Introduction to R/Bioconductor

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- R is a free, open source programming language
- R is an integrated suite of software facilities for data manipulation, simulation, calculation and graphical display
- R runs on a wide variety of platforms such as Linux, Windows, and macOS
- The R project web page http://www.r-project.org http://www.r-project.org

- Handles and analyzes data very effectively
 - Contains a suite of operators for calculations on arrays and matrices
- Has the graphical capabilities for very sophisticated graphs and data displays
- An elegant, object-oriented programming language
- The use of variables, loops, merging operations and data sets, as well as downloadable add-on packages to enhance functionality, make the R programming options powerful and endless
- R is one of the most widely used language in Bioinformatics

Rstudio

RStudio is a convenient interface and allows the user to run R in a more user-friendly environment

http://www.rstudio.com/products/rstudio/download/



R Studio is a IDE with 4 Windows on the main screen

- Code: for coding / code checker
- Console: coding calculations and display results
- Work Space: list of objects and calculations that are created
- Five tab window
 - Files: stored and imported files
 - Plot: displayed area, graph view selection, export option
 - Packages: list of packages installed on system, option to search and install other packages
 - Help: more information about functions, arguments, user manuals, etc
 - Viewer: used to view local web content such as widgets, rCharts, and applications

RStdio

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RStdio



• R is a case-sensitive, interpreted language

- For example, a and A are two different objects.
- Two ways to run R command
 - Enter commands into the R console window at the command prompt (>)
 - Create R-scripts in an editor and save them in a file (filename.R) for later re-use.

Getting Started with R

Data management

- Data importing.
- Write data into files.
- Save data as an image.

Data process

- Types of data.
- Arithmetic of data.
- Functions
- Packages

- Data can be entered from the console.
- Larger data often be read as values from external files rather than entered at the keyboard.
- Different data formats that can be imported into R and different functions to call them.
 - .CSV
 - .txt
 - HTML table
 - Excel

Data Import

CSV

• TXT

- R also contains many datasets that are built-in to the software
- These datasets are stored as data frames. To see the list of datasets, use

> data()

• Then, a window will open and the available datasets are listed

Building-in data set in R : an example

Example: to open the dataset called Orange

> data (Orange)

After doing so, the data frame Orange is now in your workspace.

```
#
    To learn more about this data, type
    ?Orange
  > Orange
     Tree age circumference
    1 118 30
  1
    1 484 58
  2
  3 1 664 87
    1 1004 115
    1 1231 120
  5
    1 1372 142
  6
       582 145
Instructor: Mary Yang, PhD (Graduate Assist
```

Data Export

• CSV:

write.csv(data, file = "path/file_name.csv")

TXT

```
write.table(data, file = "path/file_name.txt", row.names =
    FALSE, col.names = TRUE)
```

• The argument **row.names** = **FALSE** makes that no row names are written to the file. Because nothing is specified about col.names, the default option **col.names** = **TRUE** is chosen and column names are written to the file.

help

More options

help (write.csv) ?write.csv ?write.table

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>			write.table	e(x, file =	"", append = FALSI	E, quote = TRUE, sep = " ",
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> ?writ	e.table			fileEncodi	ng = "")	
>		0	write.csv()		

Save the data into image for reuse by R

save(data, file = "data.RData")
load("data.RData")

Data process: computing with R

- When variables are used, they need to be **initialized** with numbers.
- R can take as arguments for its functions single numbers, vectors, matrices, or data frames.
- The assignment operator is "←". Alternatively, as of R version 1.4.0, you can use "=" as the assignment operator.

A = 5 A <- 5

Туреѕ	Examples
Integer: Natural numbers	1, 2, 3
Numeric: Decimal values	1.5, 2.2, 3.7
Logical: Boolean values	TRUE or FALSE (T or F)
Character: Text or string values	"cat" "blue"

Data Structures

• R has a wide variety of data structures:

- vector
- matrix
- data frame
- list

	Homogeneous	Heterogeneous
ld	Vector	List
2d	Matrix	Data frame
nd	Array	

```
# numeric vector
x = c(1,2,5.3,6,-2,4)
#character vector
y = c("one","two","three")
#logical vector
z = c(TRUE,TRUE,TRUE,FALSE,TRUE,FALSE)
```

Vector

```
#replicates the values in x
> a = rep (1, 6)
> a
[1] 1 1 1 1 1 1
```

```
#Generate regular sequences.
> a = seq (1, 6)
> a
[1] 1 2 3 4 5 6
#Or
> a = 1:7
> a
[1] 1 2 3 4 5 6 7
```

Vector

[1] 2

You can refer to elements of a vector using subscripts. > a [1] 1 2 3 4 5 6 > a[2] #Access the second element of the vector

> a[c(2,4)] #Access the second and fourth elements of the vector [1] 2 4

> a[1:3] #Access the first three elements of the vector [1] 1 2 3

• All columns in a matrix must have the same mode(numeric, character, etc.) and the same length. The general format is

matrix(vector, nrow = r, ncol = c, byrow = FALSE, dimnames = list(char_vector_rownames, char_vector_colnames))

- byrow = TRUE indicates that the matrix should be filled by rows.
- byrow = FALSE indicates that the matrix should be filled by columns (the default).
- dimnames provides optional labels for the columns and rows.

```
# generates 3 * 5 numeric matrix
> x = matrix(1:15, nrow=3,ncol=5)
```

```
#Default byrow=FALSE
```

> x

	[,1]	[,2]	[,3]	[,4]	[,5]
[1,]	1	4	7	10	13
[2,]	2	5	8	11	14
[3.]	3	6	9	12	15

Matrix



> m

C1 C2 R1 1 26

R2 24 68

Matrix

You can identify rows, columns or elements using subscripts.

#4th column of matrix
> x[,4]

3rd row of matrix
> x[3,]

rows 2,3 of columns 1,2,3
> x[2:3,1:3]

Data Frame

A data frame is more general than a matrix, in that different columns can have different modes (numeric, character, factor, etc.)

```
> d = c(7,9,5, 20)
> e = c("red", "white", "red", NA)
> f = c(TRUE,TRUE,TRUE,FALSE)
> mydata = data.frame(d,e,f)
#Assign columns name
> colnames (mydata) = c("ID", "Color", "Status")
```

- > mydata
 - ID Color Status
- 1 7 red TRUE
- 2 9 white TRUE
- 3 5 red TRUE
- 4 20 <NA> FALSE

Data Frame

There are a variety of ways to access the elements of a data frame.

#	column	s 2,3 of	data	frame
>	mydata	[,2:3]		
	Color a	Status		
1	red	TRUE		
2	white	TRUE		
3	red	TRUE		
4	< N A >	FALSE		

```
# columns ID and Color from data frame
> mydata[,c("ID","Color")]
#Column ID
> mydata$ID
```

- A list is an ordered collection of objects (components).
- A list allows you to gather a variety of (possibly unrelated) objects under one name

example of a list with 4 components a string, a numeric vector
 , a matrix, and a scalar
> mylist <- list(name="Fred", my.numbers=a, my.matrix=x, age=5.3)</pre>

List

> mylist \$name [1] "Fred"

\$my.numbers
[1] 1 2 3 4 5 6 7

\$my.m	atriz	ĸ			
	[,1]	[,2]	[,3]	[,4]	[,5]
[1,]	1	4	7	10	13
[2,]	2	5	8	11	14
[3,]	3	6	9	12	15
\$age	. 3				

Identify elements of a list using the [[]] convention.

```
# 2nd component of the list
> mylist[[2]]
[1] 1 2 3 4 5 6 7
# component named my.numbers in list
> mylist[["my.numbers"]]
[1] 1 2 3 4 5 6 7
```

Useful Functions

```
# number of elements or components in a vector or a list
> length (object)
```

```
# structure of an object
```

```
> str(object)
```

```
# class or data type of an object
```

```
> class(object)
```

```
# list the variables in an object
> names(object)
```

```
# dimensions of an object, such as matrix or data frame
> dim (object)
```

```
# combine objects into a vector
```

```
> c(object,object,...)
```

```
# combine objects as columns
```

> cbind(object, object, ...)

combine objects as rows
> rbind(object, object, ...)

Data Conversion

Display the data type

> class (m)
[1] "matrix"
> class (x)
[1] "matrix"

Conversion from one type of data structures to another can easily be done

```
> m1 = as.data.frame(m)
> x1 = as.vector (x)
```

If unsure about the type, you can write

```
> is.data.frame(m1)
[1] TRUE
> is.data.frame(m)
[1] FALSE
> is.vector(x)
[1] FALSE
```

Why data conversion

heatmap {stats}

R Documentation

Draw a Heat Map

Description

A heat map is a false color image (basically <u>image(t(x)</u>)) with a dendrogram added to the left side and to the top. Typically, reordering of the rows and columns according to some set of values (row or column means) within the restrictions imposed by the dendrogram is carried out.

Usage

```
heatmap(x, Rowv = NULL, Colv = if(symm)"Rowv" else NULL,
distfun = dist, hclustfun = hclust,
reorderfun = function(d, w) reorder(d, w),
add.expr, symm = FALSE, revC = identical(Colv, "Rowv"),
scale = o("row", "column", "none"), na.rm = TRUE,
margins = c(5, 5), ColSideColors, RowSideColors,
cexRow = 0.2 + 1/log10(nr), cexCol = 0.2 + 1/log10(nc),
labRow = NULL, labCol = NULL, main = NULL,
xlab = NULL, ylab = NULL,
keep.dendro = FALSE, verbose = getOption("verbose"), ...)
```

Arguments

x	numeric matrix of the values to be plotted.
Rowv	determines if and how the row dendrogram should be computed and reordered. Either a <u>dendrogram</u> or a vector of values used to reorder the row dendrogram or Na to suppress any row dendrogram. (and reorderica) or by default NULL see

R function: Control structure

 R includes the usual control-flow statements (like conditional execution and looping) found in most programming languages. These include (the syntax can be found in the help file accessed by ?Control):

```
if(cond) expr
if(cond) cons.expr else alt.expr
for(var in seq) expr
while(cond) expr
repeat expr
break
next
```

Loop: Perform the same process several times or on several variables

> for (i in 1:5){print (i)}
[1] 1
[1] 2
[1] 3
[1] 4
[1] 5

Example: Control structure and matrix

> mat = matrix (sample (100, 50), nrow = 10)
> mat

	[,1]	[,2]	[,3]	[,4]	[,5]
[1,]	93	37	16	52	75
[2,]	87	90	21	100	46
[3,]	67	27	88	33	17
[4,]	32	91	55	56	9
[5,]	26	36	31	38	47
[6,]	59	57	62	83	18
[7,]	60	66	10	79	99
[8,]	82	40	69	70	84
[9,]	74	15	35	94	97
[10,]	5	48	65	85	6

```
> m1 = rowMeans (mat)
> m1
[1] 54.6 68.8 46.4 48.6 35.6 55.8 62.8 69.0 63.0 41.8
> m2 = apply (mat, 1, mean)
> m2
[1] 54.6 68.8 46.4 48.6 35.6 55.8 62.8 69.0 63.0 41.8
```

Example: Control structure and matrix

```
> for (i in 1:10){
+ m3 = mean(mat[i,])
+ print (m3)
+ }
[1] 54.6
[1] 68.8
[1] 46.4
[1] 48.6
[1] 35.6
[1] 55.8
[1] 62.8
[1] 69
[1] 63
[1] 41.8
```

Example: Control structure and matrix

Tip: when performing loop on a matrix, Basic function (fastest) \rightarrow apply (faster) \rightarrow for loop (slow)

```
#fastest
> m1 = rowMeans (mat)
#faster
> m2 = apply (mat, 1, mean)
#slow
> for (i in 1:10){
+ m3 = mean(mat[i,])
+ print (m3)
+ }
```

Example 2: Control structure and matrix

Create a matrix

```
> height = c(6,5,5.4,6.1,6.5,5.5,5.9)
> student = c("boy", "girl", "girl", "boy", "boy", "girl" )
> mat = data.frame (height, student)
```

>	mat	
	height	student
1	6.0	boy
2	5.0	girl
З	5.4	girl
4	6.1	boy
5	6.5	boy
6	5.5	boy
7	5.9	girl

```
> for (i in 1:nrow(mat)){
   if (mat[i,2] == "girl"){
+
 print (mat[i,])
+
+ }
+ }
 height student
2
  5 girl
 height student
3
    5.4 girl
 height student
7
  5.9 girl
```

• Many of these statements require the evaluation of a logical statement, and these can be expressed using logical operators:

Operator	Meaning
==	Equal to
!=	Not equal to
<,<=	Less than, less than or equal to
>,>=	Greater than, greater than or equal to
&	Logical AND
	Logical OR

• R users can define their own function. The general format for creating a function is

functionName <- function(arg1, arg2, ...) { R code }</pre>

- In the above, **functionName** is any allowable object name and **arg1**, **arg2**, ... are function arguments.
- As with any R function, they can be assigned default values.
- When you write a function, it is saved in your workspace as a function object.

A user-written function

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<pre>1 * f1 = function (a, b){</pre>	Rue the surget line or calenting (M	Global Environment +		(Q,
2 #This function returns the maximum of two scale	ans kun the current line or selection (w) mydata	4 obs. of 3 variables	
3 #or the statment that they are equal		mygeneticprofile	7 obs. of 6 variables	
5. if (is numeric (c(a, b))){		💿 Orange	35 obs. of 3 variables	
6 if (a < b) return (b)		x	int [1:3, 1:5] 1 2 3 4 5 6 7 8	8 9 10 🔲
7 if (a > b) return (a)		Values		
8 else print ("The values are equal")		a	int [1:7] 1 2 3 4 5 6 7	
9 }		cells	num [1:4] 1 26 24 68	
10 else print ("character inouts are equal")		cnames	chr [1:2] "C1" "C2"	
*** *		d	num [1:4] 7 9 5 20	
11:2 (Top Level) 0	R Script 0	data_df	List of Ø	
	_	e	chr [1:4] "red" "white" "red"	NA
Console ~/ 🔗	La	f	logi [1:4] TRUE TRUE TRUE FALS	E
>		mycancerstudy	"hnsc_tcga_pub"	
> data()		mycaselist	"hnsc_tcga_pub_3way_complete"	
S TI - Turction (a b)C	_	mycgds	Classes 'CGDS', 'Object' atomi	c [1:1] NA
+ #This function returns the maximum of two scalars		O mylist	List of 4	
+ #or the statment that they are equal		rnames	chr [1:2] "R1" "R2"	
+		0 t	List of 4	
<pre>+ if (is.numeric (c(a, b))){</pre>		table	List of 0	
+ if $(a < b)$ return (b) + if $(a > b)$ return (a)		tables	List of Ø	
+ else print ("The values are equal")		u	" html \n <html class="</td"><td><pre>\"client-nojs\" lang=\"en\" di</pre></td></html>	<pre>\"client-nojs\" lang=\"en\" di</pre>
+ }		ulr	" <html>\r\n<head><title>400 Ba</title></head></html>	d Request\r\n <b< td=""></b<>
 + else print ("Character inouts are equal") 		url	"https://en.wikipedia.org/wiki	/List_of_countries_and_depende
+ }		Functions		
> T1(9, 10)		f1	function (a, b)	
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- The function object **f1** will remain in your workspace until you remove it or quit R session.
- You can save these commands in R script (f1.R) for later-use.

Save user-written function for later-use



Instructor: Mary Yang, PhD (Graduate Assist

```
> calcu = function (a, b){
+ result = (a + b * 5) ^ 2
+ return (result)
+ }
> calcu (4, 2)
[1] 196
> calcu (0.5, 0.1)
[1] 1
> calcu ("A" , "B")
Error in b * 5 : non-numeric argument to binary operator
```

- It is recommended that you write and edit all of your R code in a script before you run it in the console.
- It creates a reproducible record of your work.
- You can save your script and then use it to rerun your entire analysis.
- Scripts are also very handy for editing and proofreading your code

- When you open an R Script (File → New File →R Script in the menu bar), RStudio creates a fourth pane above the console where you can write and edit your code.
- RStudio comes with many built-in features that make it easy to work with scripts.
 - R will run whichever line of code your cursor is on by clicking the **Run** button
 - If you have a whole section highlighted, R will run the highlighted code by clicking the **Run** button
 - Alternatively, you can run the entire script by clicking the **Source** button.
- $\bullet\,$ To save a script, click the scripts pane, and then go to File $\to\,$ Save As in the menu bar

Built-In Function: numeric function

Function	Description
abs(x)	absolute value
sqrt(x)	square root
ceiling(x)	ceiling(3.475) is 4
floor(x)	floor(3.475) is 3
trunc(x)	trunc(5.99) is 5
<pre>round(x, digits=n)</pre>	round(3.475, digits=2) is 3.48
<pre>signif(x, digits=n)</pre>	signif(3.475, digits=2) is 3.5
$\cos(x)$, $\sin(x)$, $\tan(x)$	
log(x)	natural logarithm
log10(x)	common logarithm
exp(x)	e^x

Built-In Function: character function

Function	Description			
<pre>substr (x, start=n1, stop=n2)</pre>	Extract or replace substrings in a			
	character vector.			
	x <- "abcdef"			
	substr(x, 2, 4) is "bcd"			
	<pre>substr(x, 2, 4) <- "22222" is "a222ef"</pre>			
grep(pattern, x)	Search for pattern in x.			
gsub(pattern,	perform replacement of matches			
replacement, x)	determined by regular			
	expression matching			
<pre>strsplit(x, split)</pre>	Split the elements of character vector			
	Split the elements of character vector x at <i>split</i> .			
	strsplit("abc", "") returns 3 elemen			
	vector "a","b","c"			
paste(, sep="")	Concatenate strings after using sep			
	Split the elements of character vectors x at split. vector "a","b","c" Concatenate strings after using sep string to seperate them. pasto("y" 143 seperate them.			
	<pre>paste("x",1:3,sep="") returns</pre>			
	c("x1","x2" "x3")			
	<pre>paste("x",1:3,sep="M") returns</pre>			
	c("xM1", "xM2" "xM3")			
	<pre>paste("Today is", date())</pre>			
toupper(x)	Uppercase			
tolower(x)	Lowercase			

Built-In Function: statistical function

Function Description		
mean (x)	mean of object x	
sd (x)	standard deviation of object (x)	
median (x)	median	
range (x)	range	
sum(x)	sum	
min (x)	minimum	
max(x)	maximum	

 In R, missing values are represented by the symbol NA Test for missing values:

```
#Test for missing values
> is.na (x)
#Excluding Missing Values from Analyses
> mean (x, na.rm = TRUE)
#Create a new dataset without missing data
> newData = na.omit (myData)
```

R packages

• R started with basic packages. To see the list of all available packages on systems, you can type into the R console window

> library()

 To install a new package, you can either click on Packages-install package(s), or type commands into the console window:

```
> install.packages ("gplots") # install a package called
    gplots
> library (gplots)} #load gplots packages
```

• To update a package called gplots

> update.packages ("gplots")

R Package

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- All variables or "objects" created in R are stored in what's called the workspace
- To see what variables are in the workspace, you can use the function ls() to list them.
- To remove objects from the workspace use the rm() function:

```
# delete a object called
> rm (X)
```

Manipulating file paths in R

Pathnames in R are written with forward slashes "/", although in windows backslashes, " \backslash ", are used.

```
#To set a working directory in R:
> setwd("Directory name")
#To print the current working directory:
> getwd()
#To show the files in the current working directory:
> dir()
#To create a directory for your project - good way to organize
   your files
> dir.create("R Workshop 2017")
```

Manipulating file paths in RStudio

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>				bitops	Bitwise Operations	1.0-6	0

Getting Help

- R has an extensive help facility
- Apart from the Help window launched from the Help menu, it is also available from the command line prompt
- For instance

```
#explain about square root function
> help(sqrt)
> ?sqrt
```

• Most of the help files also include examples. You can run all of them by using the **example()**

```
#run all the examples from the matrix help file
> example (matrix)
```

#run all the examples from the plot help file
> example (plot)

- Bioconductor is an open source and open development software project for the analysis of biomedical and genomic data.
- The project was started in the Fall of 2001 and includes developers in many countries

- Provide access to powerful statistical and graphical methods for the analysis of genomic data.
- Facilitate the integration of biological metadata (GenBank, GO, Entrez Gene, PubMed) in the analysis of experimental data.
- Allow the rapid development of extensible, interoperable, and scalable software.
- Promote high-quality documentation and reproducible research.
- Provide training in computational and statistical methods.

Bioconductor packages

- General infrastructure
 - Biobase, Biostrings, biocViews
- Annotation:
 - annotate, annaffy, biomaRt, AnnotationDbi
- Graphics/GUIs:
 - geneplotter, hexbin, limmaGUI, exploRase
- Pre-processing:
 - affy, affycomp, oligo, makecdfenv, vsn, gcrm, limma
- Differential gene expression:
 - genefilter, limma, ROC, siggenes, EBArrays, factDesign
- GSEA/Hypergeometric Testing
 - GSEABase, Category, GOstats, topGO

Bioconductor packages

- Graphs and networks:
 - graph, RBGL, Rgraphviz
- Flow Cytometry:
 - flowCore, flowViz, flowUtils
- Protein Interactions:
 - ppiData, ppiStats, ScISI, Rintact
- Sequence Data:
 - Biostrings, ShortRead, rtracklayer, IRanges, GenomicFeatures, VariantAnnotation
- Other data:

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

#Install Bioiconductor package Rintact
> biocLite ("Rintact")