Introduction to R

Anindya Bhattacharya, PhD | abhatta3@ucla.edu CBI fellow | Xia Yang lab

Workshop 3: Introduction to R

Day 2

- Statistical methods
 - Probability distributions
 - Random number generation
 - Common statistical tests
 - ☐ Fisher's exact test, chi square, qvalue, etc...
- Visualization
 - Plotting functions
 - Customizing plots
 - Saving plots for publication
 - IGV
 - CummeRbund



Statistics

- Many distributions and tests built in
- Others added as packages
- Interpreting output, names, pvalues



Basic stat functions

- summary()
- var()
- ▶ sd()
- mean()
- median()

Probability distributions

- For every statistical distribution in R, these functions are available (Four fundamental items for a statistical distribution)
 - Cumulative distribution function
 - Probability of "hitting" x or less in a distribution. >pnorm(160, mean=132, sd=13) [1] 0.9843739 dnorm(0)
 - Density or point probability
 - relative probability of getting a value close to x
 >x<-seq(-4,4,0.1)
 >plot(x, dnorm(x), type="l")
 #letter l=line curve
 - Quantiles
 - Opposite to Cumulative distribution qnorm(0.01)
 - Pseudo-random numbers
 - #10 random number
 rnorm(10)
 rnorm(10,mean=7,sd=5)



Example distributions

- Gaussian or normal
 - ▶ dnorm, pnorm, qnorm, and rnorm
- binomial
- poisson
- gamma
- beta
-) t
- chi-square



Common statistical tests

- cor.test()
- t.test()
- fisher.test()
- chisq.test()
- wilcox.test()
- ks.test()
- p.adjust()
- ightharpoonup regression: $Im(y \sim x)$
- anova:aov()



Try it out: ALL data

- x = read.table("ALL_subset.txt", header=T)
- Compare two samples by t.test
- t.test(x[,1],x[,2])
- Compute the correlation between two samples
- cor.test(x[,1],x[,2])
- ▶ Compute all pairwise correlations, returns matrix
- cor(x)



A chisquare example

```
> mat = matrix( c(1463, 48486, 7892, 334758), nrow=2, ncol=2)
> mat
      [,1] [,2]
[1,] 1463 7892
[2,] 48486 334758
> chisq.test(mat)
        Pearson's Chi-squared test with Yates' continuity
correction
data: mat
X-squared = 73.1195, df = 1, p-value < 2.2e-16
# Critical value. Quantile function from Chi-square distribution
>achisa(0.95,1)
```



Simple Regression

```
> data(iris)
> head(iris)
> # attach(iris) - did not run
> names(iris)
[1] "Sepal.Length" "Sepal.Width" "Petal.Length" "Petal.Width"
"Species"
> lm(iris$Sepal.Length ~ iris$Petal.Length)
Call:
lm(formula = Sepal.Length ~ Petal.Length)
Coefficients:
 (Intercept) Petal.Length
      4.3066 0.4089
\# Sepal.Length = 4.3066 + 0.4089 * Petal.Length
```

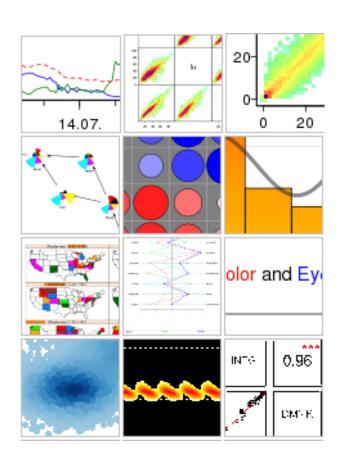


Simple Regression

```
> a = lm(iris$Sepal.Length~ iris$Petal.Length)
> names(a)
[1] "coefficients" "residuals" "effects"
                                                  "rank"
                                                                 "fitted.values" "assign"
"df.residual" "xlevels"
[10] "call"
                    "terms"
                                   "model"
> summary(a)
Call:
lm(formula = Sepal.Length ~ Petal.Length)
Residuals:
    Min
              10 Median 30
                                       Max
-1.24675 -0.29657 -0.01515 0.27676 1.00269
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 4.30660 0.07839 54.94 <2e-16 ***
Petal.Length 0.40892
                       0.01889 21.65 <2e-16 ***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 '' 1
Residual standard error: 0.4071 on 148 degrees of freedom
Multiple R-squared: 0.76, Adjusted R-squared: 0.7583
F-statistic: 468.6 on 1 and 148 DF, p-value: < 2.2e-16
```

Plotting

- A great strength of R is visualization
- Many type of plots:
 - line
 - histogram
 - scatter plot
 - topographical
- A great display of R graphs at http://gallery.r-enthusiasts.com/



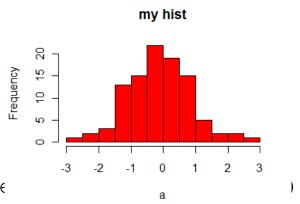


Very useful!

```
> a = rnorm(100)
```

- > hist(a)
- > hist(a, breaks=10, col="red", main="my hist")

hist(a, breaks=c(-30, 0, 1, 4), col="re



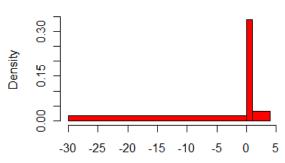
breaks can be customized

$$> br = c(-30, 0, 1, 4)$$

> br

$$[1]$$
 -30 0 1

> res = hist(a, breaks=br, col="red")



Histogram of a

returns an object of class "histogram"

```
> result.a = hist(a)
> class(result.a)
[1] "histogram"
> names(result.a)
[1] "breaks" "counts" "intensities" "density"
"mids"
[6] "xname" "equidist"
```



```
> result.a
$breaks
[1] -3 -2 -1 0 1 2 3
$counts
[1] 3 16 37 34 7 3
$intensities
[1] 0.03 0.16 0.37 0.34 0.07 0.03
$density
[1] 0.03 0.16 0.37 0.34 0.07 0.03
$mids
[1] -2.5 -1.5 -0.5 0.5 1.5 2.5
$xname
[1] "a"
$equidist
[1] TRUE
attr(,"class")
[1] "histogram"
>
```



```
> help(hist)
hist(x, breaks = "Sturges",
       freq = NULL,
       probability = !freq,
       include.lowest = TRUE,
       right = TRUE,
       density = NULL,
       angle = 45,
       col = NULL,
       border = NULL,
       main = paste("Histogram of", xname),
       xlim = range(breaks),
       ylim = NULL,
       xlab = xname, ylab, axes = TRUE,
       plot = TRUE,
       labels = FALSE,
       nclass = NULL,
       warn.unused = TRUE, ...)
```

Try it out

```
#Read in the ALL dataset
> x = read.table("ALL subset.txt")
# how many expts are there? how many probes?
> dim(all)
# plot histogram
> hist(x[,1], xlim=c(0,25), xlab = "tryout workshop2")
> hist(x[,2], xlim=c(0,25), col="blue", add=T)
> hist(x[,3], xlim=c(0,25), col="red", add=T) \# add=T: overlap the
plot.
# box plot of all expts; three expts
> boxplot(x)
boxplot(x[, c(1,12,3)])
```

Plotting

plots

- histogram
- boxplot
- barplot
- scatter plot
- heatmap
- cluster
- points, lines, segments
- abline
- qqplot
- & more

customizations

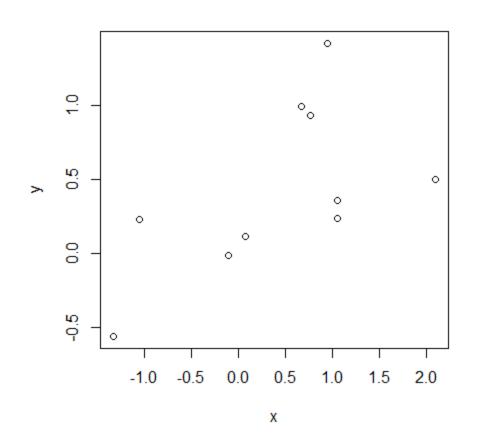
- axes
- header text/title
- margin text
- identify
- display symbols
- layout
- colors



A plain scatter plot

```
x = rnorm(10)
y = rnorm(10)
plot(x,y)

plot(x,y,pch="&")
```





Graphical parameters

adj	controls text justification with respect to the left border of the text so that
	0 is left-justified, 0.5 is centred, 1 is right-justified, values > 1 move the text
	further to the left, and negative values further to the right; if two values are
	given (e.g., c(0, 0)) the second one controls vertical justification with respect
	to the text baseline
bg	specifies the colour of the background (e.g., bg="red", bg="blue"; the list of
	the 657 available colours is displayed with colors())
bty	controls the type of box drawn around the plot, allowed values are: "o",
	"l", "7", "c", "u" ou "]" (the box looks like the corresponding character); if
	bty="n" the box is not drawn
cex	a value controlling the size of texts and symbols with respect to the default; the
	following parameters have the same control for numbers on the axes, cex.axis,
	the axis labels, cex.lab, the title, cex.main, and the sub-title, cex.sub
col	controls the colour of symbols; as for cex there are: col.axis, col.lab,
	col.main, col.sub
font	an integer which controls the style of text (1: normal, 2: italics, 3: bold, 4:
	bold italics); as for cex there are: font.axis, font.lab, font.main, font.sub
las	an integer which controls the orientation of the axis labels (0: parallel to the
	axes, 1: horizontal, 2: perpendicular to the axes, 3: vertical)
lty	controls the type of lines, can be an integer (1: solid, 2: dashed, 3: dotted,
	4: dotdash, 5: longdash, 6: twodash), or a string of up to eight characters
	(between "0" and "9") which specifies alternatively the length, in points or
	pixels, of the drawn elements and the blanks, for example 1ty="44" will have
	the same effet than lty=2
lwd	a numeric which controls the width of lines
mar	a vector of 4 numeric values which control the space between the axes and the
	border of the graph of the form c(bottom, left, top, right), the default
	values are c(5.1, 4.1, 4.1, 2.1)
mfcol	a vector of the form c(nr,nc) which partitions the graphic window as a ma-
	trix of nr lines and nc columns, the plots are then drawn in columns (see
	section 4.1.2)
mfrow	id. but the plots are then drawn in line (see section 4.1.2)
pch	controls the type of symbol, either an integer between 1 and 25, or any single
-	character within "" (Fig. 2)
ps	an integer which controls the size in points of texts and symbols

pty	a character which specifies the type of the plotting region, "s": square, "m": maximal
tck	a value which specifies the length of tick-marks on the axes as a fraction of the smallest of the width or height of the plot; if tck=1 a grid is drawn
tcl	id. but as a fraction of the height of a line of text (by default tcl=-0.5)
xaxt	if xaxt="n" the x-axis is set but not drawn (useful in conjunction with axis(side=1,))
yaxt	if yaxt="n" the y-axis is set but not drawn (useful in conjunction with axis(side=2,))

R for Beginners E. Paradis



Printing character

- you can specify the pch parameter
 - a number (pch=1 gives a circle)
 - a text character
 (pch="v" uses the
 letter "v")

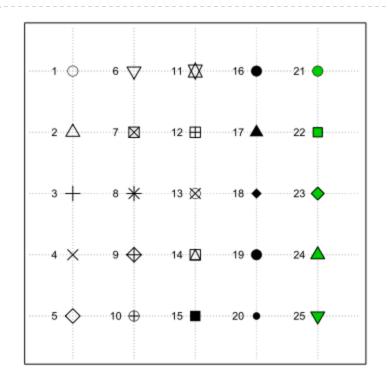


Figure 5: Plotting symbols



Try it out

```
#Read in the ALL dataset
> x = read.table("ALL subset.txt")
# how many expts are there? how many probes?
> dim(all)
# plot the first three expts against the first on the same page
> par(mfrow=c(1,3)); # speicify the space for three plot.
> plot(x[,1], x[,2], pch=8);
> plot(x[,1], x[,3]);
> plot(x[,1], x[,4],col="red",pch=4)
#close the window
> dev.off()
# plot one graph again, what's different?
plot(x[,1], x[,2])
# change some plotting options
> plot(x[,1], x[,2], pch=".", col="blue", xlab="expt1", ylab="expt2")
# add a title with the correlation in it
> x.cor = cor.test(x[,1], x[,2])
> title(paste("Expt 1 versus Expt 2\n cor=", round(x.cor$estimate, 2))) # 2\n mean new line.
# log transform
   plot(log2(x[,1]), log2(x[,2]), pch=".", col="blue", xlab="expt1", ylab="expt2", main="Expt1 versus Expt2")
   X=1:10
  Y=11:20
  Plot(x,y)
  Plot(x,y, type="1")
  Plot(x,y, type="b")
  Plot(x,y, type="h")
```

Scatter plots, line plots

- the type parameter controls the scatter plot type
 - options include points, lines, points+lines and histogram

```
> somedata = rnorm(25)
> plot(somedata, type="p")
> plot(somedata, type="l")
> plot(somedata, type="b")
> plot(somedata, type="h")
  xI=rnorm(100000)
  y1=rnorm(100000)
 x1=rnorm(100000, mean=2, sd=1.5)
  x2=rnorm(100000, mean=2, sd=1.5)
  y2=rnorm(100000, mean=2, sd=1.5)
  xI=rnorm(100000)
  Xy=rbind(cbind(x1,y1), cbind(x2,y2))
  smoothScatter(xy)
```

Id=sample(1:100, 25)



A few tips

- If you have many points (100-1000's), the plot may become obscured
- Potential remedies:
 - downsample your data: suppose you have 1000 rows of data in a data frame, you can pick some indices to display

```
# this will pick 25 numbers between 1 and 100
ids = sample(1:100, 25)
```

try a smaller plotting symbol: pch="." will use a dot
plot(x[ids, 1], x[ids, 2], pch=".")



smoothScatter

```
x1 = rnorm(100000)
y1 = rnorm(100000)
x2 = rnorm(100000, mean=2, sd=1.5)
y2 = rnorm(100000, mean=3, sd=1.5)
xy = rbind(cbind(x1, y1), cbind(x2, y2))
smoothScatter(xy)
```

Alpha values

```
# colors are given as amount of
# Red, Green, Blue and Alpha with range [0,1].
# you can specify maxColorValue=255 if you want to use [0,255]
plot(x,y, col=rgb(0,0.5,0,0.1))
```

- ▶ lines()
- abline(lm (y ~x))
- points()
- legend()
- qqnorm
- identify()
- barplot(), stacked barplot
- pie()
- boxplot()

Adding additional points to a plot

points() lets you add points to an existing plot

```
x = rnorm(10); y=rnorm(10)
xx = rnorm(10); yy = rnorm(10)
plot(x, y)
points(xx, yy, col="green", pch="v")
```

If nothing shows up, your axes might not contain the data area



More plots

Hierarchical clustering

```
all = read.table("ALL_subset.txt")
hier1 = hclust( dist(1-cor(all)))
plot(hier1)
```

Heatmap

```
heatmap(cor(all), symm=T)
```

Multiple plots per page

Use par

```
par(mfrow=c(2,1))
```

Another method is to designate matrix of slots

```
layout( matrix(c(1,2,3,4), ncol=2, nrow=2))
for(i in 1:4) {
    plot(rnorm(10))
}
# compare with
layout(matrix(c(1,1,2,3),2,2)
```

 Once you close a window, settings disappear



Graphics devices

- R will "write" plots to a graphics device
- If you don't have one, for example you ssh into a remote machine, it will write to a file called Rplots.pdf in your current directory.

```
# show your current graphic device
dev.cur()
# show all devices
dev.list()
# select a device (from already open)
dev.set()
# create a new window
x11()
```



Output formats

- pdf(), png(), bmp(), tiff(), jpeg(), formats all supported
- png(filename="file.png", width=800, height=800)
- dev.cur(), dev.off(), dev.set()
- To create a graphic, think of it as writing an image to a file

```
pdf(file="foo.pdf")
plot(rnorm(100))
dev.off()
```

Don't forget to dev.off() your device, otherwise your figure will get corrupted!

▶ pl



Fancier graphics

- There are more sophisticated plotting paradigms for multipanel, multidimensional data
 - lattice
 - trellis
 - ggplot



Extending R

- Many distributions and tests built in
- Others added as packages
 - available from CRAN
 - bioconductor is specialized for life sciences
- Packages essentially add new functionality to R

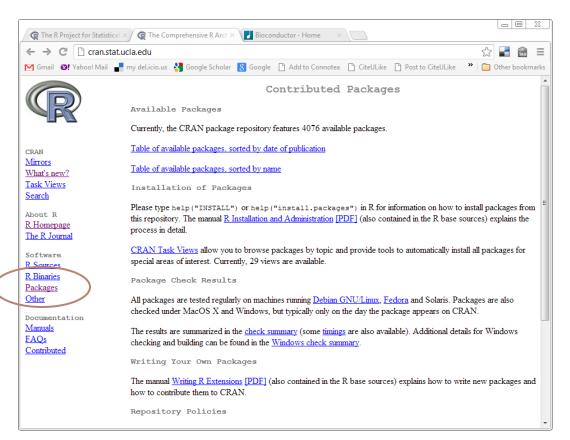


Installing package from CRAN

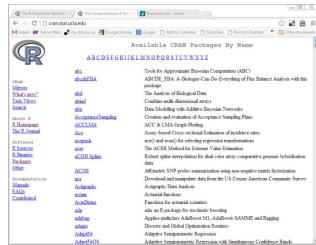
- installing packages is usually easy
- biggest problems are due to incompatibility of versions of R with versions of a package
 - eg MASS ver 2 requires R version >= 2.13
- source code is always available
- install.packages()
- Where do files get stored?
 - > .libPaths()
 - [1] "/Library/Frameworks/R.framework/Resources/library"



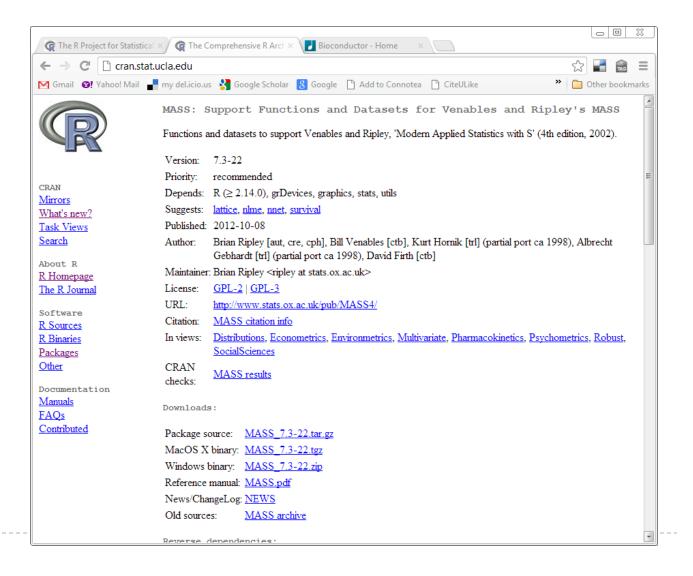
Finding packages







Finding packages



Using the GUI

- Use "Package" menu
- select a mirror (aka geographically close repository)
- Repo should include CRAN
- Install packages allows you to select
- Update packages allows you to select which packages to update



Troubleshooting install problems

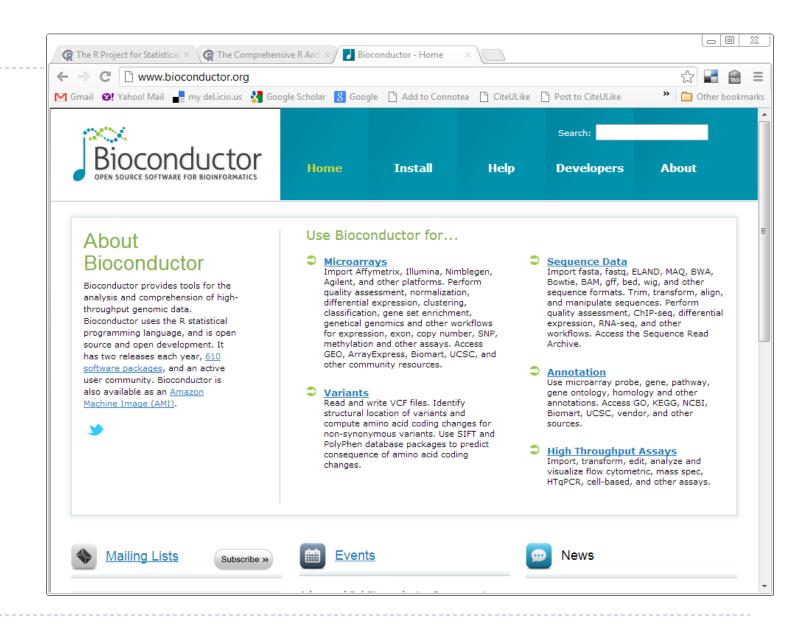
- Usually a dependency is missing
 - missing dev package
 - out of date package
 - > sometimes R needs access to a C or Fortran compilier
 - need administrator access



Installing from bioconductor

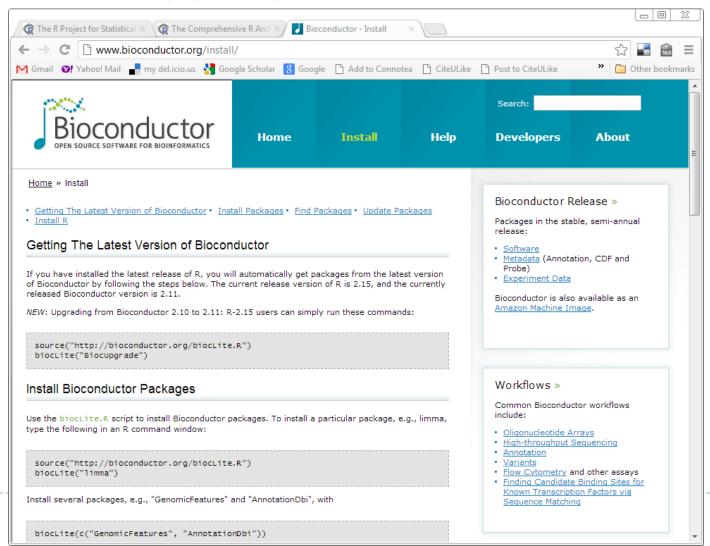
- set of packages tailored to life sciences
- requires its own installation method but relies on many tools from CRAN
- navigating the bioconductor website
- source("http://bioconductor.org/biocLite.R")
- example datasets





C:\Users\KGong\AppData\Local\Temp\RtmpcIximR\downloaded_packages

nloaded binary packages are in



Exercise

Try and install bioconductor packages

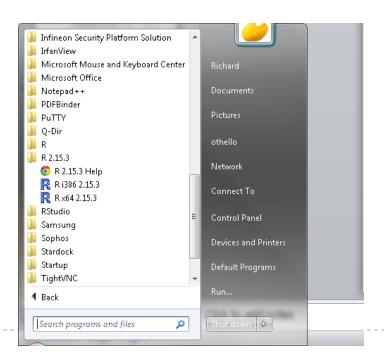
- GenomicFeatures
- cummeRbund
- DEseq
- Both will require several (many?) additional packages, so let's them install

```
source("http://bioconductor.org/biocLite.R")
# this installs core bioconductor packages
biocLite()
# install Rsamtools
biocLite("Rsamtools")
# GenomicFeatures and gene positions
biocLite("GenomicFeatures")
biocLite("TxDb.Hsapiens.UCSC.hg19.knownGene")
# this installs DEseq2
biocLite("DESeq2")
# other useful data sets
biocLite(c("pasilla", "parathyroidSE"))
# this would install cummeRbund
biocLite("cummeRbund")
```



Exercises

- If you run into trouble installing packages
 - On a PC running Windows
 - Try running R in "administrator mode"
 - From the start menu, right click on the R program and select "Run as administrator"





Exercises

- If you run into trouble installing packages
 - On a Mac
 - You may need administrator privileges
 - On Unix/Linux
 - You may need to become root to install to the correct directory
 - ▶ Try running sudo either
 - > sudo R
 - ▶ OR
 - > sudo su
 - > R
 - > And then installing packages



Additional resources

- Many resources but sometimes difficult to access
 - R-bloggers.com
 - R-project.org FAQ and manuals
 - bioconductor.org
 - UCLA ATS (http://www.ats.ucla.edu/stat/r/)
 - learning modules
 - Quick R http://www.statmethods.net/index.html
 - http://onertipaday.blogspot.com/
 - http://wiki.stdout.org/rcookbook/
 - http://gettinggeneticsdone.blogspot.com/
 - manuals.bioinformatics.ucr.edu/workshops

Good free books

- ▶ An Introduction to R (on r-project.org) Venables, Smith, R Core
- Using R
- R for Beginners by Emmanuel Paradis



Some R books

- R in Action, Robert I. Kabacoff
- R Cookbook, Paul Teetor
- ▶ The R book, Michael Crawley
- Introduction to the R Project for Statistical Computing for Use at the ITC, Rossiter



More Resources

- software-carpentry.org
- Google developer R lectures on Youtube
 - http://www.youtube.com/watch?v=iffR3fWv4xw&list=PLOU2X LYxmsIK9qQfztXeybpHvru-TrqAP



ggplot2

- library(ggplot2)
- > install.packages("ggplot2")