# **Basic Introduction to R and Bioconductor**

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R (www.r-project.org) is an open source interactive computer system for visualization and analysis of statistical data. Bioconductor (www.bioconductor.org) is a project within R. The Bioconductor project is a source of many resources relevant to data analysis for high-throughput biology.

## Installing R and Bioconductor

1. Download R from <u>http://www.r-project.org</u>. The primary portal for R is <u>http://cran.r-project.org</u>. Many local mirrors exist and these may provide quicker downloads.

R can be download precompiled for Linux, Mac OS and Windows. If you are using Linux, R can be installed using yum install.

The Comprehensive R Archive Network
ntly used pages
Download and Install R
Precompiled binary distributions of the base system and contributed packages, Windows and Mac users most likely want
One
• <u>Linux</u>
• <u>MacOS X</u>
• <u>Windows</u>
Source Code for all catforms
Windows and Mac users most likely want the precompiled binaries listed in the upper box, not the source code. The
sources have to be compiled before you can use them. If you do not know what this means, you probably do not want to
do it!
• The latest release (2011-04-13): R-2.13.0.tar.gz (read what's new in the latest version).
<ul> <li>Sources of R alpha and beta releases (daily snapshots, created only in time periods before a planned release).</li> </ul>
• Sources of <u>reading and beta releases</u> (dairy shapshots, created only in unit periods before a plauned release).
• Daily snapshots of current patched and development versions are <u>available here</u> . Please read about <u>new features and</u>
bug fixes before filing corresponding feature requests or bug reports.
<ul> <li>Source code of older versions of R is <u>available here</u>.</li> </ul>
Contributed extension packages
Questions About R
• If you have questions about R like how to download and install the software, or what the license terms are, please
read our <u>answers to frequently asked questions</u> before you send an email.

To install R simply, click on download and select the version appropriate for your operating system.

For the most users, this precompiled version of R is sufficient. However if you wish to write an extension package for R/Bioconductor, you need to install R from source. Please refer to the R FAQ for directions on this. Windows users will need to install unix tools/perl/latex, please refer to the excellent guide at <u>http://www.murdoch-sutherland.com/Rtools/</u>.

### **R** Extension Packages

On <u>http://cran.r-project.org/</u> or the mirror you are using. To find out about contributed package please click on the link **Contributed extension <u>packages</u>** at the bottom of the main download page. This will link to a page with lots of information on contributed packages and to "**CRAN Task Views**".

#### Contributed Packages

Installation of Packages

Please type help ("INSTALL") or help ("install.packages") in R for information on how to install packages from this directory. The manual <u>R</u> <u>Installation and Administration</u> (also contained in the R base sources) explains the process in detail.

CRAN Task Views allow you to browse packages by topic and provide tools to automatically install all packages for special areas of interest. Currently, 28 views are available.

CRAN task views, provide over 25 categories of contributed packages which makes installation of these packages simpler.

		CRAN Task Views
	Bayesian	Bayesian Inference
	ChemPhys	Chemometrics and Computational Physics
	<b><u>ClinicalTrials</u></b>	Clinical Trial Design, Monitoring, and Analysis
CRAN	Cluster	Cluster Analysis & Finite Mixture Models
Mirrors	Distributions	Probability Distributions
What's new?	Econometrics	Computational Econometrics
<u>Task Views</u> Search	<b>Environmetrics</b>	Analysis of Ecological and Environmental Data
	ExperimentalDesign	Design of Experiments (DoE) & Analysis of Experimental Data
About R	Finance	Empirical Finance
<u>R Homepage</u> The R Journal	Genetics	Statistical Genetics
The resound	Graphics	Graphic Displays & Dynamic Graphics & Graphic Devices & Visualization
Software	gR	gRaphical Models in R
<u>R Sources</u> R Binaries	HighPerformanceComputi	ing High-Performance and Parallel Computing with R
Packages	MachineLearning	Machine Learning & Statistical Learning
Other	MedicalImaging	Medical Image Analysis
Documentation	<u>Multivariate</u>	Multivariate Statistics
Manuals	<u>NaturalLanguageProcessi</u>	ng Natural Language Processing
FAQs	<b>OfficialStatistics</b>	Official Statistics & Survey Methodology
Contributed	Optimization	Optimization and Mathematical Programming
	<b>Pharmacokinetics</b>	Analysis of Pharmacokinetic Data
	<b>Phylogenetics</b>	Phylogenetics, Especially Comparative Methods
	Psychometrics	Psychometric Models and Methods
	<u>ReproducibleResearch</u>	Reproducible Research
	Robust	Robust Statistical Methods
	SocialSciences	Statistics for the Social Sciences
	Spatial	Analysis of Spatial Data
	Survival	Survival Analysis
	TimeSeries	Time Series Analysis

To automatically install these views, the ctv package needs to be installed, e.g., v install.packages("ctv") library("ctv") For example to install genetics packages type

```
install.packages("ctv")
library(ctv)
install.views("Genetics")
```

Note these categories contain many packages, for example this Genetics contains 29 packages.

## Installing Individual Contributed or Extension Packages

Although a team of statisticians and programmers maintain R, many independent groups submit contributed packages. This is a very extensive resource of hundreds of programs and will not be install by default.

R extensions can be installed easily. R extensions are stored in repositories. Select the package repositories using **Packages -> Select repositories** 



The R repositories are:

**CRAN**: basic R distribution

CRAN (extras)- Contributed R packages. There are several hundred.

**Bioconductor:** The <u>Bioconductor Project</u> produces an open source software framework that will assist biologists and statisticians working in bioinformatics, with primary

emphasis on inference using DNA microarrays. A CRAN style R package repository is available via <u>http://www.bioconductor.org/</u>.

**Omegahat:** The Omegahat Project for Statistical Computing provides a variety of opensource software for statistical applications, with special emphasis on web-based software, Java, the Java virtual machine, and distributed computing. But recently many other plugins including are available, into RGoogleStorage, RGoogleDocs,ROpenOffice, RAmazonDBRest, RAmazonS3, R2GoogleMaps etc. CRAN style R package repository is available via <u>http://www.omegahat.org/</u>

Select one repository, it may provide a select of CRAN mirrors, select one close by. You can now download extension packages.

There are several ways to install extension packages

- 1. From the GUI, select **Packages -> Install Packages**. Note only the packages from the selected repositories will be shown.
- 2. From command line type install.packages("packagename")
- 3. Download a compressed copy of the package from the R website and install from local zip or .tar.gz file





Alternatively one can install a package and specific the repository using the following command within R:

## Installing Bioconductor

The recommended method to install Bioconductor is using the following script, which can be sourced directly:

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

This will automatically download core bioconductor packages. Due to the number and size of Bioconductor, many useful packages will not be installed, so these can be installed as described above.

## Using a script to simplify Installation of R and Bioconductor

Once you are familiar with R and Bioconductor, you may wish to script the above progress so that updates to R are simpler ;-)

I use the following script, which I saved to a plain text file called bioC\_install.R

```
### Packages for R.
install.packages("ade4")
install.packages("scatterplot3d")
install.packages("Rcurl")
install.packages("DBI")
install.packages("RMySQL")
install.packages("impute")
install.packages("fastICA")
install.packages("e1071")
### BioC Packages for R.
source("http://www.bioconductor.org/biocLite.R")
biocLite()
biocLite("made4")
biocLite("hgu133plus2")
biocLite("hgu133plus2cdf")
biocLite("hgu133plus2probe")
biocLite("Heatplus")
biocLite("biomaRt")
```

To install these packages, I simply type command

source(``./bioC\_install.R")

and then go for coffee ;-)

R and Bioconductor are pretty "intelligent", if you wish to install a package that has dependencies (needs other packages) it will automatically install these.

## Introduction to Bioconductor Packages

Bioconductor (<u>http://www.bioconductor.org</u>) has a really nice "task views" interface to ipacakges in Bioconductor. To find out more about these click on **Packages link** or click **Install** and this will give you a link to **BioCViews** 

				Search:	
DEN SOURCE SOFTWARE FOR BIOINFORMATICS	Home	Install	Help	Developers	About
About Bioconductor provides tools for the analysis and comprehension of high-throughput genomic data. Bioconductor uses the R statistical programming language, and is open source and open development. It has two releases each year, more than <u>460 packages</u> , and an active user community.	<ul> <li>Use Bioconductor for</li> <li>Microarrays</li> <li>Import Affymetrix, Illumina, Nimblegen, Agilent, and other platforms. Perform quility assessment, normalization, differential expression, clustering, classification, gene set enrichment, genetical genomics and other workflows for expression, exon, copy number, SNP, methylation and other assays. Access GEO, ArrayExpress, Biomart, UCSC, and other community resources.</li> <li>High Throughput Assays Import, transform, edit, analyze and visualize flow cytometric, mass spec, HTqPCR, cell-based, and other assays.</li> </ul>		mblegen, erform ion, ng, nent, workflows mber, SNP, Access JJCSC, and is e and s spec,	<ul> <li>Sequence Data Import fasta, fastq, ELAND, MAQ, BWA, Bowtie, BAM, gff, bed, wig, and other sequence formats. Trim, transform, align, and manipulate sequences. Perform quality assessment, ChIP-seq, differential expression, RNA-seq, and other workflows. Access the Sequence Read Archive.</li> <li>Annotation Use microarray probe, gene, pathway, gene ontology, homology and other annotations. Access GO, KEGG, NCEI, Biomart, UCSC, vendor, and other sources.</li> </ul>	
Mailing Lists Subscribe >> BiocViews are grouped into a Annotation Data Experiment Data. Software	Even 3 categories			Dews	
Bioconductor OPEN SOURCE SOFTWARE FOR BIOINFORMATICS	Home	Instal	He	Search: Search:	pers About
Home » Help » BiocViews					
All Packages					
Bioconductor version 2.8 (Release)	to explore	the package	es in Bioc	onductor	

## **Bioconductor Software Packages**

Software packages are sub divided into the categories:

Bioconductor version 2.8 (Release)
AnnotationData (594)
ExperimentData (79)
▼ Software (467)
Annotation (61)
AssayDomains (182)
AssayTechnologies (289)
Bioinformatics (270)
Classification (34)
Clustering (38)
MultipleComparisons (31)
Preprocessing (83)
QualityControl (46)
SequenceMatching (7)
TimeCourse (11)
BiologicalDomains (46)
Infrastructure (194)
DataImport (40)
DataRepresentation (10)
GraphsAndNetworks (40)
GUI (8)
Visualization (78)

Each contains a long list of contributed packages. For example clicking on Bioinformatics -> PreProcessing returns a long list of packages with methods for handling many different data types

### Packages

Software > Bioinformatics > Preprocessing

```
    ABarray affy affycomp AffyExpress affyILM affyImGUI affyPara affypdnn affyPLM
    AffyTiling Aqi4x44PreProcess AqiMicroRna altcdfenvs aroma.light ArrayTools beadarray
    beadarraySNP BUS CALIB cellHTS2 CGHcall CGHnormaliter codelink crimm DEGseq
    dyebias ExiMiR farms frma frmaTools gcrma genArise Harshlight HELP HTqPCR
    imageHTS limma limmaGUI LMGene lumi LVSmiRNA maCorrPlot maigesPack
    makecdfenv makePlatformDesign MANOR marray MBCB MEDIPS methylumi Mfuzz
    MiChip multiscan nnNorm NTW oligo OLIN OLINgui oneChannelGUI puma gpcrNorm
    grqc rama RBioinf RefPlus Ringo rMAT RNAinteract RNAither Rolexa RPA SAGx
    simpleaffy snapCGH snm spotSeqmentation Starr stepNorm TargetSearch tilingArray
```

and each package page give details, help and a link to a vignette (tutorial).

### Methods for Affymetrix Oligonucleotide Arrays

Bioconductor version: Release (2.8)

The package contains functions for exploratory oligonucleotide array analysis. The dependence on tkWidgets only concerns few convenience functions. 'affy' is fully functional without it.

Author: Rafael A. Irizarry, Laurent Gautier, Benjamin Milo Bolstad, and Crispin Miller with contributions from Magnus Astrand, Leslie M. Cope, Robert Gentleman, Jeff Gentry, Conrad Halling, Wolfgang Huber, James MacDonald, Benjamin I. P. Rubinstein, Christopher Workman, John Zhang

Maintainer: Rafael A. Irizarry

To install this package, start R and enter:

```
source("http://www.bioconductor.org/biocLite.R")
biocLite("affy")
```

#### Documentation

PDF	R Script	1. Primer
PDF	R Script	2. Built-in Processing Methods
PDF	R Script	3. Custom Processing Methods
PDF	R Script	4. Import Methods
Reference	e Manual	

Reference Manual

#### Details

biocViews	Microarray, OneChannel, Preprocessing
Depends	R, <u>Biobase</u>
Imports	affyio, Biobase, graphics, grDevices, methods, preprocessCore, stats, utils
Suggests	tkWidgets, affydata
System Requirements	
License	LGPL (>= 2.0)
URL	
Depends On Me	AffyExpress, AqiMicroRna, ArrayTools, ExiMiR, LMGene, LVSmiRNA, MLP, RPA, RefPlus, Starr, affyContam, affyPLM, affyPara, affyQCReport, affycoretools, affyImGUI, affypdnn, altcdfenvs, arrayMvout, bqx, dualKS, farms, frmaTools, gcrma, logitT, maDB, panp, plw, puma, gpcrNorm, rHVDM, simpleaffy, sscore, webbioc
Imports Me	AffyTiling, ArrayExpress, ArrayTools, GEOsubmission, HTgPCR, Harshlight, TurboNorm, affyILM, affyQCReport, arrayQualityMetrics, farms, frma, gcrma, lumi, makecdfenv, plier, plw, puma, pvac, simpleaffy, tilingArray, vsn
Suggests Me	<u>AnnotationDbi, BiocCaseStudies, Biostrings, BufferedMatrixMethods, ExpressionView, GeneRegionScan, TurboNorm, beadarraySNP, beadarray, factDesign, hexbin, limma, made4, oneChannelGUI, siggenes</u>
Version	1.30.0

## **Bioconductor Annotation Data Packages**

Almost 600 bioconductor packages are annotation packages. These are an incredible value resource in bioconductor. These packages provide annotation on the genes on microarrays.

This resource can be searched by organism, chip manufacturer, chip name etc

For example click on chip manufacturer -> AffymetrixChip or Organism -> homo sapiens to get a list of relevant annotation packages.

Each Affymetrix array is represented by 3 annotation packages, .db, cdf and probe. For example the human GeneChip Human Genome U133 Plus 2.0 Array has the following

hgu133plus2.db	annotation data (chip hgu133plus2) assembled using data from public repositories
hgu133plus2cdf	chip description file (cdf)
hgu133plus2probe	probe sequence data (probe).

The latter two are compiled from Affymetrix or manufacturer information

To install an annotation package, follow the same guidelines as package installation

```
source("http://www.bioconductor.org/biocLite.R")
biocLite("hgu133plus2.db")
```

To find out about a package:

```
> library(hgu133plus2.db)
> hgu133plus2()
> hqu133plus2()
Quality control information for hgu133plus2:
This package has the following mappings:
hgu133plus2ACCNUM has 54675 mapped keys (of 54675 keys)
hqu133plus2ALIAS2PROBE has 73685 mapped keys (of 110538 keys)
hgu133plus2CHR has 40772 mapped keys (of 54675 keys)
hgu133plus2CHRLENGTHS has 93 mapped keys (of 93 keys)
hgu133plus2CHRLOC has 39212 mapped keys (of 54675 keys)
hgu133plus2CHRLOCEND has 39212 mapped keys (of 54675 keys)
hgu133plus2ENSEMBL has 37294 mapped keys (of 54675 keys)
hgu133plus2ENSEMBL2PROBE has 18042 mapped keys (of 19887 keys)
hgu133plus2ENTREZID has 40801 mapped keys (of 54675 keys)
hgu133plus2ENZYME has 4616 mapped keys (of 54675 keys)
hgu133plus2ENZYME2PROBE has 928 mapped keys (of 936 keys)
```

hgu133plus2GENENAME has 40801 mapped keys (of 54675 keys)

hgu133plus2GO has 35250 mapped keys (of 54675 keys) hgu133plus2GO2ALLPROBES has 13288 mapped keys (of 13360 keys) hgu133plus2GO2PROBE has 10091 mapped keys (of 10161 keys) hgu133plus2MAP has 40571 mapped keys (of 54675 keys) hgu133plus2OMIM has 27795 mapped keys (of 54675 keys) hgu133plus2PATH has 11297 mapped keys (of 54675 keys) hgu133plus2PATH2PROBE has 214 mapped keys (of 214 keys) hgu133plus2PFAM has 39581 mapped keys (of 54675 keys) hgu133plus2PFAM has 39581 mapped keys (of 54675 keys) hgu133plus2PMID has 40160 mapped keys (of 54675 keys) hgu133plus2PMID2PROBE has 276342 mapped keys (of 283543 keys) hgu133plus2PROSITE has 39581 mapped keys (of 54675 keys) hgu133plus2REFSEQ has 40248 mapped keys (of 54675 keys) hgu133plus2REFSEQ has 40248 mapped keys (of 54675 keys) hgu133plus2SYMBOL has 40801 mapped keys (of 54675 keys) hgu133plus2UNIGENE has 40632 mapped keys (of 54675 keys) hgu133plus2UNIFROT has 37123 mapped keys (of 54675 keys)

Additional Information about this package:

DB schema: HUMANCHIP\_DB DB schema version: 2.1 Organism: Homo sapiens Date for NCBI data: 2010-Sep7 Date for GO data: 20100904 Date for KEGG data: 2010-Sep7 Date for Golden Path data: 2010-Mar22 Date for IPI data: 2010-Aug19 Date for Ensembl data: 2010-Aug5

### **Bioconductor Experiment Data Packages**

Experiment data package contain published data pre-prepared for Bioconductor. For example these packages include data from leukemia gene expression studies (Golub et al., 1999), colon cancer gene expression profiling (Alon et al. 1999), etc. Many of the tutorials (vignettes) in Bioconductor use these data in exercises.

## Bioconductor and R Help

The first place to find help on R and Bioconductor is their website. These provide an excellent source of information.

In particular it is worth examining the manuals, FAQ and searchable archives of the mailing lists. **Do subscribe to the Bioconductor and R mailing lists.** When subscribing click the option "daily digest" as these mailing list as busy with >20 emails a day and could quickly fill up your email inbox. The daily digest option, send 1 email a day which contains all the correspondence from that day.

### <u>R Help</u>

My favorite beginners guide to R is from Emmanuel Paradis. Its available from <u>http://cran.r-project.org/doc/contrib/Paradis-rdebuts\_en.pdf</u>

Introduction to R classes and objects http://cran.r-project.org/doc/manuals/R-intro.html

Tom Short's R reference card (<u>http://cran.r-project.org/doc/contrib/Short-refcard.pdf</u>) and other contributed are useful <u>http://cran.r-project.org/other-docs.html</u>

I have taught several R courses (Bio503, HSPH) and the course notes are online. I hope you find them useful also. The 2011 course is available at <a href="http://isites.harvard.edu/icb/icb.do?keyword=k76038">http://isites.harvard.edu/icb/icb.do?keyword=k76038</a> but I will this the most recent course from my HSPH website homepage <a href="http://www.hsph.harvard.edu/research/aedin-culhane/">http://www.hsph.harvard.edu/research/aedin-culhane/</a>

### **Bioconductor Help**

I found the Bioconductor course and workshop examples very useful when I was learning. These are available at <u>http://www.bioconductor.org/workshops</u>

For more information on getting started in R and Bioconductor, please see Vince Carey's guide at <u>http://bosbioc.wordpress.com/</u>

Thomas Girke, UC Riverside has also written excellent online tutorials on R and bioconductor available from <u>http://manuals.bioinformatics.ucr.edu/</u>

## Interfaces for R

I have used all of these below at one stage or another and all color R code, highlighting brackets and are useful

Rstudio (my current favorite)

Cross platform- available on Windows, Linux, MacOS etc <u>http://www.rstudio.org/</u>

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€) Instanticus III ×         Dennes +           B)         Envires in faire % ∠ × ±           A mare Prices IIII faires         Mare Arrison Michigan           Instanticus IIII faires         Mare Arrison Michigan           Instanticus IIII faires         Instanticus IIII faires           Instanticus IIII faires         Instanticus IIII faires           Instanticus IIII faires         Instanticus IIII faires           Instanticus IIIII faires         Instanticus IIIII faires           Instanticus IIIII faires         Instanticus IIIII faires	en) Enterlande (Enterlat	Warkspace Minkery (************************************	n 20
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V. And Second Se	<ul> <li>Conditions.</li> <li>Formation of the additional helps:</li> </ul>	n, mantoos or data θωτικό κλοβλ πουτ [1] = 193,345,	

### TinnR

Only available on Windows http://www.sciviews.org/Tinn-R/



### NotePad++ (with the plugin NpptoR)

Only available on Windows http://notepad-plus-plus.org/ http://sourceforge.net/projects/npptor/



On Linux I have also used **Kate** the KDE text editor which has inbuilt highlighting of R code. On Mac, many users simply use the standard R GUI which is pretty good and has some features that are better than the version that comes on Windows/Linux.