenomicRanges' and various statistics: platforms (all), downloads (top 5%), posts (28 / 1 / 3 / 10), in Bioc (6 years), build warnings, commits (14.17), and test coverage (unknown). There are social media icons for Facebook and Twitter. The description states: 'Representation and manipulation of genomic intervals and variables defined along a genome'. It also provides the Bioconductor version (Release (3.3)), author information (P. Aboyoun, H. Pagès, and M. Lawrence), maintainer information (Bioconductor Package Maintainer), and a citation. The installation instructions are: 'To install this package, start R and enter:'. A code block shows the R commands:

```
## try http:// if https:// URLs are not supported
source("https://bioconductor.org/biocLite.R")
biocLite("GenomicRanges")
```

. The documentation section says: 'To view documentation for the version of this package installed in your system, start R and enter:'. On the right side, there are sections for Documentation and Support.

```
source("https://bioconductor.org/biocLite.R")
```

```
biocLite("GenomicRanges")
```

Learn

Vignettes 文档说明

- Each package!

Courses 相关课程

Support site

The screenshot shows the Bioconductor website for the ComplexHeatmap package. The page has a teal header with the Bioconductor logo and navigation links: Home, Install, Help, Developers, and About. A search bar is located in the top right. The main content area is titled 'ComplexHeatmap' and includes a breadcrumb trail: Home > Bioconductor 3.3 > Software Packages > ComplexHeatmap. Below the title are several statistics: platforms (all), downloads (top 5%), posts (3 / 1 / 0.7 / 0), in Bioc (1 year), build (ok), commits (9.33), and test coverage (5%). There are social media icons for Facebook and Twitter. The main heading is 'Making Complex Heatmaps'. The text describes the package's purpose: 'Complex heatmaps are efficient to visualize associations between different sources of data sets and reveal potential structures. Here the ComplexHeatmap package provides a highly flexible way to arrange multiple heatmaps and supports self-defined annotation graphics.' It lists the author (Zuguang Gu) and maintainer (Zuguang Gu <z.gu at dkfz.de>). A citation is provided: 'Gu Z (2016). ComplexHeatmap: Making Complex Heatmaps. R package version 1.10.2, https://github.com/joker00/ComplexHeatmap.' The 'Installation' section instructs users to start R and enter a code block:

```
## try http:// if https:// URLs are not supported
source("https://bioconductor.org/biocLite.R")
biocLite("ComplexHeatmap")
```

 The 'Documentation' section lists various resources: HTML and R Script links for 1. Introduction to ComplexHeatmap package, 2. Making a single heatmap, 3. Making a list of Heatmaps, 4. Heatmap Annotations, 5. Heatmap and Annotation Legends, 6. Heatmap Decoration, 7. Interactive with Heatmaps, 8. OncoPrint, 9. More Examples, a PDF Reference Manual, and a Text NEWS file. The 'Details' section is partially visible at the bottom. On the right side, there are two boxes: 'Documentation' with links to vignettes, workflows, course and conference material, videos, and community resources; and 'Support' with a posting guide and links to support site and Bioc-devel mailing list.

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Vignettes 文档说明

- Each package!

Courses 相关课程

Support site 支持网站

Differential analysis of count data – the DESeq2 package

Michael I. Love¹, Simon Anders^{2,3}, Wolfgang Huber³

¹ Department of Biostatistics, Dana-Farber Cancer Institute and Harvard TH Chan School of Public Health, Boston, US;

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³ European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

May 15, 2016

Abstract

A basic task in the analysis of count data from RNA-seq is the detection of differentially expressed genes. The count data are presented as a table which reports, for each sample, the number of sequence fragments that have been assigned to each gene. Analogous data also arise for other assay types, including comparative ChIP-Seq, HiC, shRNA screening, mass spectrometry. An important analysis question is the quantification and statistical inference of systematic changes between conditions, as compared to within-condition variability. The package *DESeq2* provides methods to test for differential expression by use of negative binomial generalized linear models; the estimates of dispersion and logarithmic fold changes incorporate data-driven prior distributions¹. This vignette explains the use of the package and demonstrates

typical workflows. An R notebook version of this vignette but at a slower pace is available at [https://github.com/mikelove/DESeq2-notebook](#).

DESeq2 version: 1.12.0

Differential analysis of count data – the DESeq2 package

4

1 Standard workflow

1.1 Quick start

Here we show the most basic steps for a differential expression analysis. These steps require you have a *RangedSummarizedExperiment* object `se` which contains the counts and information about samples. The `design` indicates that we want to measure the effect of condition, controlling for batch differences. The two factor variables `batch` and `condition` should be columns of `colData(se)`.

```
dds <- DESeqDataSet(se, design = ~ batch + condition)
dds <- DESeq(dds)
res <- results(dds, contrast=c("condition", "trt", "con"))
```

If you have a count matrix and sample information table, the first line would use `DESeqDataSetFromMatrix` instead of `DESeqDataSet`, as shown in Section 1.3.3.

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Bioconductor - Course x

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Courses & Conferences

Bioconductor provides training in computational and statistical methods for the analysis of genomic data. You are welcome to use material from previous courses. However, you may not include these in separately published works (articles, books, websites). When using all or parts of the Bioconductor course materials (slides, vignettes, scripts) please cite the authors and refer your audience to the Bioconductor website.

[Upcoming events](#) are advertised 6 to 8 weeks in advance.

Show entries

Search:

Keyword	Title	Course	Materials	Date	Bioc/R Version
Introduction	A1: R Intro, Martin Morgan, Lori Shepherd	BiocIntroRPCI	HTML R Rmd	2016-05-16	3.3/3.3
Introduction	A2: Input and Manipulation, Martin Morgan, Lori Shepherd	BiocIntroRPCI	HTML R Rmd	2016-05-16	3.3/3.3
Introduction	A3: Statistical Analysis, Martin Morgan, Lori Shepherd	BiocIntroRPCI	HTML R Rmd	2016-05-16	3.3/3.3
Introduction	A4: Visualization, Martin Morgan, Lori Shepherd	BiocIntroRPCI	HTML R Rmd	2016-05-16	3.3/3.3
Introduction	B1: Bioconductor Intro, Martin Morgan, Lori Shepherd	BiocIntroRPCI	HTML R Rmd	2016-05-16	3.3/3.3
Introduction	B2: Common Operations, Martin Morgan, Lori Shepherd	BiocIntroRPCI	HTML R Rmd	2016-05-16	3.3/3.3
Introduction	B3: RNASeq Workflow, Martin Morgan, Lori Shepherd	BiocIntroRPCI	HTML R Rmd	2016-05-16	3.3/3.3
Introduction	B4: Next Steps, Martin Morgan, Lori Shepherd	BiocIntroRPCI	HTML R Rmd	2016-05-16	3.3/3.3
Introduction	Introduction to High Throughput DNA Sequence Data Analysis Using R / Bioconductor, Martin Morgan	ENAR 2016	Rmd R ; github	2016-04-08	3.2/3.2

Content: [html](#)

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Support site 支持网站

The screenshot shows the 'Latest Posts' page on the Bioconductor support site. The page features a navigation bar with the Bioconductor logo and links for 'ASK QUESTION', 'LATEST 2', 'NEWS', 'JOBS', 'TUTORIALS', 'TAGS', and 'USERS'. Below the navigation bar, there are filters for 'Limit' and 'Sort', and a search box. The main content area displays a list of posts, each with a title, view count, and a list of tags. The posts are sorted by the number of answers, with the most recent post having 2 answers and 85 views. The right sidebar contains sections for 'Recent...', 'Replies', 'Votes', 'Awards', and 'Locations', each with a list of recent activity.

Limit	Sort	Search
0 votes	0 answers	26 views
0 votes	0 answers	15 views
0 votes	0 answers	21 views
4 votes	2 answers	78 views
1 vote	0 answers	28 views
2 votes	1 answer	35 views
1 vote	2 answers	41 views
4 votes	4 answers	63 views
3 votes	2 answers	85 views

Recent...
Replies
• C: limma paired design with... by meeta.mistry • 10
• C: limma paired design with... by meeta.mistry • 10
• A: Filtering of RNA-seq dat... by alireza_sharifian_10 • 0
• C: DESeq2 multifactorial de... by Michael Love ♦ 7.8k
• A: inconsistent results fro... by Martin Morgan ♦♦ 17k
Votes
• A: limma paired design with...
• A: inconsistent results fro...
• A: Filtering of RNA-seq dat...
• Summer Studentship in Data ...
• C: DESeq2 multifactorial de...
Awards • All »
• Scholar to Aaron Lun • 9.2k
• Scholar to Michael Lawrence ♦ 7.8k
• Commentator to Aaron Lun • 9.2k
• Scholar to Steve Lianoglou ♦ 11k
• Appreciated to Aaron Lun • 9.2k
• Scholar to Dan Tenenbaum ♦♦ 8.0k
Locations • All »
• United States, 15 minutes ago
• Genentech, 1 hour ago

https://support.bioconductor.org/p/83141/

... for differential binding above a log2 fold change threshold using

Learn

Essential Software 基础软件包

- *Biostrings*
- ***GenomicRanges***
- *SummarizedExperiment*

Annotation Resources 注释资源

- Packages
 - org.*
 - TxDb.*
 - BSgenome.*
- On-line
 - [biomaRt](#)
 - [AnnotationHub](#)

```
> 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

```
seqlengths:
      chr1      chr2 ... chrUn_g1000249
249250621 243199373 ... 38502
```

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)

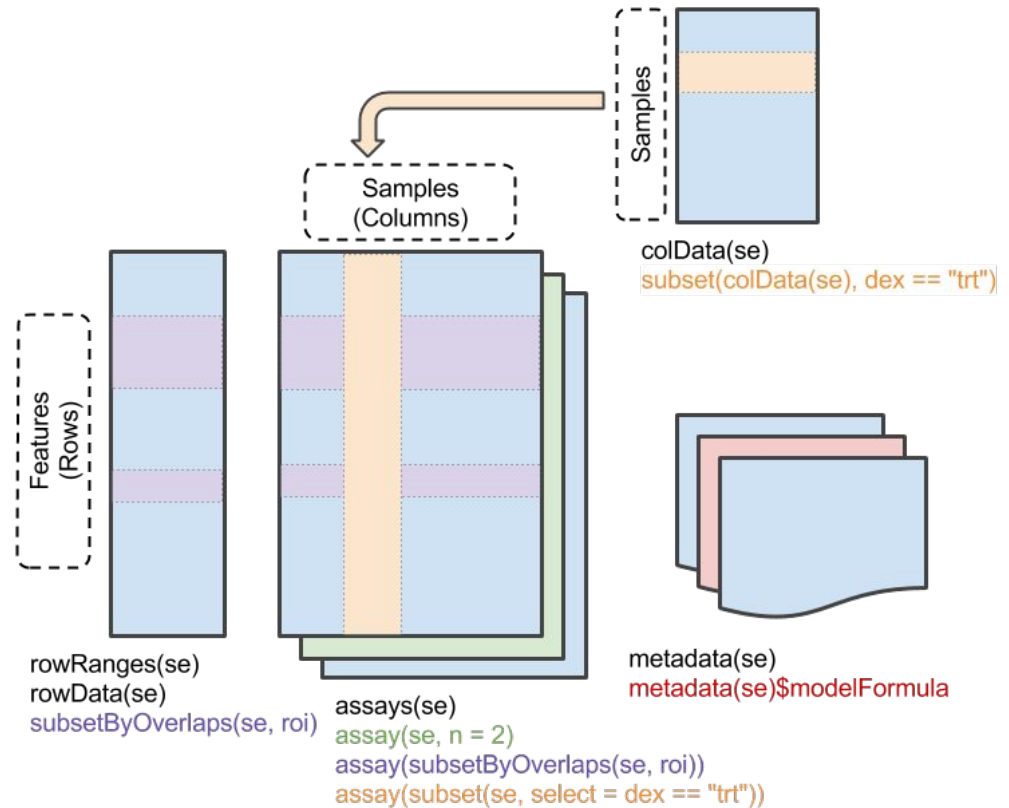
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Essential Software 基础软件包

- *Biostrings*
- *GenomicRanges*
- ***SummarizedExperiment***

Annotation Resources 注释资源

- Packages
 - org.*
 - TxDb.*
 - BSgenome.*
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 - [biomaRt](#)
 - [AnnotationHub](#)



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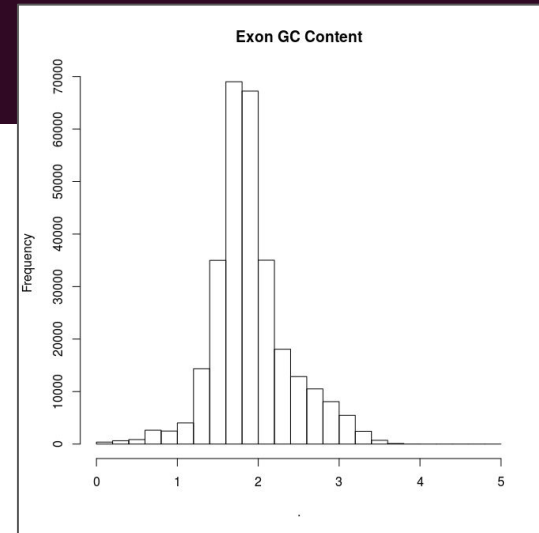
Essential Software 基础软件包

- *Biostrings*
- *GenomicRanges*
- *SummarizedExperiment*

Annotation Resources 注释资源

- Packages
 - Identifier mapping
 - Gene models
 - Genome sequence
- On-line
 - [biomaRt](#)
 - [AnnotationHub](#)

```
> library(Homo.sapiens)
> head(id <- select(Homo.sapiens, "BRCA1", c("GENENAME", "TXID"), "SYMBOL"), 3)
'select()' returned 1:many mapping between keys and columns
  SYMBOL      GENENAME  TXID
1  BRCA1 breast cancer 1 63594
2  BRCA1 breast cancer 1 63595
3  BRCA1 breast cancer 1 63596
> brca1tx <- exonsBy(Homo.sapiens, "tx")[id$TXID]
> library(BSgenome.Hsapiens.UCSC.hg19)
> head(dna <- getSeq(BSgenome.Hsapiens.UCSC.hg19, brca1tx))
DNAStringSetList of length 6
[["63594"]] TTCATTGGAACAGAAAGAAATGGATTTATCTGCTCTTCGCGTTGAAGAAGTACAAAATGTCATTA...
[["63595"]] AGATAACTGGGCCCTGCGCTCAGGAGGCCTTACCCTCTGCTCTGGGTAAAGGTAGTAGAGTCC...
[["63596"]] AGATAACTGGGCCCTGCGCTCAGGAGGCCTTACCCTCTGCTCTGGGTAAAGGTAGTAGAGTCC...
[["63597"]] CTTAGCGGTAGCCCCTTGGTTTCCGTGGCAACGGAAAAGCGCGGGAATTACAGATAAATTAAC...
[["63598"]] CTTAGCGGTAGCCCCTTGGTTTCCGTGGCAACGGAAAAGCGCGGGAATTACAGATAAATTAAC...
[["63599"]] GTACCTTGATTCGATTCTGAGAGGCTGCTGCTTAGCCGTAGCCCCTTGGTTTCCGTGGCAACG...
> library(magrittr)
> getSeq(BSgenome.Hsapiens.UCSC.hg19, exons(Homo.sapiens)) %>%
+   letterFrequency("GC") %>% log10 %>% hist(main="Exon GC Content")
>
```



Use

The screenshot displays the RStudio interface with two main panels. The left panel shows the R console and a R Markdown report. The right panel shows a volcano plot.

Console:

```
> dds = DESeq(DESeqDataSet(airway, ~ cell + dex))
estimating size factors
estimating dispersions
gene-wise dispersion estimates
mean-dispersion relationship
final dispersion estimates
fitting model and testing
> plotMA(dds, alpha=.01)
```

Report.Rmd:

```
1 ---
2 title: "Bioinformatics Report"
3 author: "Martin Morgan"
4 date: "March 12, 2016"
5 output: html_document
6 ---
7
8 # Introduction
9
10 It is very easy to create advanced reports in a fully
11 reproducible way. Reports can be distributed as PDF, HTML, ...
12 Reports can include figures, tables, analytic results,
13 interactive applets., etc.
14 ```{r, warning=FALSE, message=FALSE, echo=FALSE}
15 ## R code evaluated when report produced
16 library(DESeq2)
17 library(airway) # example data set
18 data(airway)
19 dds = DESeq(DESeqDataSet(airway, ~ cell + dex))
20 plotMA(dds, alpha=.01)
21 ```
```

Volcano Plot:

The volcano plot shows the relationship between the mean of normalized counts (x-axis, log scale from 1e-01 to 1e+05) and the log fold change (y-axis, from -1.5 to 1.5). A horizontal red line is drawn at log fold change = 0. Points are colored red if they are significantly differentially expressed (|log fold change| > 1.5 and mean of normalized counts > 10). The plot shows a clear separation between the red points (differentially expressed) and the grey points (not differentially expressed).

Use

Gene expression 基因表达

- RNA-seq: *DESeq2*, *edgeR*, *scde*, ...
- Microarray: *limma*

Gene regulation 基因调控

- ChIP-seq: *csaw*, *DiffBind*
- Methylation arrays: *minfi*, *missMethyl*
- Gene set enrichment: *topGO*, *limma*

Variants 变异

- *VariantAnnotation*
- *VariantFiltering*

Flow cytometry 流式细胞仪

- *flowCore*

Data access 数据访问

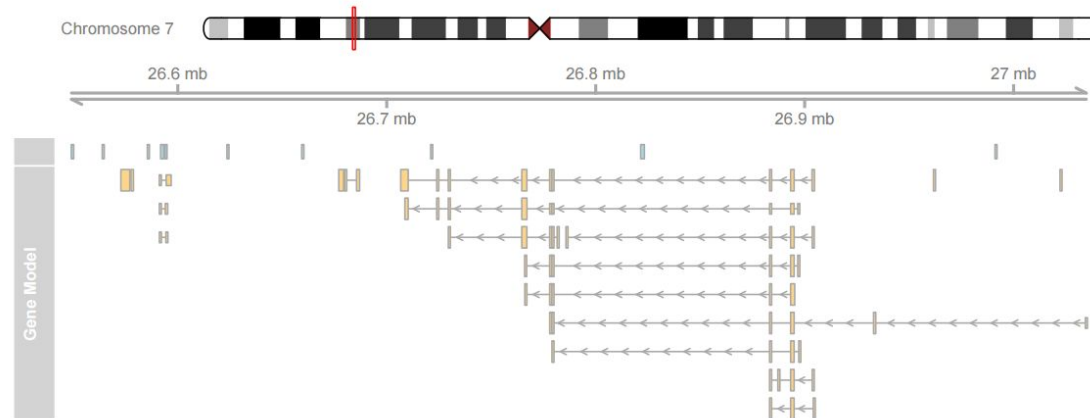
- *biomaRt*
- *GEOquery* / *SRADB*
- *TCGAbiolinks*
- *AnnotationHub* / *ExperimentHub*

Visualization 可视化

- *Gviz*, *ComplexHeatmap*, *ggbio*, *ggtree*, ...

Many other packages!

```
> plotTracks(list(itrack, gtrack, atrack, grtrack))
```



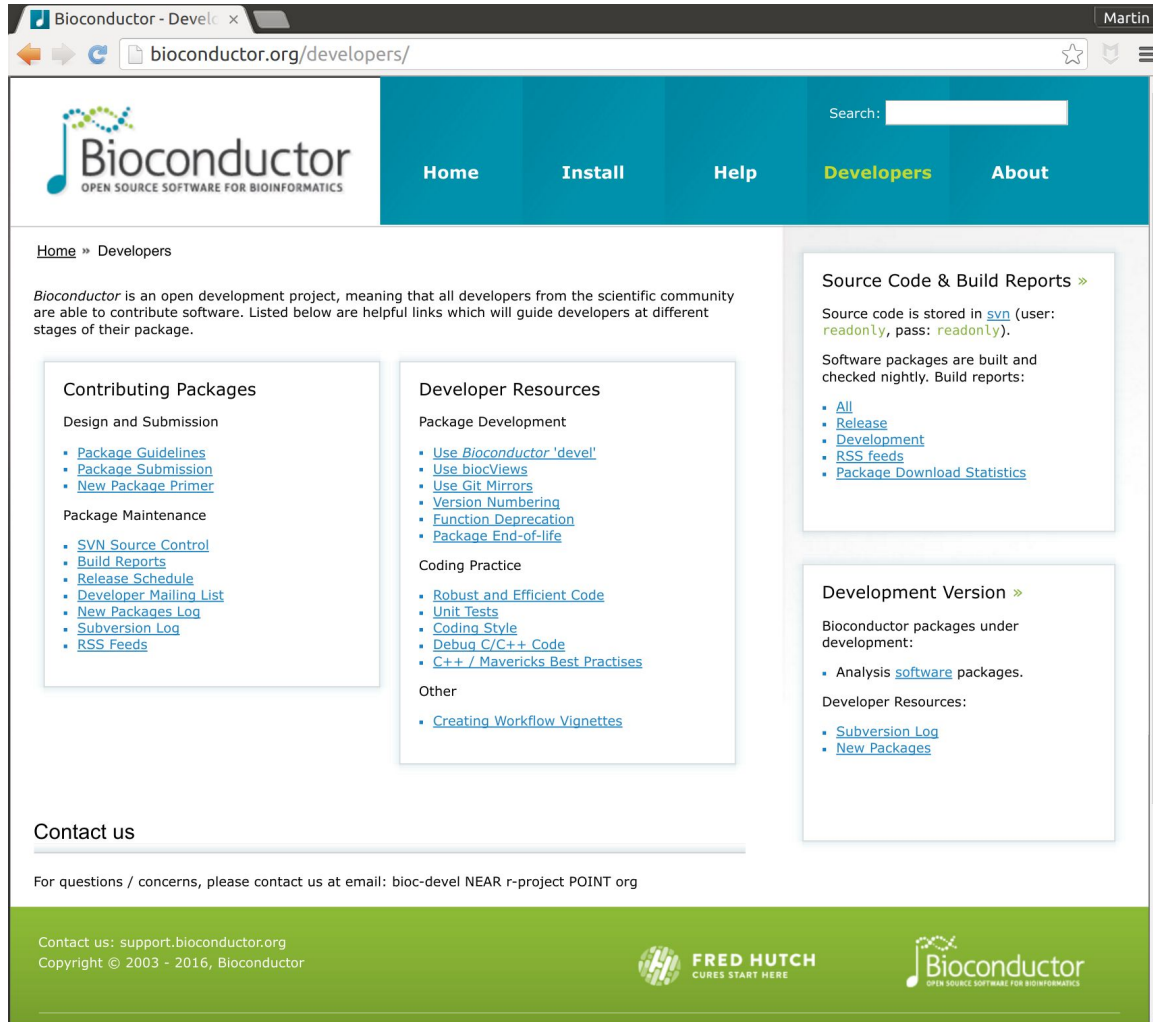
Develop

Create a package 创建包

- Use existing classes -- *DNAStrngSet*, *GRanges*, *SummarizedExperiment*
- Use existing packages -- *rtracklayer*, *Rsamtools*, *Biostrings*, ...
- Full vignette and examples
- [Unit tests](#) and other best practices

Submit to *Bioconductor* 提交

- Technical review
- Long-term [support](#) & maintenance
- Introduced to 'devel' branch, new release every April and October



The screenshot shows the Bioconductor website's 'Developers' page. The page has a teal header with the Bioconductor logo and navigation links: Home, Install, Help, Developers (highlighted), and About. A search bar is located in the top right. Below the header, the page content is organized into several sections:

- Home » Developers**: A breadcrumb trail.
- Bioconductor is an open development project...**: A paragraph explaining the open development process.
- Contributing Packages**: A section with sub-sections:
 - Design and Submission**: [Package Guidelines](#), [Package Submission](#), [New Package Primer](#)
 - Package Maintenance**: [SVN Source Control](#), [Build Reports](#), [Release Schedule](#), [Developer Mailing List](#), [New Packages Log](#), [Subversion Log](#), [RSS Feeds](#)
- Developer Resources**: A section with sub-sections:
 - Package Development**: [Use Bioconductor 'devel'](#), [Use biocViews](#), [Use Git Mirrors](#), [Version Numbering](#), [Function Deprecation](#), [Package End-of-life](#)
 - Coding Practice**: [Robust and Efficient Code](#), [Unit Tests](#), [Coding Style](#), [Debug C/C++ Code](#), [C++ / Mavericks Best Practises](#)
 - Other**: [Creating Workflow Vignettes](#)
- Source Code & Build Reports**: A section with sub-sections:
 - Source code is stored in svn**: [\(user: readonly, pass: readonly\)](#)
 - Software packages are built and checked nightly. Build reports:**
 - [All](#)
 - [Release](#)
 - [Development](#)
 - [RSS feeds](#)
 - [Package Download Statistics](#)
- Development Version**: A section with sub-sections:
 - Bioconductor packages under development:**
 - [Analysis software packages.](#)
 - Developer Resources:**
 - [Subversion Log](#)
 - [New Packages](#)

At the bottom, there is a **Contact us** section with the text: "For questions / concerns, please contact us at email: bioc-devel NEAR r-project POINT org". The footer contains contact information: "Contact us: support.bioconductor.org", "Copyright © 2003 - 2016, Bioconductor", and logos for Fred Hutch (CURES START HERE) and Bioconductor (OPEN SOURCE SOFTWARE FOR BIOINFORMATICS).

Acknowledgements

Core team (current & recent)

- Valerie Obenchain, Herve Pages, Dan Tenenbaum, Lori Shepherd, Marcel Ramos, Brian Long, Jim Hester, Jim Java, Sonali Arora, Nate Hayden, Paul Shannon, Marc Carlson

Technical advisory board

- Vincent Carey, Wolfgang Huber, Robert Gentleman, Rafael Irizzary, Levi Waldron, Michael Lawrence, Sean Davis, Aedin Culhane

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- Simon Tavare (CRUK), Paul Flicek (EMBL/EBI), Simon Urbanek (AT&T), Vincent Carey (Brigham & Women's), Wolfgang Huber (EBI), Raphael Irizzary, Robert Gentleman (23andMe)



SOUND



Research reported in this presentation was supported by the National Human Genome Research Institute and the National Cancer Institute of the National Institutes of Health under award numbers U41HG004059 and U24CA180996. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Resources

Web sites

- <https://bioconductor.org>
- <https://support.bioconductor.org>

Events

- Annual Conference, Stanford, CA, USA, 24-26 June
- *Bioconductor* Asia-Pacific Conference, Brisbane, Australia 3-4 November
- *Bioconductor* European Developer Workshop, Basel, Switzerland, 6-7 December