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")) {
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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?

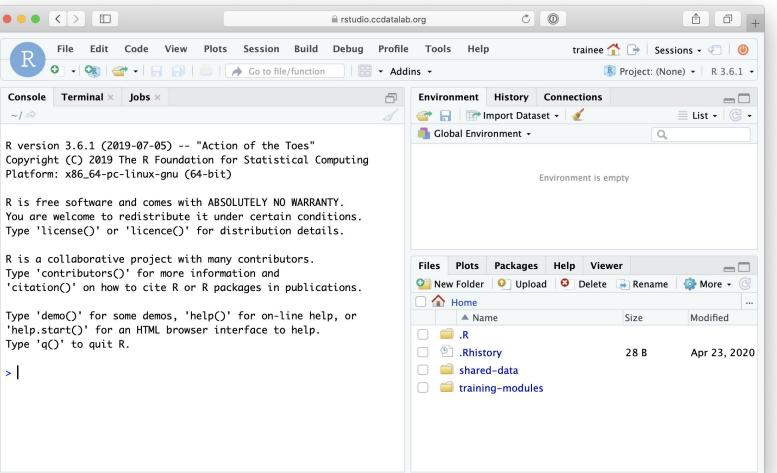
...feel would be easier to reproduce?

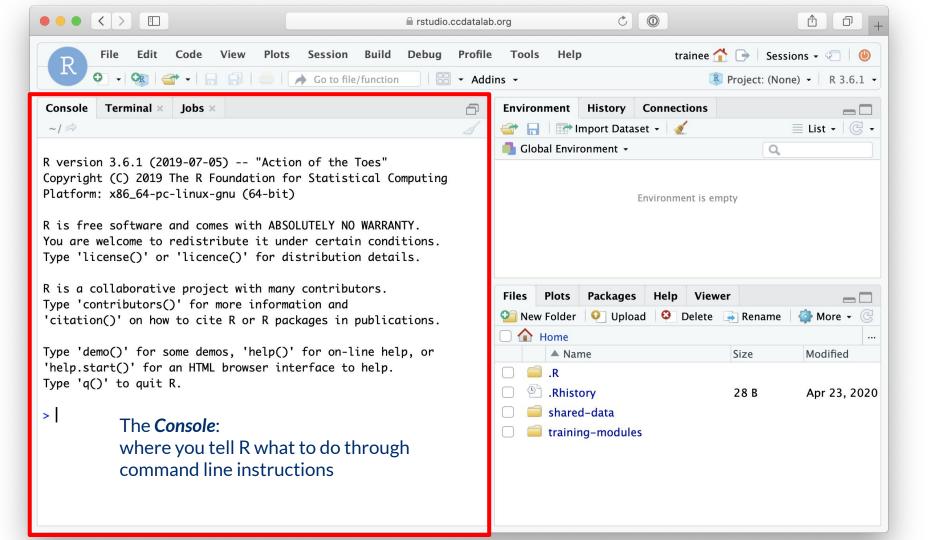
Command line vs GUI (graphics user interface)

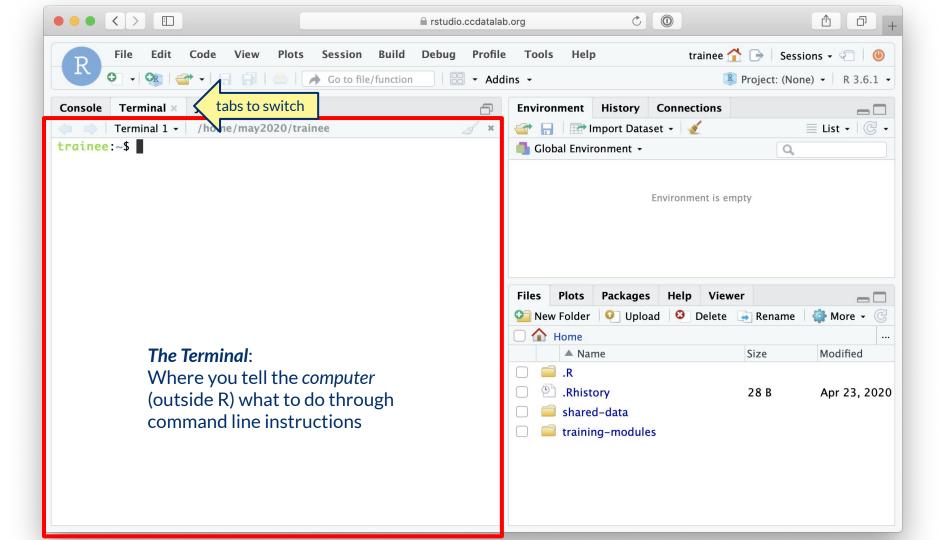
- An interface is how you interact with a program

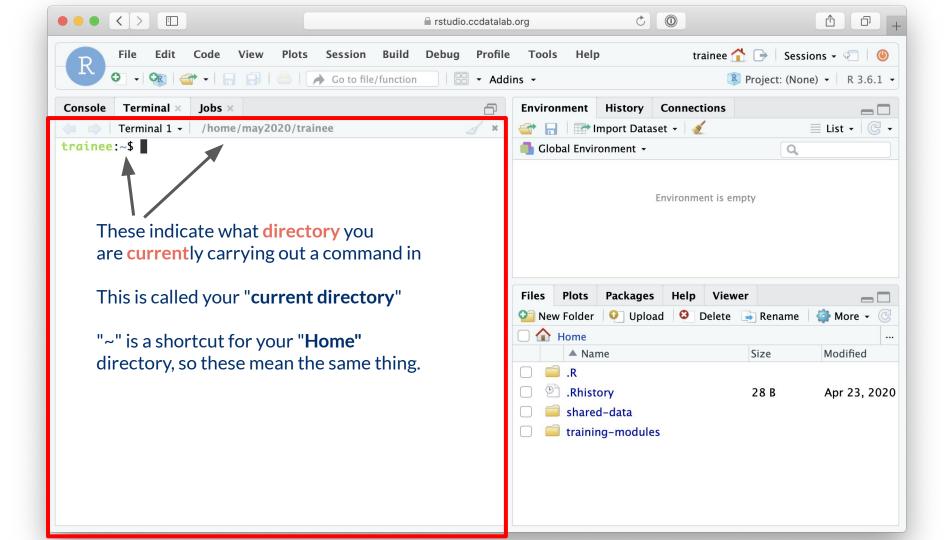
- GUI's have buttons you can *click* to do things,
- Command-line interfaces have you type out things to do them

RStudio Server: A basic guide



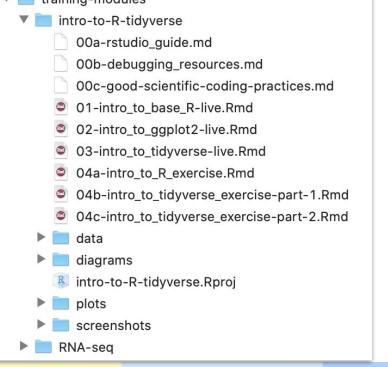


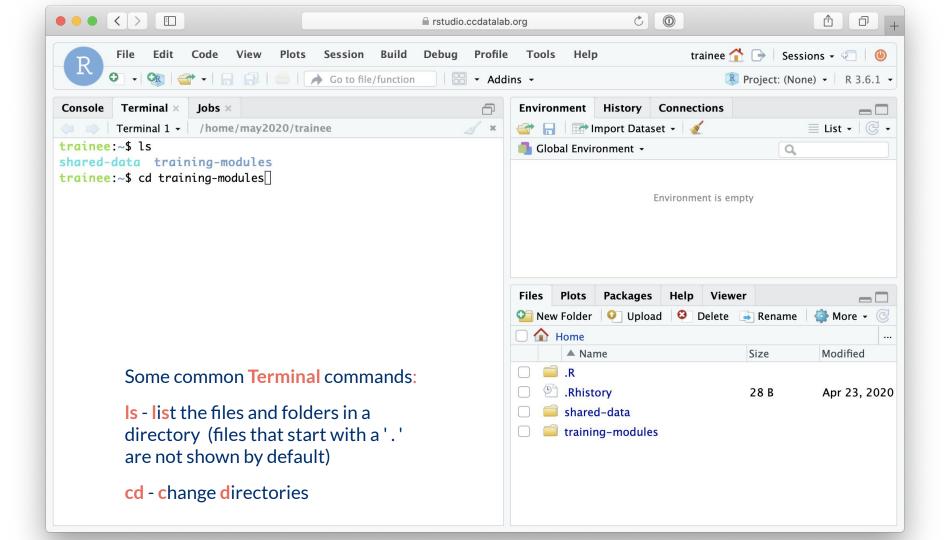


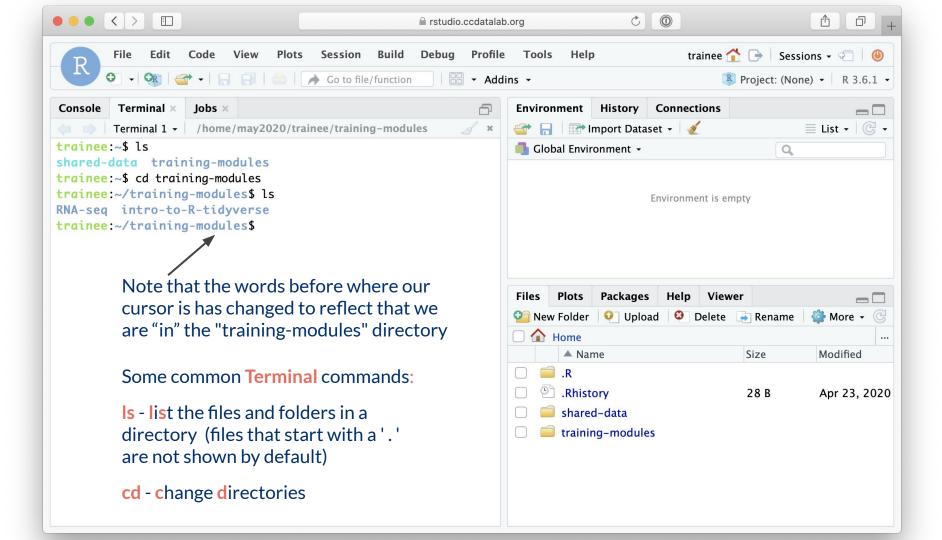


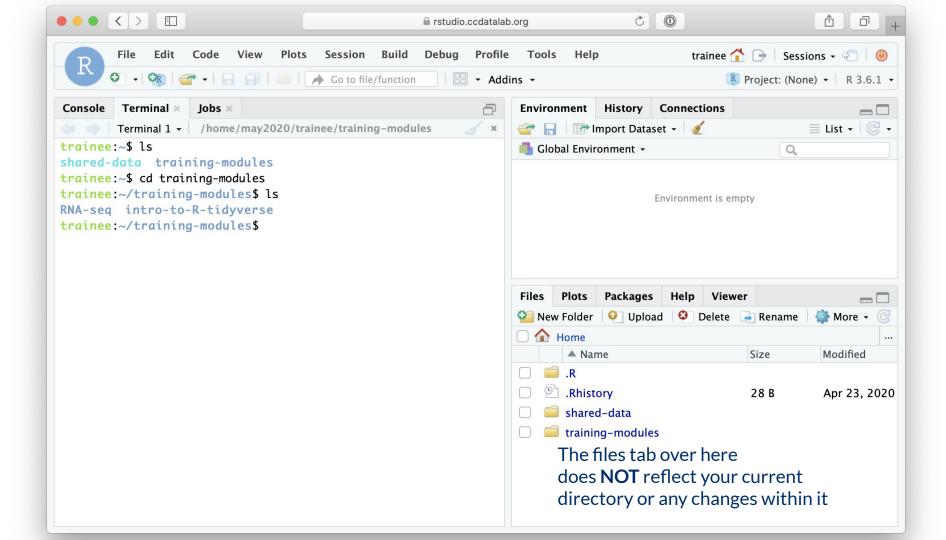
Directories = Folders

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
🕨 🚞 data
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 ∨
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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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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

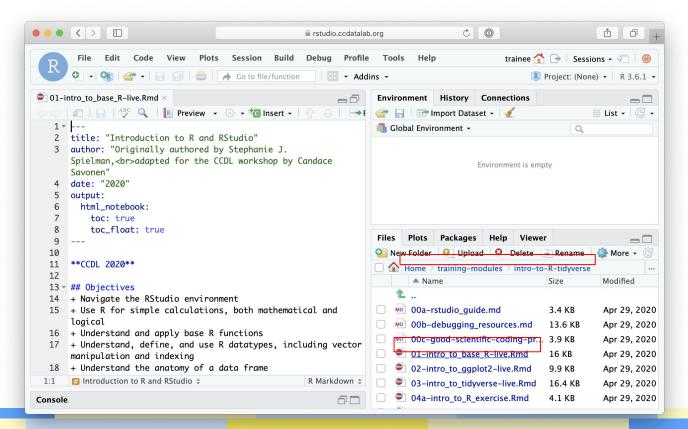
By Jessica Boddy | Aug. 29, 2016, 1:45 PM

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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

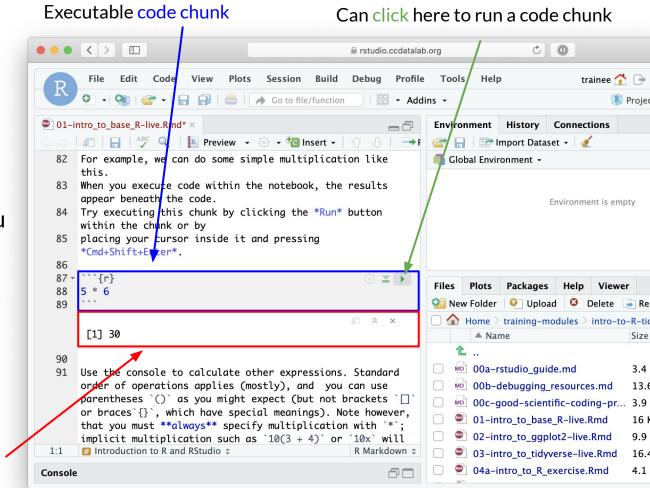
Use the "Files" tab to open: training-modules/intro-to-R-tidyverse/01-intro_to_base_R-live.Rmd



R Notebooks

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

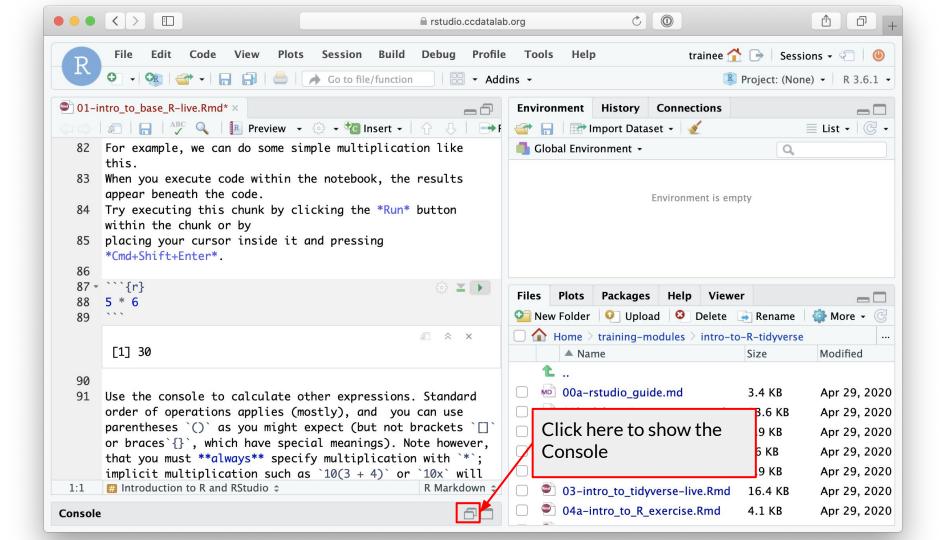
Output from above code chunk



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

