# Introduction to RStudio Server

The CCDL

The following two analysis examples are both microarray differential expression analyses.

#### Differential Analysis Example 1: "100% Up to Date Analysis"

**Methods Documentation:** ~200 words describe the general methods in a publication. The step-by-step recount of how these data came to be were handwritten, with one copy located in a lab closet.

**Data availability:** The original data are on a flashdrive, in a desk drawer, in a lab. The already processed version is on <u>GEO</u>.

**Project organization:** Many files with various nebulous terms like "MasterSheet" and "Sorted" and "Edit" and "100% Up to Date".

Software versions: No idea what software package(s), regardless of version.

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#### Data Analysis Example 2: "GitHubbed Analysis"

Methods Documentation: Publicly available, online notebooks show step-by-step process that can be re-run.

Data availability: Data that was originally used is available for download online.

Project organization: Folders labeled, most recent is present, with prior versions tracked on GitHub.

Software versions: Specific requirements and version numbers shown in notebook and README.

#### 1) Install libraries

2) Import and set up data
 3) Set up design matrix

4) Apply linear model

5) Explore fitness of model

6) Write statistics to output results file

#### Differential Expression Analysis: Microarray

#### ALSF CCDL - Candace Savonen

Purpose: This notebook takes data and metadata from refine.bio and identifies differentially expressed genes. This script is generally applicable to microarray data.

#### 1) Install libraries

Cn

This script uses the bioconductor R package limma to identify differentially expressed genes.

The full guide on limma shows examples of limma functions. *Citation:* Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK (2015). "limma powers differential expression analyses for RNA-sequencing and microarray studies." Nucleic Acids Research, 43(7), e47.

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Code -

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    # Create the plots folder if it doesn't exist
    if (ldir.create("plots")) {
        dir.create("plots")
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#### refine.bio Example Workflow: Differential expression

refine.bio includes both microarray and RNA-seq experiments. The methods for performing differential gene expression (DGE) analysis for each technology differ. In this module, we include simple two-group comparison examples for each.

#### Contents

- Microarray notebook: takes microarray data and metadata from refine.bio and identifies genes that are differentially expressed between two groups.
- RNA-seq notebook: takes RNA-seq data without quantile normalization from refine.bio and identifies genes that are differentially expressed between two groups. Read more about skipping quantile normalization here in our documentation.
- GenePattern differential expression analysis: GenePattern modules can be run via a GUI. To use refine.bio data with GenePattern, you will need to change the format as described in this section.

#### **Requirements and usage**

This module requires you to install the following software to run examples yourself:

• R

- · RStudio for working with R Notebooks.
- Bioconductor
- tidyverse

These requirements can be installed by following the instructions at the links above. The example R Notebooks are designed to check if additional required packages are installed and will install them if they are not.

"100% Up to Date Analysis" OR "GitHubbed Analysis"

Which analysis would you...

...be more inclined to borrow the methods from?

...trust the methods of more?

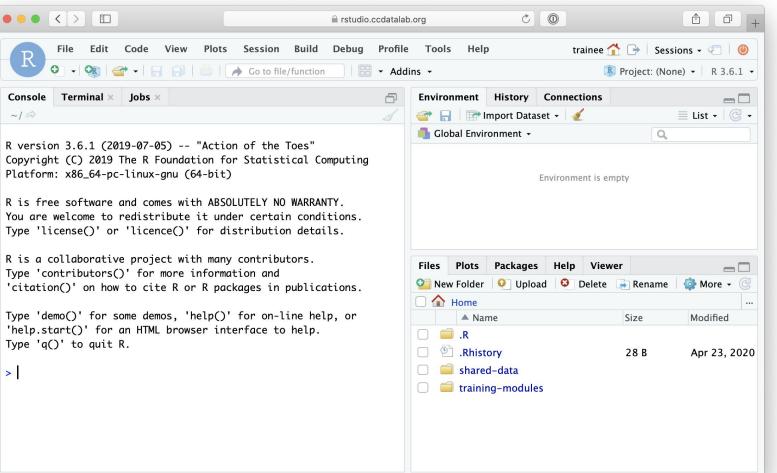
...feel would be easier to reproduce?

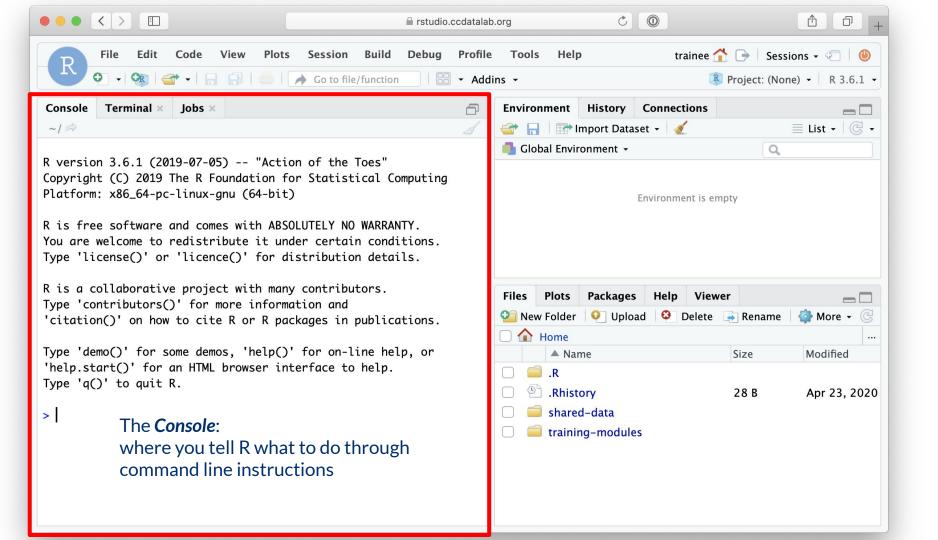
## Command line vs GUI (graphics user interface)

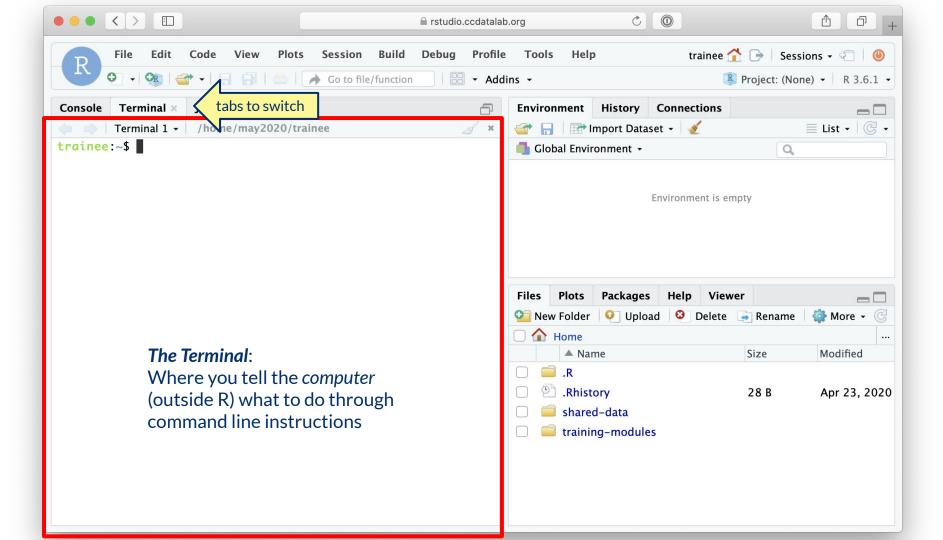
- An interface is how you interact with a program

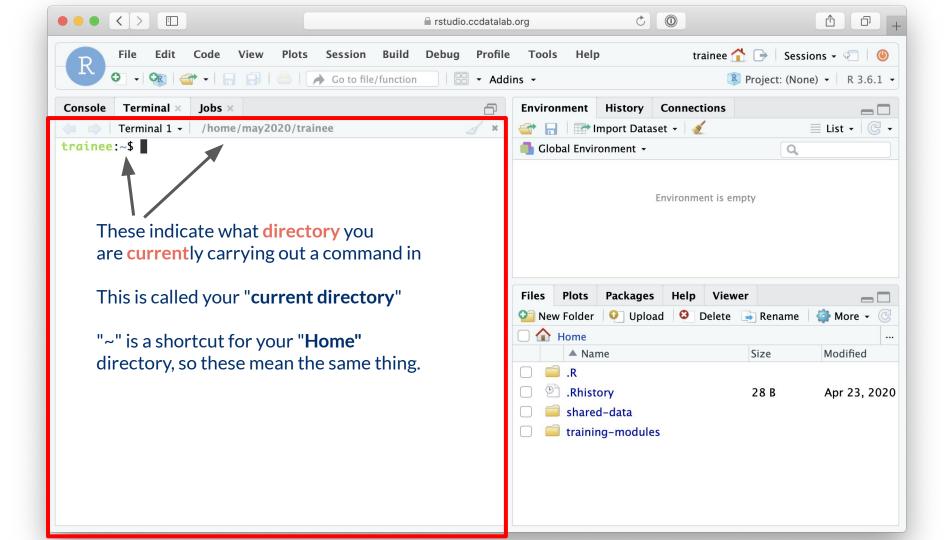
- GUI's have buttons you can *click* to do things,
- Command-line interfaces have you type out things to do them

## RStudio Server: A basic guide



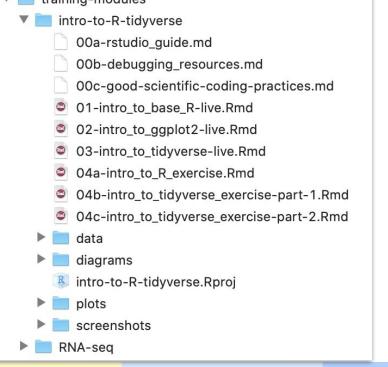


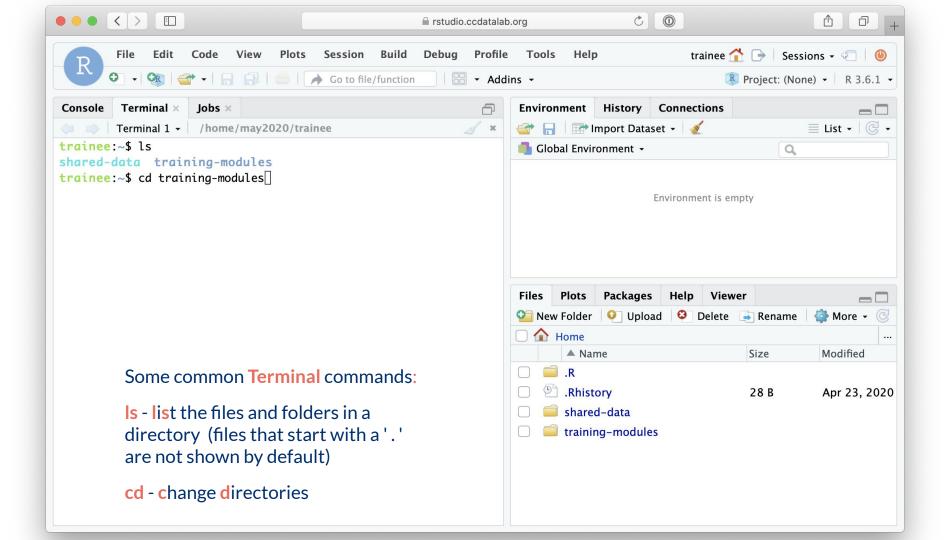


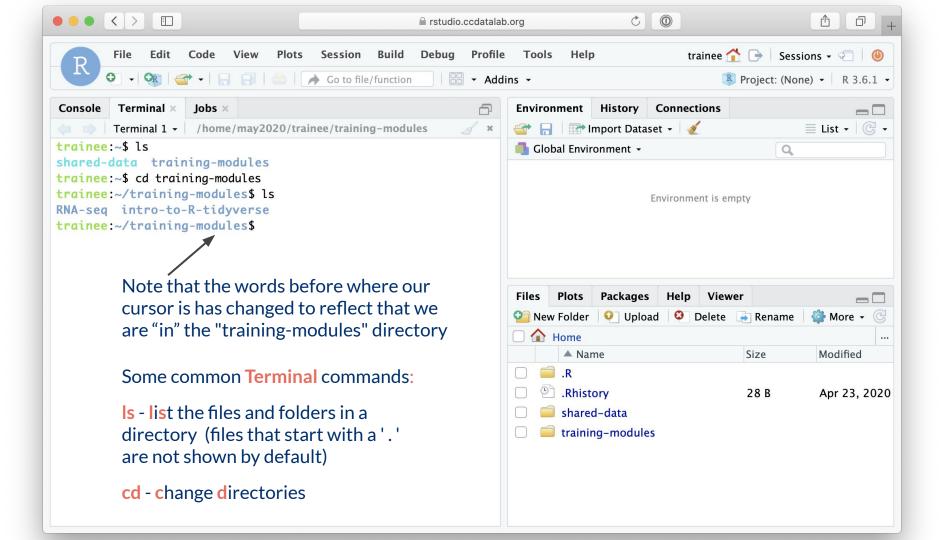


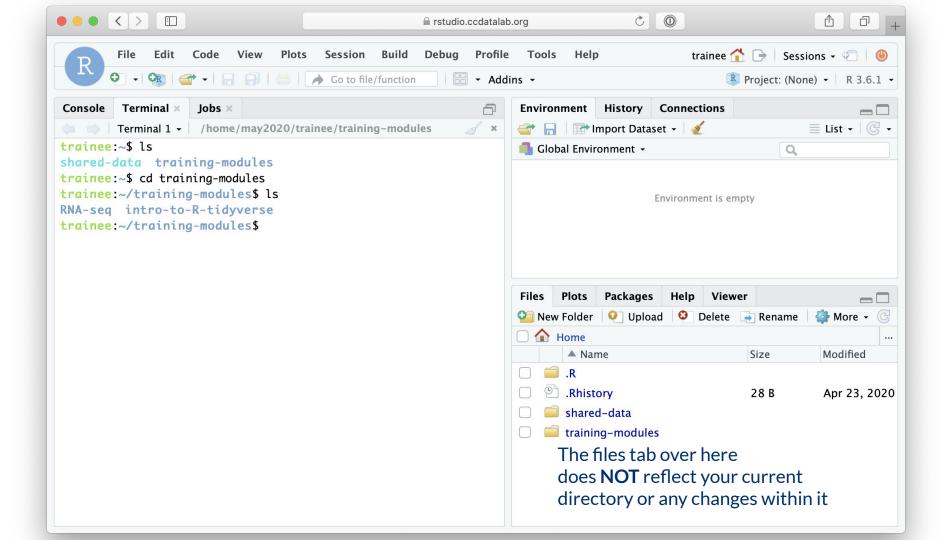
## **Directories = Folders**

When we are working on the command line, we have to keep track of where the files we are using are being kept. Training-modules









## File paths: Directions to a file or folder

Let's say we want access to "01-intro\_to\_base\_R-live.Rmd"

▲  home > training-modules ∨
Name ^
intro-to-R-tidyverse
00a-rstudio_guide.md
00b-debugging_resources.md
00c-good-scientific-coding-practices.md
01-intro_to_base_R-live.Rmd
02-intro_to_ggplot2-live.Rmd
03-intro_to_tidyverse-live.Rmd
04a-intro_to_R_exercise.Rmd
04b-intro_to_tidyverse_exercise-part-1.Rmd
04c-intro_to_tidyverse_exercise-part-2.Rmd
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diagrams
intro-to-R-tidyverse.Rproj
plots
screenshots
▶ 📃 RNA-seq

Current directory = "training-modules"

File path = "intro-to-R-tidyverse/01-intro\_to\_base\_R-live.Rmd"

## File Paths can be relative

#### Let's say we want access to "01-intro\_to\_base\_R-live.Rmd"

▲  home > training-modules ∨	▲   home > training-modules > intro-to-R-tidyverse ∨
Name A	Name ^
intro-to-R-tidyverse	00a-rstudio_guide.md
00a-rstudio_guide.md	00b-debugging_resources.md
00b-debugging_resources.md	00c-good-scientific-coding-practices.md
00c-good-scientific-coding-practices.md	01-intro_to_base_R-live.Rmd
01-intro_to_base_R-live.Rmd	02-intro_to_ggplot2-live.Rmd
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03-intro_to_tidyverse-live.Rmd	O4a-intro_to_R_exercise.Rmd
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*Current directory* = "training-modules"

Relative file path = "intro-to-R-tidyverse/01-intro\_to\_base\_R-live.Rmd" Current directory = "training-modules/intro-to-R-tidyverse"

Relative file path = "01-intro\_to\_base\_R-live.Rmd"

## Introduction to R

The CCDL

## R programming

Programming: making executable scripts for accomplishing a task (in this case, data analysis is our task)

Scripts allow others to see, step-by-step, what you did.

#### Why we use R:

- It's free
- People make cool packages that do stuff for us
- Many researchers in genomics use it (as well as Python)

## One in five genetics papers contains errors thanks to Microsoft Excel

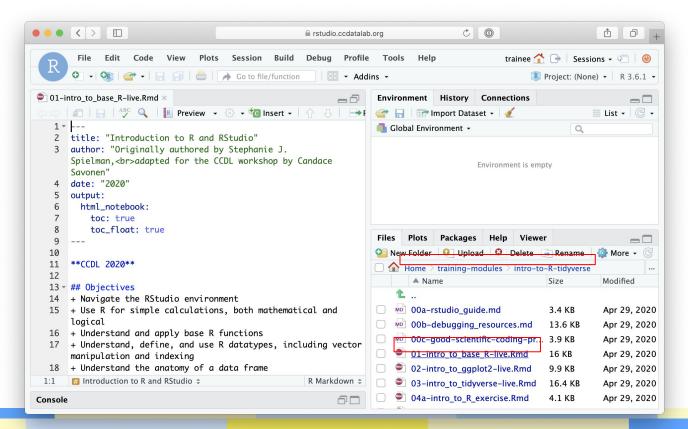
By Jessica Boddy | Aug. 29, 2016, 1:45 PM

What you type	What you see	How Excel stores it
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SEPT2	2-SEP	42615

https://www.sciencemag.org/news/2016/08/one-five-genetics-papers-contains-errors-thanks-microsoft-excel Ziemann et al. Genome Biology (2016) 17:177 DOI 10.1186/s13059-016-1044-7

## **R** Notebooks

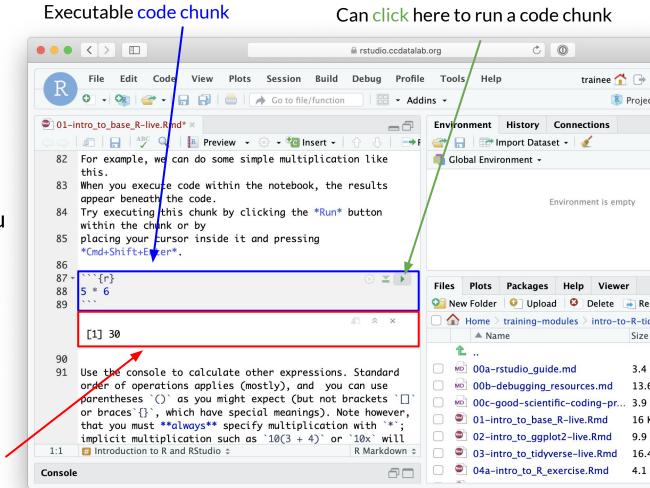
Use the "Files" tab to open: training-modules/intro-to-R-tidyverse/01-intro\_to\_base\_R-live.Rmd



## **R** Notebooks

 R Notebooks allow you to have files that show both your code and results

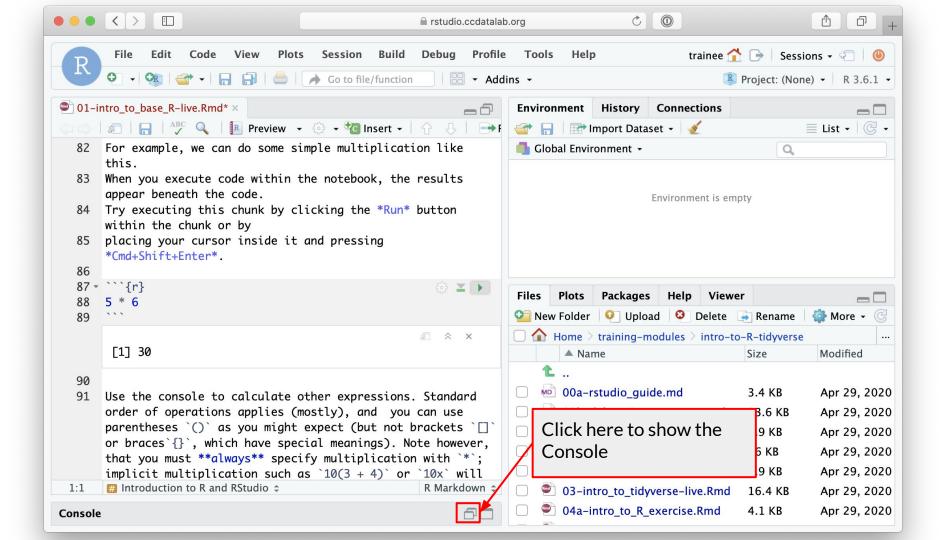
Output from above code chunk



## **R** Notebooks

- Code that runs in R Notebooks uses <u>wherever the file is</u> <u>saved</u> as its current directory
- Warning! That may not be the directory shown in the files pane *or* the console!

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113	$\frac{1}{2} = \frac{1}{2} = \frac{1}$	Environment       History       Connections         Import Dataset -       Import Dataset -       Import Dataset -         Global Environment -       Import Dataset -       Import Dataset -         Values       X       5.5
110	[1] 5.5	Other Assistance Tabs: Things that help you in your coding
119 120 121	We can modify an existing variable by reassigning it to its same name. Here we'll add $2^{t}$ to $x^{t}$ and reassign the result back to $x^{t}$ .	Files       Plots       Packages       Help       Viewer         Image: New Folder       Image: Open content of the second s
1:1 Console ~/ ~ > x <- > x [1] 5.5 >	5.5	1          00a-rstudio_guide.md       3.4 KB       Apr 29, 2020         00b-debugging_resources.md       13.6 KB       Apr 29, 2020         00c-good-scientific-coding-pr       3.9 KB       Apr 29, 2020         01-intro_to_base_R-live.Rmd       16 KB       Apr 29, 2020         02-intro_to_ggplot2-live.Rmd       9.9 KB       Apr 29, 2020         03-intro_to_tidyverse-live.Rmd       16.4 KB       Apr 29, 2020         04a-intro_to_R_exercise.Rmd       4.1 KB       Apr 29, 2020

## **RStudio Sessions**

- On the server, R is running many times at once
  - Each user has their own "Session" running, with its own memory and processes
  - It is possible for a user to have more than one session at a time
- We will usually want to start new sessions between notebooks to keep the environment clean

