

Introduction to Data Analysis With R

Gladstone Institutes

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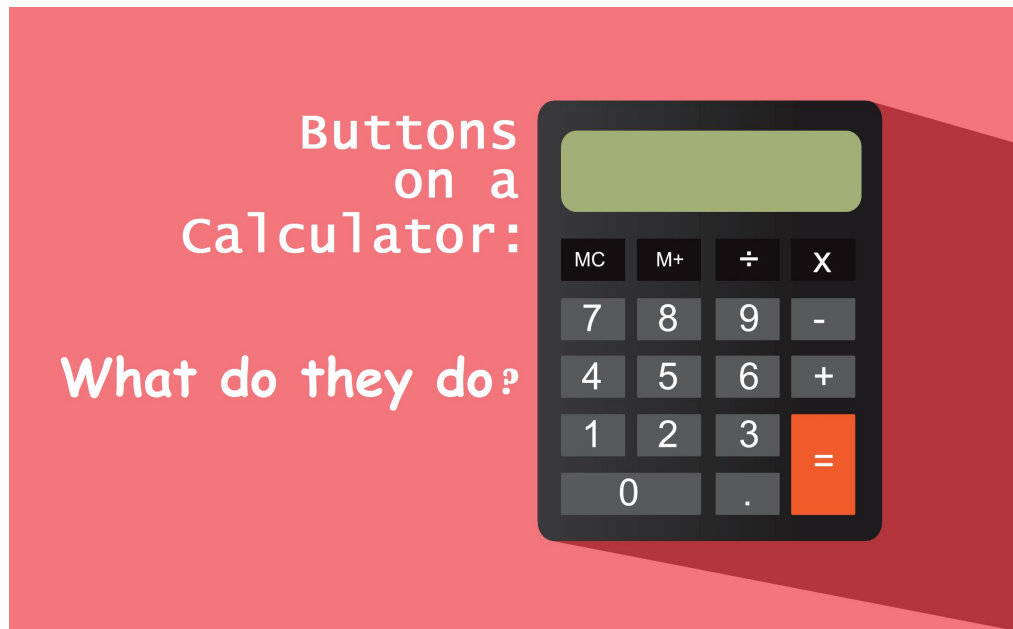
Contents

- ✦ Motivation
- ✦ Hands-on work in RStudio
- ✦ Exercises
- ✦ Conclusions

R topics covered

- ✦ RStudio interface.
- ✦ Addition, subtraction, basic math operations.
- ✦ Assigning values to variables.
- ✦ Commenting in a script.
- ✦ Logical operators.
- ✦ Intro to functions and libraries.
- ✦ Reading data.
- ✦ Troubleshooting error messages.
- ✦ Exploring data. (Basic summaries such as mean, median, etc.)
- ✦ Selecting subsets of data.
- ✦ Plotting data.
- ✦ Data structures available in R.

R:= A calculator with more “buttons” than you will ever use.



- ♦ R can do all that a calculator can.
 - ♦ Open R.
 - ♦ Try $2+3$.
 - ♦ Try $2*3$.
 - ♦ Try $(2+3)/5$.
- ♦ Conclusion: Off with the calculators!

What is R?

- ◆ Collection of “buttons” that will perform mathematical calculations when pressed
 - ◆ The way to press “buttons” in R is by writing a command. Examples:
 - ◆ `sum(2, 3)`
 - ◆ `prod(2,3)`
 - ◆ `sqrt(4)`
- ◆ Links for useful R commands:
 - ◆ <https://www.calvin.edu/~scofield/courses/m143/materials/RcmdsFromClass.pdf>
 - ◆ <https://www.personality-project.org/r/r.commands.html>
 - ◆ <https://www.rstudio.com/wp-content/uploads/2016/10/r-cheat-sheet-3.pdf>

Excel: A boat to explore the surface of “Data-lantic ocean”.
R: A submarine for deep exploration.

Excel



R



R offers a lot more than Excel.

Excel

- ◆ Familiarity.
- ◆ Simple visualizations only.
- ◆ Simple statistics only. Limited support for advanced needs.
- ◆ You know it's falling short. That's why you're here.
- ◆ Pay for an inferior option. Why?

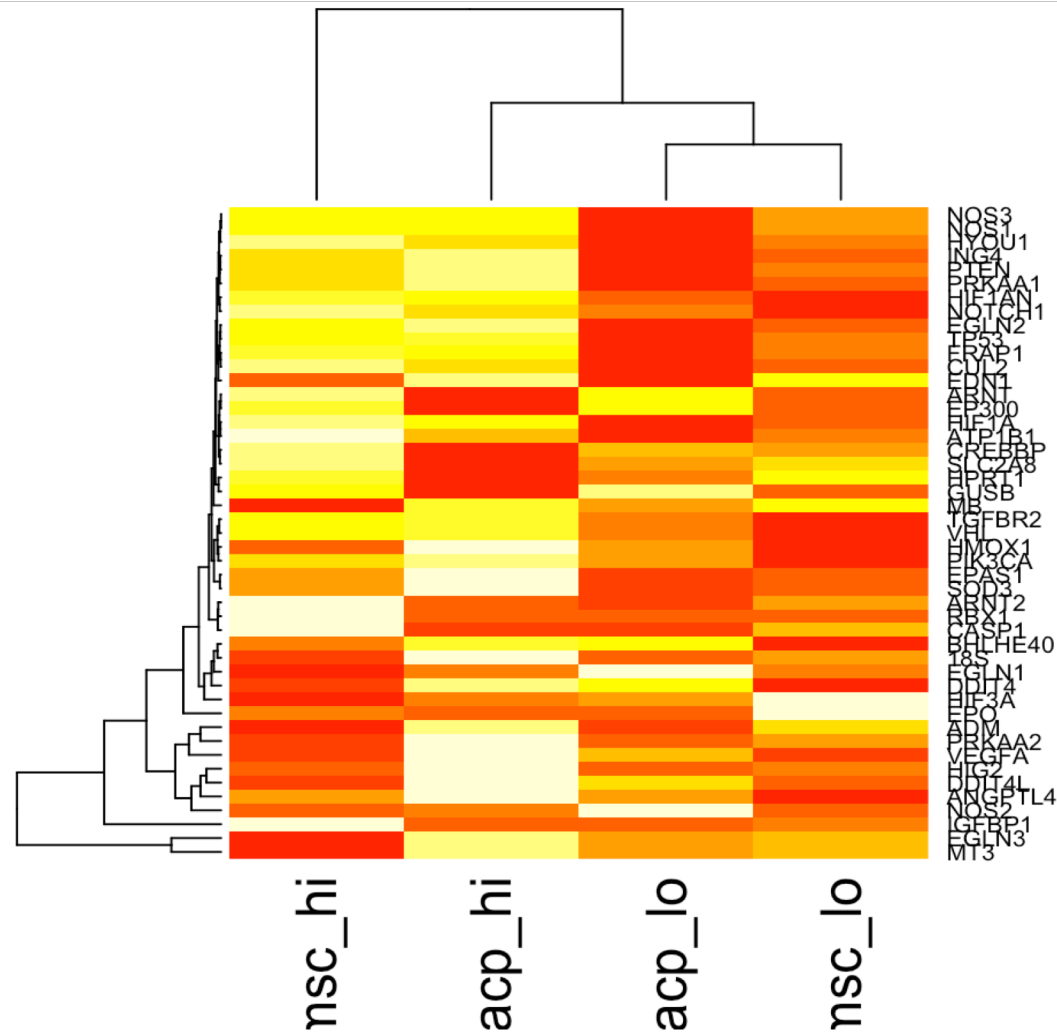
R

- ◆ Unknown territory.
- ◆ Visualization heaven.
- ◆ If there is a statistical method out there, you'll likely find it in R. All you need is to "hit a button".
- ◆ You will need it ever more.
- ◆ Free of cost.

R: designed specifically to support variety of biological data analysis.

- ✦ Bioconductor: Software for all kinds of biological data.
 - ✦ Understands file formats commonly used in biology.
 - ✦ Vetted by large community of users.
 - ✦ Frequently updated.
 - ✦ <https://www.bioconductor.org/>

R: easy visualization of biological data. Supports interactive visualization.



R: Increasingly becoming more central to biology.

- ✦ Biological software tools are being integrated with R.
- ✦ Upcoming training workshops may require R.

R vs Python: depends on what we need

- ◆ <https://www.datacamp.com/community/tutorials/r-or-python-for-data-analysis>
 - ◆ R is used by statisticians, engineers and scientists.
 - ◆ Python is popular among developers and programmers because it is faster.
 - ◆ R is primarily used in academics and research. Also common in industry for bioinformatics.
 - ◆ Unique attractions of R: ggplot2 for data visualization, Bioconductor

RStudio and basic arithmetic.

R is a console application.

- ✦ Text only interface. Inputs and outputs can be images.
- ✦ RStudio: Brings more Graphical User Interface (GUI) features to R.
 - ✦ RStudio is to R as Microsoft Word is to Notepad in Windows or TextEdit in Mac. (~kinda)
- ✦ Launch RStudio.
- ✦ Try from these:
 - ✦ `sum(2,3)`
 - ✦ `prod(2,3)`
 - ✦ `sqrt(5.5)`

Commenting inside a source script

- ♦ Looking at an old script, or someone else's script can be scary.
- ♦ Speak to machine in code.
- ♦ Leave comments in natural language (e.g., English).
 - ♦ Anything written after `#` is interpreted by R as a comment for humans.

Assignment *operators* in R

- ◆ Results of one calculation may be input to another.
- ◆ Save intermediate results by assigning them to variables.
- ◆ `a <- sum(2,3)`
- ◆ `a = sum(2,3)`
 - ◆ Variables store values.
 - ◆ Called so because they can be assigned any value.
- ◆ Both work but prefer to use '`<=`' for now.
- ◆ Change '`=`' to '`<=`' in the script and rerun.

Rules for naming variables

- ✦ Names can be a combination of letters, digits, period (.) and underscore (_).
- ✦ It must start with a letter or a period. If it starts with a period, it cannot be followed by a digit.
- ✦ Reserved words in R must not be used as variable names.

Empty workspace/environment



Data analysis creates objects that store data.

(top-right pane in RStudio)



Scientific calculations may require checking criteria.

- ♦ Is p-value less than 0.05?
- ♦ Is organism named *Homo sapiens*?
- ♦ What percent of patients have heart rate greater than 130 bpm?

Scientific calculations may require checking criteria.

- ♦ Is p-value less than or equal to 0.05?
 - ♦ $p \leq 0.05$
- ♦ Is organism named Homo sapiens?
 - ♦ `name == "Homo sapiens"`
- ♦ What percent of patients have heart rate greater than 130 bpm?
 - ♦ `bpm > 130`

Might need to check multiple criteria simultaneously.

- ✦ `p <= 0.05 & bpm > 130`
- ✦ `bpm == 120 & name == "Homo sapiens"`

Useful *operators* in R

- ◆ ! means 'NOT'
- ◆ != means 'NOT EQUAL'
- ◆ == means 'EQUAL'
- ◆ & means 'AND'
- ◆ | means 'OR'

Thus far...

- ✦ R console
- ✦ Source scripts
- ✦ Commenting
- ✦ Environment
- ✦ Simple calculations
- ✦ Variables

Coming up...

- ♦ Intro to functions and libraries.
- ♦ Reading data.
- ♦ Troubleshooting error messages.
- ♦ Exploring data. (Basic summaries such as mean, median, etc.)
- ♦ Selecting subsets of data.
- ♦ Plotting data.
- ♦ Data structures available in R.

Functions

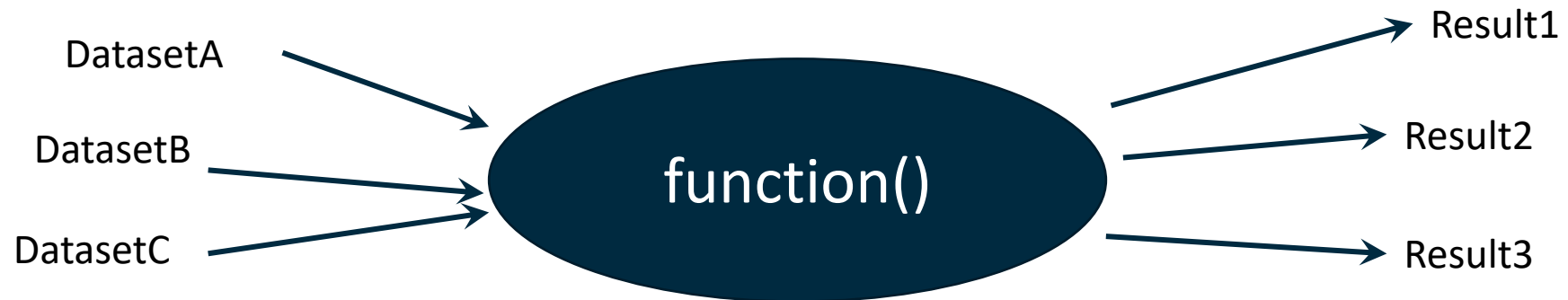
Working with buttons for modern scientific calculations?

- ♦ The best of calculators => 10s of buttons for specific tasks.
- ♦ Modern science needs many times more than 10s.



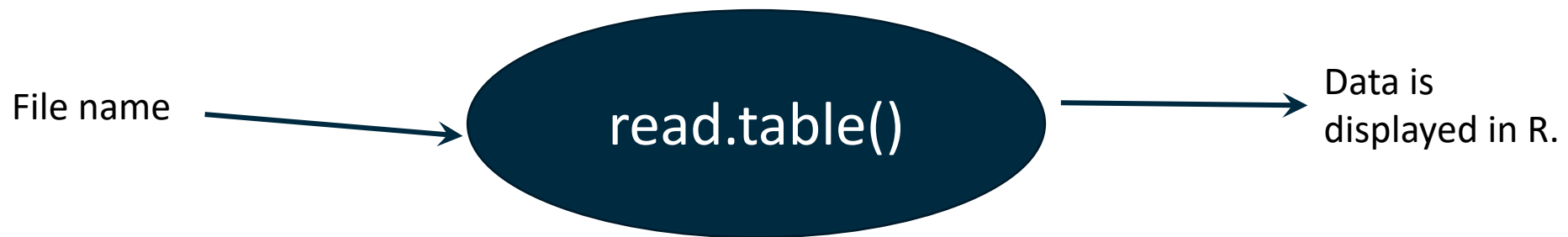
Functions are to R what buttons are to calculators.

- ♦ R can perform 100s of 1000s of tasks.
- ♦ Tasks are performed by using functions.
 - ♦ Examples: `sum()`, `prod()`, `mean()`, `t.test()`.



There are functions for all sorts of things.

- ◆ Example to read a table saved in a file:
 - ◆ `read.table()`
 - ◆ Usage: `read.table("filename")`

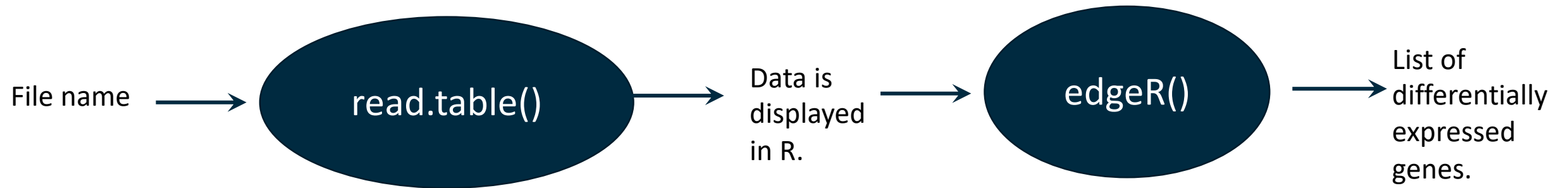


There are functions for all sorts of things.

- ♦ Example to find complementary DNA sequence.
 - ♦ `complement()`
 - ♦ Usage: `complement(sequence)`



Directing output of one function as input to another.



Library is a collection of functions.

- ✦ R works with > 100000 functions.
- ✦ More being added everyday.
- ✦ If the software were to load (i.e., make available) all of them at startup, it will take a while to launch.
- ✦ Solution: Bundle related functions together in a *library*.
 - ✦ Example: edgeR.
- ✦ Load functions only when needed. (:= Install apps on smartphone as needed)

Functions may need lots of information

- ✦ Calculators may take only numbers.
- ✦ R functions take variety of things. Numbers, characters, etc.
- ✦ Example:
 - ✦ Reading a file. Inputs required: File name and address on computer, formatting of file, type of file, etc.
 - ✦ Writing a file. Inputs required:
 - ✦ what to write?
 - ✦ where to write?
 - ✦ how to format the file?
 - ✦ Include column names, row names?
 - ✦ separate with comma?

Reading a data file.

There are functions for all sorts of things.

- ✦ Functions to read files:
 - ✦ `read.csv()`
 - ✦ `read.table()`
 - ✦ `read.xlsx()`
- ✦ Try reading a file called “iris.csv”
 - ✦ `read.table(file = “iris.csv”)`

Error!!!



How to find help?

- ♦ Execute a command name with the question mark before it. Example:
 - ♦ `?read.table`
- ♦ Read the error message!
 - ♦ Often, no need to understand every word.
 - ♦ Look for key words in error message.
- ♦ Copy-paste the error message verbatim in Google!

We need to set directory to where the file is.

- ♦ The function for this is:
 - ♦ `setwd()`
- ♦ Can also do it interactively using the menu bar.
 - ♦ In RStudio: Session -> Set Working Directory -> Choose Working Directory.
- ♦ Verify using
 - ♦ `getwd()`

Working with a dataset

(Read, Subset, Visualize)

Reading a dataset

- ◆ Function we will use: `read.table()`.
- ◆ Dataset used: Iris dataset.
 - ◆ Iris is a plant genus.
 - ◆ Species of this genus may be identified based on the dimensions of petals and sepals of its flowers.
- ◆ Try `read.table("iris.csv")`.
 - ◆ `dat <- read.table("iris.csv")`

Explore the current data

- ♦ `View(dat)` #Shows the data in a tabular format.
- ♦ `dim(dat)` #Shows the dimensions of the table, i.e. rows and columns.
- ♦ `colnames(dat)` #Shows the names of columns, if any.
- ♦ `summary(dat)` #Outputs summary statistics of data. Ex- minimum, max, median, etc.
- ♦ `head(dat)` #Displays first 6 rows of data.
- ♦ `tail(dat)` #Displays last 6 rows of data.

Extracting parts of data in another variable.

- ♦ `spl_len <- dat$Sepal.Length`
 - ♦ `$` is used to refer to a feature in the data.
 - ♦ Check what `spl_len` is.
 - ♦ `class(spl_len)`
- ♦ It contains numbers, makes sense to call it “numeric”.
- ♦ `spl_len` is an ordered series of numeric values.
 - ♦ Ordered series of values are called vectors in R. (More on that later)

Another data type: *Factor*

- ◆ `spcs <- dat$Species`
 - ◆ Check what `spcs` is.
 - ◆ Factor variable.
 - ◆ `spcs <- as.character(spcs)`
 - ◆ `spcs <- unique(spcs)`
 - ◆ How to check if `spcs` includes `sapiens`?
 - ◆ `"sapiens" %in% spcs`
- ◆ Factors are used to represent categorical variables.
- ◆ `spcs` is now a character vector.

Goal: To extract subset of data for *Iris setosa*.

- ♦ `which_setosa <- dat$Species == "setosa"`
 - ♦ `dat$Species == "setosa"` compares each value in `dat$Species` with character "setosa".
 - ♦ If a value equals "setosa", output is TRUE.
 - ♦ Check what `which_setosa` is. (a logical vector)
- ♦ To extract the subset for *Iris setosa*:
 - ♦ `dat_setosa <- dat[which_setosa, ]`

Goal: To extract subset of data for *Iris setosa*.

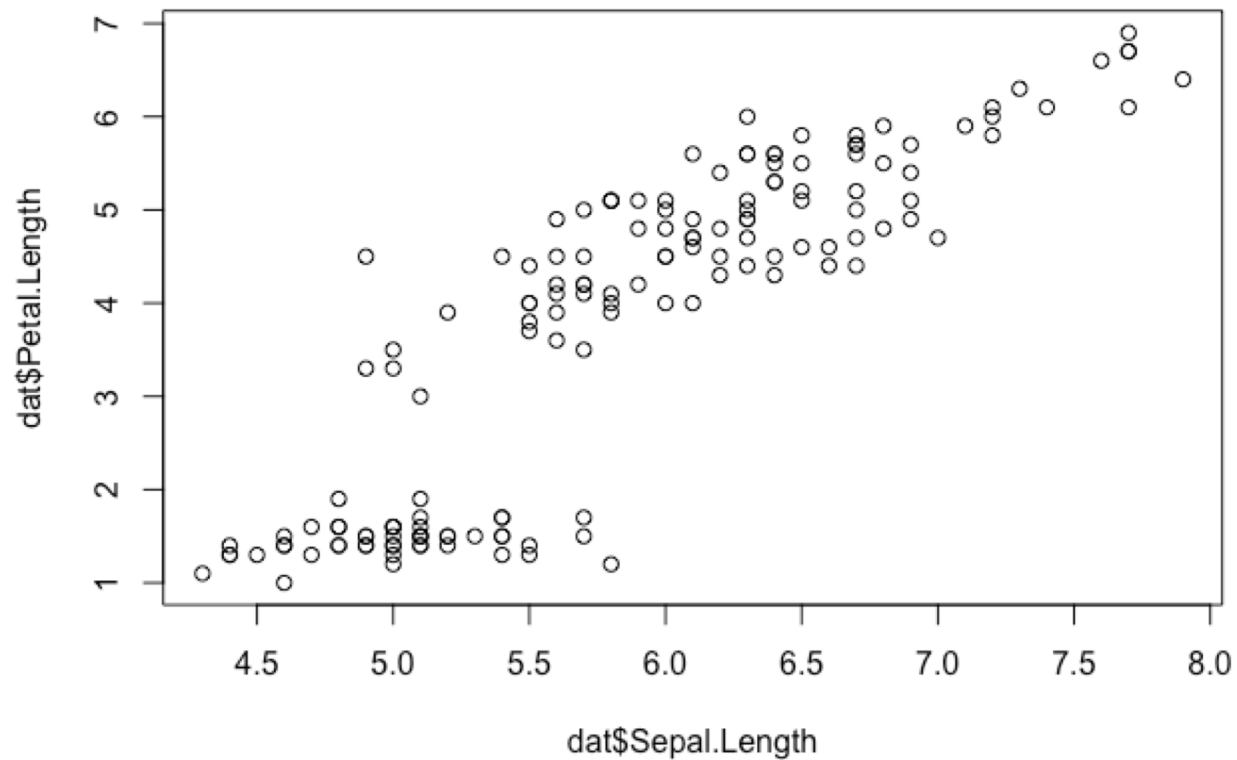
- ✦ Alternatively
 - ✦ `dat_setosa <- subset(dat, Species == "setosa")`
 - ✦ If only I had googled!!
- ✦ Wishing you could just hit enter?
- ✦ Feeling like a lot of people would want it?
- ✦ Google!
- ✦ TRUE for most anything at a beginner level.
- ✦ If you get an error, someone else would have got it too. Google!

Basic statistics.

- ◆ Mean
 - ◆ `mean(dat$Sepal.Length)`
- ◆ Median
 - ◆ `median(dat$Petal.Length)`
- ◆ Standard deviation
 - ◆ `sd(dat$Petal.Width)`
- ◆ Histogram
 - ◆ `hist(dat$Sepal.Width)`
- ◆ Boxplot
 - ◆ `boxplot(dat$Sepal.Length)`

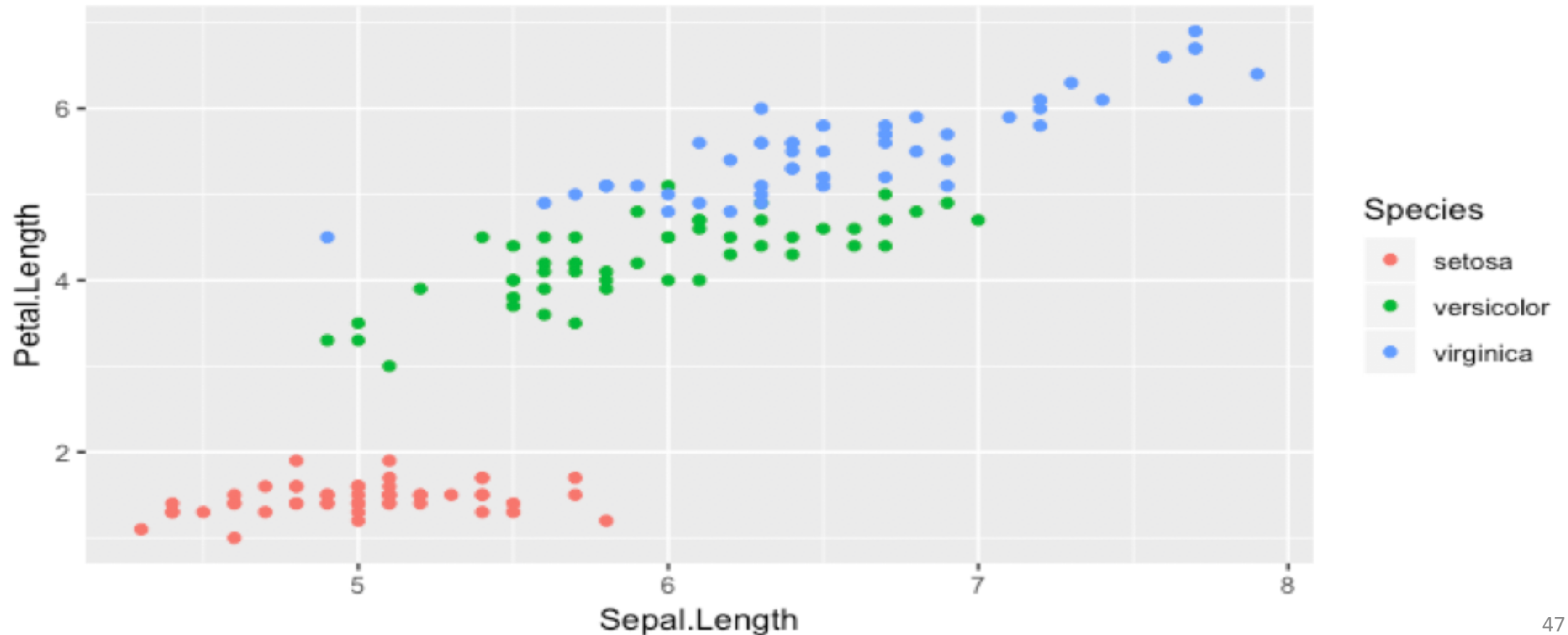
Visualizing data.

✦ `plot(x= dat$Sepal.Length, y = dat$Petal.Length)`



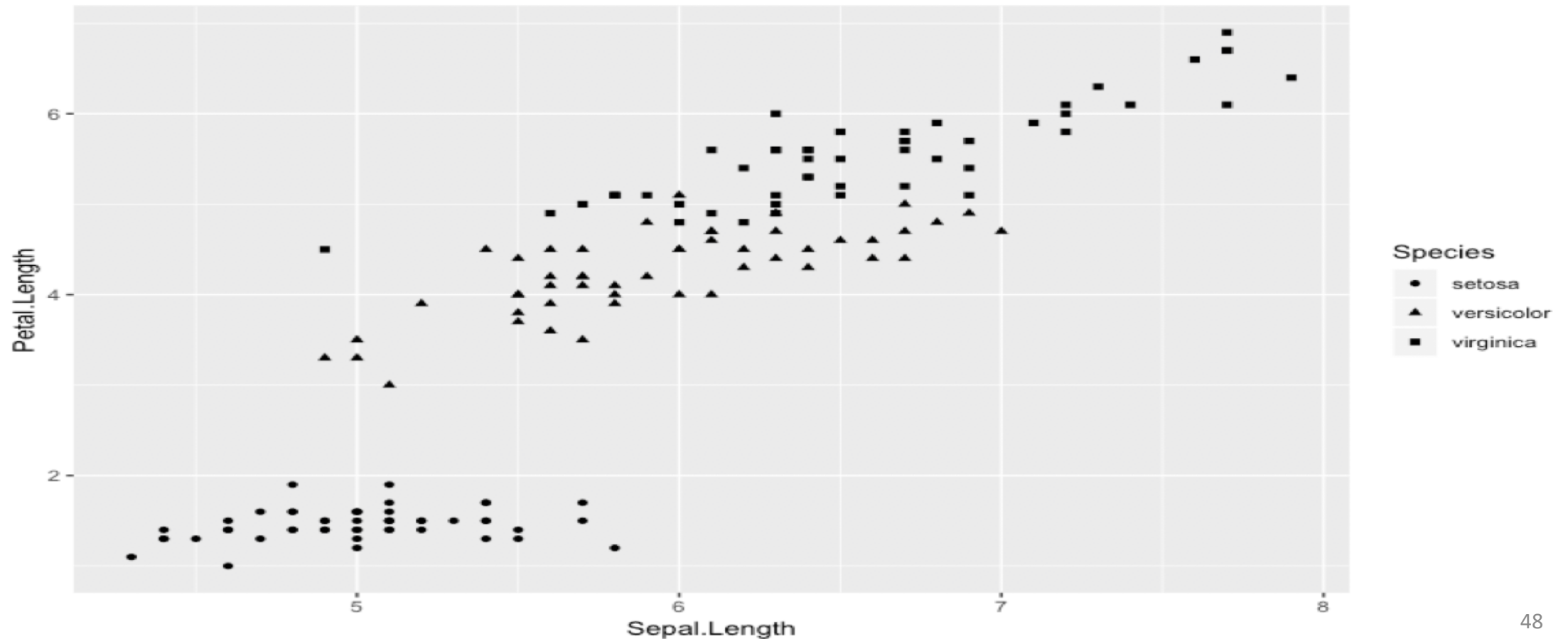
Visualizing data.

✦ `qplot(x = Sepal.Length, y = Petal.Length, data = dat, color = Species)`



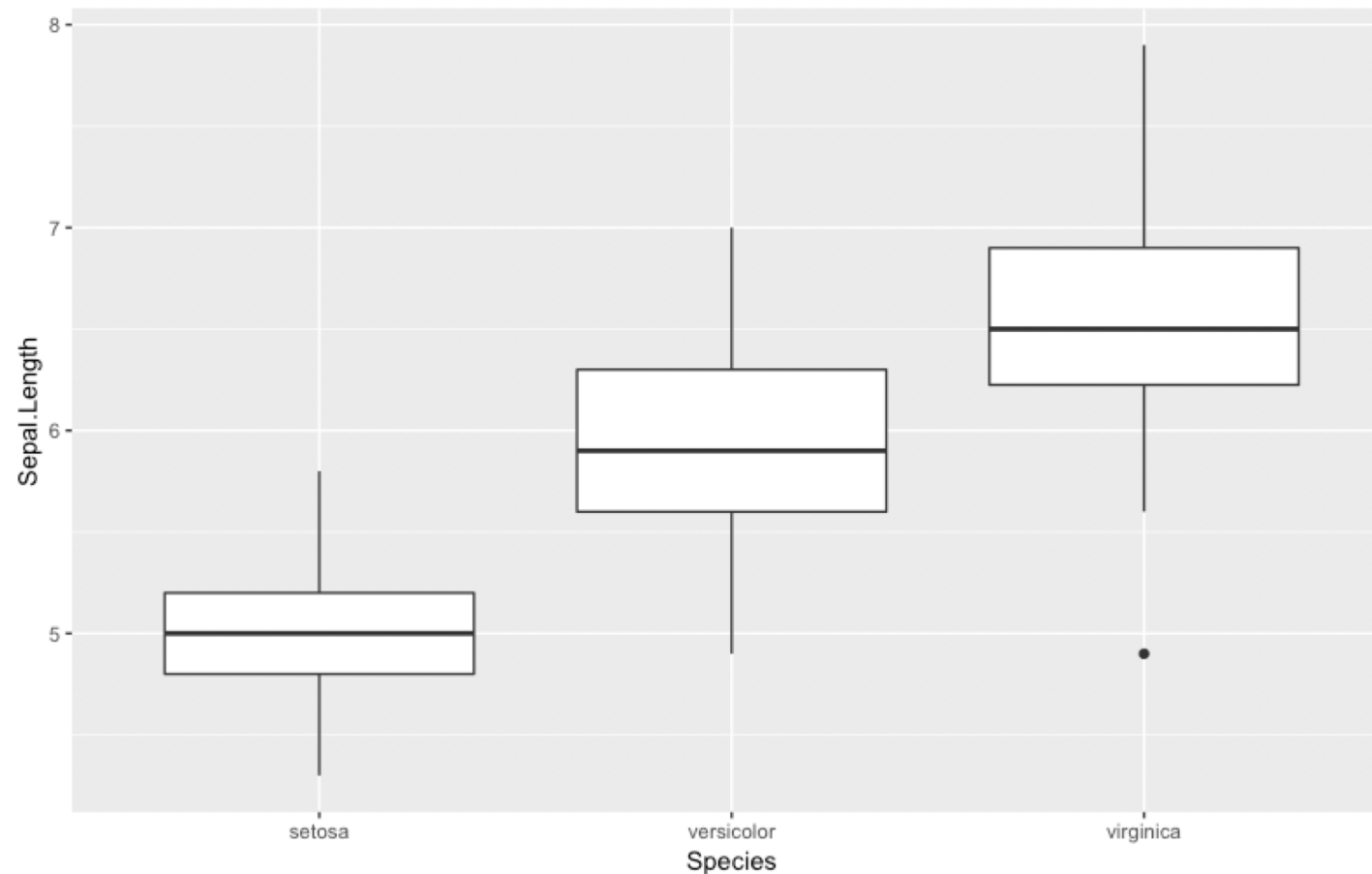
Visualizing data.

✦ `qplot(x = Sepal.Length, y = Petal.Length, data = dat, shape = Species)`



Visualizing data.

- ◆ `qplot(x = Species, y = Sepal.Length, data = dat, geom = "boxplot")`



Loops and conditional statements.

Loop over rows in a tabular data

```
◆ table <- read.table("iris.csv")
◆ for (row in table) {
    #Do some calculation for each row
    ...
    ...
}
```

Conditional statements

```
♦ if (p < 0.05) {  
    print(gene_info)  
    ...  
} else {  
    print(gene_name)  
}
```

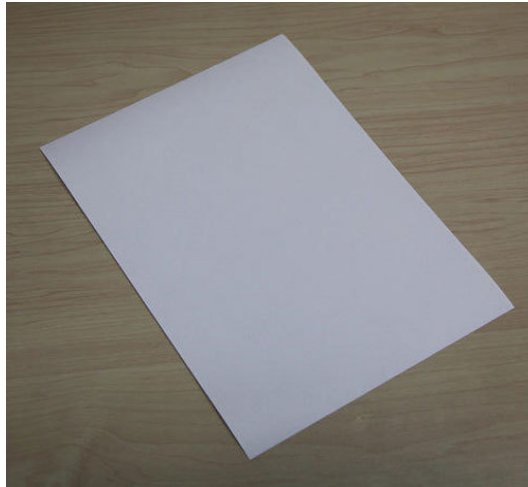
Data structures.

Data structures are data organization/storage formats.

✦ Storing data physically? Options:



Sticky note



Printer paper



Task pad



Long scroll

Data structures are data organization/storage formats.

- ♦ Working with data in R? Options:
 - ♦ Numeric vector
 - ♦ `c(2, 5, 10, 11)`
 - ♦ Character vector
 - ♦ `c("apples", "oranges", "butter", "dry yeast")`
 - ♦ Data frames
 - ♦ `dat` from Iris example.
 - ♦ Matrix
 - ♦ Example counts for genes in several samples.
 - ♦ Lists
 - ♦ Flexible data structures.
 - ♦ `mylist <- list(a = c(2, 5, 10, 11),
 b = c("apples", "oranges", "butter", "dry yeast")
)`

Data structures for special needs?

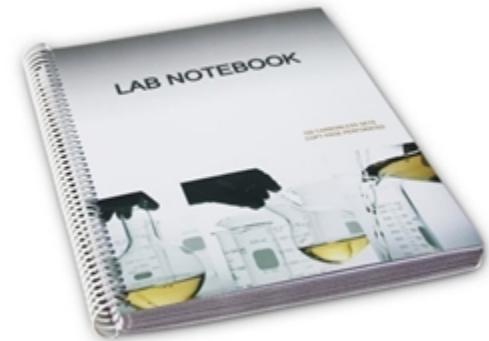
- ✦ Storing data physically? Options:



Sheet music



Ledger



Lab notebook

Data structures for special needs?

- ✦ Working with data in R?
 - ✦ Packages may have author-defined data structures.
- ✦ Example – DGEList defined in edgeR.
 - ✦ Stores counts, sample grouping information, normalization factors, etc. in one place.

Exercise

Reading unclean data.

- ✦ “dirty_data.txt” contains data that is formatted differently.
- ✦ Figure out how to read it.
- ✦ Tips:
 - ✦ Error message comes in peace. It is your friend!
 - ✦ Google!

Alternative file types

- ♦ Scientific studies may involve various kinds of files.
- ♦ Example:
 - ♦ FASTA files for sequences.
 - ♦ FASTQ files for sequencing reads.
 - ♦ SAM files for alignment.
 - ♦ GTF files for gene annotations.
 - ♦ Excel sheets with multiple sheets.
- ♦ Google for how to read FASTA file in R. (5 min)

How to read in FASTA files?

- ♦ Searching for packages returns:
 - ♦ seqinr
 - ♦ Biostrings
 - ♦ ...
- ♦ Example with seqinr
 - ♦ `install.packages("seqinr")`
 - ♦ `library(seqinr)`
 - ♦ `read.fasta("16S.fasta")`
- ♦ Check options available with `read.fasta`.
 - ♦ `?read.fasta`

Recap of R topics covered

- ◆ RStudio interface.
- ◆ Addition, subtraction, basic math operations.
- ◆ Assigning values to variables.
- ◆ Commenting in a script.
- ◆ Logical operators.
- ◆ Intro to functions and libraries.
- ◆ Reading data.
- ◆ Troubleshooting error messages.
- ◆ Exploring data. (Basic summaries such as mean, median, etc.)
- ◆ Selecting subsets of data.
- ◆ Plotting data.
- ◆ Data structures available in R.

Your feedback is important to us!

- ✦ <https://www.surveymonkey.com/r/RRTZPTC>
- ✦ ~3 min.

Possibilities

Detailed high resolution graphics : Examples

- ✦ <https://www.r-graph-gallery.com/>

Bioconductor for biological data analysis

Packages

- ♦ edgeR, DESeq2, limma, voom
- ♦ phyloseq, microbiome
- ♦ rWikipathways, KEGGgraph
- ♦ MSstats
- ♦ bsseq, methylKit
- ♦ chipseq
- ♦ CRISPRseek, gCrisprTools, CrispRVariants

Uses

- ♦ Differential gene expression analysis
- ♦ Microbiome data analysis
- ♦ Pathway analysis
- ♦ Proteomic data analysis
- ♦ DNA methylation analysis
- ♦ ChIP-Seq data analysis
- ♦ CRISPR related data analysis

Conclusions

- ✦ R is a “smart” version of calculators.
- ✦ Error message comes in peace.
- ✦ Google!

Upcoming workshops

- ✦ Advanced Cytoscape Automation (Oct 6)
- ✦ Introduction to Pathway Analysis Using R (Oct 13)
- ✦ Intro to RNA-seq analysis (Oct 26-27)





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